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Correlation Between ABCA1 Gene Polymorphism and aopA-I and HDL-C in Abdominal Aortic Aneurysm

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Background: As the most common type of aneurysm, abdominal aortic aneurysm (AAA) has an unfavorable prognosis due to the high frequency of rupture. Studies have indicated a close relationship between the pathogenesis and progression of AAA and abnormal serum lipid levels. ATP-binding cassette transport protein A1 (ABCA1) is a cell-surface protein facilitating cellular efflux of cholesterol. The single-nucleotide polymorphism (SNP) of ABCA1 gene has been suggested to be correlated with abnormal metabolism of lipids. Therefore, this study aimed to investigate the relationship between ABCA1 polymorphism and apoA-I and HDL-C in an attempt to elucidate its correlation with AAA occurrence.

Material/Methods: We included 126 AAA patients and 119 healthy controls in this study. PCR and restriction fragment length polymorphism (RFLP) were used to detect the SNP pattern of ABCA1 gene at locus rs2230806 from both AAA patients and healthy controls. The distribution pattern and correlation with apoA-I and HDL-C was analyzed.

Results: The distribution of KK/RR genotype of ABCA1 gene had significant difference between disease and control group, with lower rates of RR genotype and R allele in the disease group ($p<0.05$). Levels of apoA-I and HDL-C, but not triglyceride and LDL-C levels, in AAA patients who carried R allele in ABCA1 gene (including RR and RK genotypes) were higher than in non-carriers ($p<0.05$). The R allele of ABCA1 gene was shown to be related with the occurrence of AAA ($p<0.05$).

Conclusions: Polymorphism of ABCA1 gene is correlated with AAA occurrence, possibly via the regulation of serum lipid metabolism by R allele.

MeSH Keywords: Aortic Aneurysm, Abdominal • Apolipoprotein A-II • ATP Binding Cassette Transporter 1

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Background

As the most common aneurysm, abdominal aortic aneurysm (AAA) mainly occurs in middle-aged or elderly people, and shows an increasing trend worldwide. With the transition of demographic distribution toward an aging society, the incidence of AAA in China is also increasing [1,2]. Although having a benign nature, AAA still has relatively high mortality due to rapid progression and eventual rupture without timely diagnosis and intervention [3]. Therefore, understanding the pathogenesis of AAA can greatly benefit screening in high-risk populations for making timely diagnosis and treatment, and thus has become a research focus in recent years.

The etiology of AAA involves a complicated process containing a cascade reaction with multiple factors and steps, including genetic/environmental factors and abnormal anatomy, and alternation of hemodynamics and inflammatory response [4–6]. Among all those risk factors, atherosclerosis and abnormal blood lipid are commonly accepted as important predisposing factors for AAA [7]. ATP-binding cassette transport protein A1 (ABCA1) is the most important cell-surface protein facilitating efflux of cholesterol identified to date [8]. It exerts its function mainly through the regulation of reverse cholesterol transport (RCT), thus mediating the level of high-density lipoprotein (HDL) [9]. In patients with either Tangier disease or HDL deficiency, mutations of ABCA1 gene have been identified, causing deficits of apolipoprotein A-I (apoA-I) and HDL, leading to the insufficiency of serum HDL and further atherosclerosis [10,11]. Genetic studies have found the prominent distribution of ABCA1 mutations in the binding domain and N-terminal region of this gene [12]. In certain atherosclerosis patients, single-nucleotide polymorphism (SNP) of ABCA1 gene may also alter the function of this gene [13]. Other studies have also confirmed the correlation between SNP of ABCA1 gene and both atherosclerosis and lipid metabolic disorders [14]. Therefore, this study aimed to investigate the correlation between ABCA1 gene polymorphism of AAA patients and apoA-I and HDL-C, in an attempt to analyze its relationship with AAA pathogenesis.

Material and Methods

Patient information

A total of 126 AAA patients (including 86 males and 40 females, ages 12~89 years, average age=62.3±12.7 years old) who were diagnosed and treated in our hospital between January 2012 and December 2014 were recruited in this study. All patients received confirmed diagnosis of AAA via abdominal ultrasound, Doppler ultrasound, MSCTA, or DSA. All patients had enlargement of abdominal aorta more than 50% of normal value. None of the included patients were blood relatives. We recruited 119 healthy volunteers from routine health check-ups in our hospital. Among the control group, there were 72 males and 47 females, ages 12~78 years (average age=61.5±14.1 years old). Control group individuals had no atherosclerosis and had normal blood sugar/lipid levels as determined by ultrasound and laboratory tests. Any medications that potentially affect lipid metabolism were suspended for 2 weeks before collecting the blood samples. All participants were Chinese Han people. This study was pre-approved by the ethics committee of our hospital and we obtained written consents from all participants or their guardians.

Blood sample collection

Fasted venous blood samples (10 mL) were collected within 24 h after admission from elbow vein puncture on all patients. Samples from the control group were collected in the same way. Blood samples were aliquoted for biochemical assays and whole-genomic DNA extraction. Generation information of participants, including age, sex, and body-mass index (BMI), were recorded along with other risk factors, including blood pressure and smoking habits.

Biochemical assays

An automatic biochemical analyzer was used to detect biochemical indexes, including triglyceride (TG), HDL-C, total cholesterol (TC), and low-density lipoprotein (LDL-C) by standard enzymatic assays. ApoA-I level was determined by immunity transmission turbidity assay.

Whole-genome DNA extraction

Genomic DNA was extracted from peripheral erythrocytes using a whole-blood genomic DNA extraction kit (Shanghai Shenggong Bioengineering Technology, China) following the manual instructions. In brief, 1 mL erythrocyte lysis buffer were mixed with 0.6 mL anticoagulated blood. After centrifugation at 12 000 rpm for 11min and discarding the supernatant, 0.2 mL solution A (PBS buffer) was added in the precipitation for re-suspension, followed by the addition of 20 μL RNase A (10 mg/mL). The mixture was then incubated at room temperature for 10 min, followed by the addition of 20 μL proteinase K (10 mg/mL). The sample was then resolved by 65°C water bath and loaded onto the absorbent column, which was centrifuged and eluted by elution buffer. The extracted genomic DNA was stored at –20°C for further use.

PCR amplification and genotyping

The PCR reaction mixture included specific primers (0.1 μM; Forward, 5'-GTATT TTTGC AAGGC TACCA GTTAC ATTTG ACAA-3';

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Table 1. General information of AAA patients and controlled individuals.

	Control	Disease	P value
Age (years)	62.3±12.7	61.5±14.1	0.892
BMI(kg/m^2)	24.61±3.95	24.91±3.81	0.782
Sex (Male/Female)	86/40	72/47	0.569
Smoking (%)	27 (21%)	24 (20.1%)	0.808
Diabetes (%)	24 (19.5%)	22 (18.5%)	0.792
Hypertension (%)	46 (36.5%)	22 (18.5%)	0.002
FBG (mM)	5.42±1.21	5.31±0.92	0.951

Table 2. Blood lipid indexes between disease and control groups.

	Control	Disease	P value
TC (mM)	4.98±1.09	4.87±0.98	0.982
TG (mM)	3.05±0.69	1.25±0.31	0.024
apoA-I (g/L)	1.45±0.51	2.07±0.26	0.019
HDL-C (mM)	1.21±0.16	1.37±0.28	0.033
LDL-C (mM)	3.01±0.51	2.17±0.61	0.021

Reverse, 5'- GATTG GCTTC AGGAT GTCCA TGTTG GAA-3'; designed as previously documented [15] and synthesized by Shanghai Shenggong Bioengineering Technology, China), 0.5 µg of genomic DNA, 0.25 mM dNTPs, Tag polymerase, 2 mM $MgCl_2$ and 10X Tag buffer (Invitrogen, US). The amplification parameters were: 94°C per-denature for 5 min, followed by 30 cycles each consisting of 94°C denature for 1 min, 58°C annealing for 50 s and 72°C elongation for 25 s. The reaction ended with 72°C elongation for another 10 min. PCR products (10 µL) were digested by 2 µL restriction enzyme (Takara, China) at 37°C for 6 h. The digestion products were further amplified and sequenced by Shanghai Shenggong Bioengineering Technology, China. Distribution of alleles and genotypes at *loci* rs2230806 (R219K) of ABCA1 gene was determined by sequencing.

Statistical analysis

SPSS 20.0 software package was used to analyze all collected data, of which enumeration data, including genotype and allele frequencies, were analyzed by chi-square test. The homogeneity of the sample was further evaluated by Hardy-Weinberg (H-W) equilibrium. Measurement data are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to compare means among multiple groups. The correlation between genotype/allele frequency and occurrence of AAA was analyzed by logistic regression analysis. A statistical analysis was defined as $p<0.05$.

Results

General information of AAA patients

We compared general information, including age, sex, smoking history, diabetes, and hypertension, between disease and control groups. No statistically significant difference regarding sex or age were identified between these 2 groups ($p>0.05$). Results (Table 1) showed significantly higher incidence of hypertension in AAA patients compared to the control group. The male-to-female ratio of AAA incidence was 2.15-fold higher ($p<0.05$). Other indexes, including BMI, smoking, diabetes, and fasted blood glucose (FBG) level, showed no significant difference between the 2 groups ($p>0.05$).

Blood lipid levels of AAA patients

As shown in Table 2, AAA patients had significantly higher TG and LDL-C levels compared to those in the control group, along with lower HDL-C and apoA-I levels ($p<0.05$ in all cases). TC, however, was not different between groups ($p>0.05$).

Genotype and allele frequency of ABCA1 gene

The distribution of genotypes in both groups fit Hardy-Weinberg equilibrium, suggesting the homogeneity of both populations. In a comparison of polymorphisms at *loci* rs2230806 (R219K) of ABCA1 gene, the frequency of KK and RR genotypes was significantly different between the disease and control groups (Table 3, $p<0.05$). The RK genotype frequency, however, had no significant difference ($p>0.05$). A further analysis of allele

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Table 3. Genotype and allele frequency of ABCA1 gene.

Group	Genotype frequency (%)			Allele frequency (%)	
	RR	RK	KK	K	R
Disease	13 (10.3%)	58 (46%)	55 (43.6%)	168 (66.7%)	84 (33.3%)
Control	23 (19.3%)	60 (53.7%)	36 (30.3%)	132 (55.5%)	106 (44.5%)
χ^2 value	3.96	0.472	4.71	6.47	6.47
P value	0.046	0.49	0.03	0.011	0.011

Table 4. ABCA1 genotype and blood lipid levels.

	KK	RK	RR
TC (mM)	4.72±1.03	4.81±0.91	4.88±1.01
TG (mM)	3.09±0.21	3.04±0.67	3.05±0.11
apoA-I (g/L)	1.21±0.41	1.48±0.36*	1.56±0.62*
HDL-C (mM)	1.01±0.11	1.26±0.28*	1.29±0.22*
LDL-C (mM)	2.95±0.69	3.03±0.52	2.98±0.72

* p<0.05 compared to those in KK genotype carriers.

Table 5. Correlation between ABCA1 genotypes and AAA occurrence.

Genotype	OR value	95% CI	P value
KK	1.77	0.26–0.85	0.329
RK	0.56	0.29–0.80	0.026
RR	0.45	0.03–0.55	0.013
R allele	0.66	0.48–1.05	0.037
K allele	0.89	0.52–0.92	0.681

frequency showed significantly lower incidence of R allele in the disease group compared to the control group (p<0.05).

Correlation between ABCA1 gene polymorphism and blood lipid level

A further analysis of the correlation between ABCA1 genotype and blood lipid level showed significantly higher apoA-I and HDL-C levels in AAA patients carrying R alleles (both RR and RK genotypes at R219K *loci*). The level of TC, TG, and LDL-C, however, showed no statistically significant difference between the 2 groups (Table 4, p>0.05).

The correlation between ABCA1 genotype and AAA incidence

Logistic regression analysis was used to analyze the correlation between ABCA1 genotypes and the occurrence of AAA. As shown in Table 5, no significant correlation was discovered between KK genotype and AAA occurrence. RR genotype, however, was correlated with AAA occurrence (OR=0.45, p<0.05), as was RK genotype (OR=0.56, p<0.05). The R allele, therefore was correlated with AAA pathogenesis (OR=0.66, p<0.05).

Discussion

As one of the most common dilated vascular diseases, AAA can be classified as symptomatic and asymptomatic sub-types. The former has relatively high diagnostic rate and treatment efficacy, but the asymptomatic AAA presents an insidious disease onset, impeding timely treatment and causing a higher mortality rate after aneurysm rupture and hemorrhagic shock. Therefore, both etiology and treatment of AAA are now receiving considerable research interest [16]. Under influences from various predisposing factors, AAA may weaken the basal layer of the aortic wall, causing elevated degradation and decreased synthesis of elastin. The hemodynamic force further expands the aneurysm, aggravating the disease condition due to atherosclerosis and vascular dilation [17]. In previous studies, controversial opinions existed regarding related factors of both pathogenesis and progression of AAA. The present study revealed the male-biased

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occurrence of AAA, which is consistent with previous reports. A significantly higher percentage of hypertension existed in the disease group when compared to the control group, suggesting the relationship between hypertensive atherosclerosis and AAA occurrence. Other studies reported smoking, obesity, and abnormal blood glucose as factors affecting AAA [18,19]. This study, however, did not find any significant difference regarding BMI, smoking, or diabetes between AAA patients and control individuals.

Abnormal blood lipid level usually induces atherosclerosis, which further facilitates the occurrence of AAA. This study found significantly elevated blood TG and LDL-C in AAA patients, as well as lower HDL-C and apoA-I levels. As the most important gene regulating human lipid metabolism, ABCA1 gene locates at 9q31 region and contains more than 50 exons plus 49 introns, making the total length of the gene over 150 kb. The promoter region, which is localized 1453 bp upstream of the start codon, has multiple binding sites for transcriptional factors involved in lipid metabolism regulation. ABCA1 gene has been suggested to be related with atherosclerosis and lipid metabolic disorders [20,21], both of which contribute to the occurrence and progression of AAA. The SNP pattern of ABCA1 has been reported, especially at R219K *loci*, which locates at 1051 nt of 7th exon, which has significant relationship with various diseases, including hyperlipidemia, coronary heart disease, and atherosclerosis [11,12], further suggesting the important role of ABCA1 gene polymorphism in AAA. This study revealed a statistically significant difference in frequency of KK and RR genotypes between AAA patients and the control group, as supported by lower incidence of RR genotype and R alleles in the disease group. Further correlation analysis showed higher apoA-I and HDL-C levels in R allele carriers within AAA patients compared to non-carrier patients. Moreover, both RR/RK genotypes and R allele were closely correlated with AAA pathogenesis.

Conclusions

This study for the first time demonstrates the close relationship between R219K polymorphism of ABCA1 gene and occurrence of AAA; R allele at this locus may regulate the pathogenesis and progression of AAA. Therefore, further studies are needed on the detailed mechanism of R219K mutation-induced blood lipid abnormalities. Such studies are expected to provide evidence useful for developing large-scale screening, early diagnosis and treatment of AAA.

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