Endothelial Lipase-384A/C Polymorphism Is Associated with Acute Coronary Syndrome and Lipid Status in Elderly Uygur Patients in Xinjiang

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Objective: To explore the relationship between the endothelial lipase (*EL*) gene promoter –384A/C polymorphism and acute coronary syndrome (ACS) and lipid status in elderly Uygur patients in Xinjiang. **Methods:** The polymerase chain reaction–restriction fragment length polymorphism method was used to detect the *EL* gene promoter –384A/C genotype in 341 cases of elderly patients with ACS and 380 healthy subjects. **Results:** In an elderly Chinese Han population, the *EL*-384A/C genotype and allele frequency distribution were significantly different between the ACS group and the control group ($p < 0.05$); the frequency of the CC genotype in the ACS group was significantly higher than that in the control group ($p < 0.05$). After adjusting for gender, age, diabetes, hypertension, smoking, hyperlipidemia, and other cardiovascular risk factors, the difference remains statistically significant ($p < 0.05$). In the ACS group, C allele carriers had significantly higher serum triglycerides, total cholesterol, and low-density lipoprotein cholesterol concentrations compared to AA genotypes (all $p < 0.05$). **Conclusion:** *EL*-384A/C polymorphism was significantly associated with the ACS and lipids profile in an elderly Uygur population in Xinjiang.

Introduction

ENDOTHELIAL LIPASE (*EL*) is a recently discovered new member of the triglyceride (TG) lipase family, which is involved in atherosclerosis and coronary heart disease possibly through cleavage of high-density lipoprotein cholesterol (HDL-C) and a number of other ways (Adeyo *et al.*, 2012). In recent years, several studies indicated that *EL* gene polymorphism was closely associated with coronary heart disease (Jensen *et al.*, 2009; Cai *et al.*, 2012). Yamakawa-Kobayashi *et al.* (2003) and Hutter *et al.* (2006) found that –384A/C polymorphism (rs3813082) in the *EL* promoter was associated with plasma HDL-C levels. However, so far, whether the *EL*-384A/C gene polymorphism is associated with acute coronary syndrome (ACS) in the elderly Uygur population in Xinjiang has not been reported. In the present study, we utilized polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) to detect the genotypes of *EL*-384 A/C in 341 cases of elderly patients with ACS and 380 healthy subjects in a Uygur population. Therefore, we can understand the *EL*-384A/C polymorphism frequency distribution and explore the relationship between ACS and lipids and *EL*-384 A/C polymorphism in the elderly Xinjiang Uygur population.

Subjects and Methods

Ethnics

The present study has been performed with the approval of the ethics committee of the First Affiliated Hospital of Xinjiang Medical University and is in compliance with the Declaration of Helsinki. Informed consents were collected from all the candidate subjects.

Subjects

All the participants were selected from January 2008 to March 2013 at the Cardiology Department of the First Affiliated Hospital of Xinjiang Medical University. The ACS group enrolled 341 patients, including 256 males and 85 females, who were aged from 61 to 89 years with a mean age of 66.47 ± 5.74 years. ACS was defined in accordance with the American Heart Association standards; most of the patients underwent coronary angiography. The results were judged by two experienced cardiac catheterization physicians.

Three hundred eighty hospitalized patients were enrolled as the control group; earlier coronary angiography examination had been performed in these subjects to exclude coronary artery disease. Two hundred ninety-one cases were male and 79 were female, and they were aged from 63 to 90

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years with a mean age of 67.44 ± 6.75 years. The enrolled patients were all Xinjiang Uygur people. Exclusion criteria were as follows: infectious diseases, rheumatic heart disease, severe liver dysfunction, cancer, and other diseases.

General information collection

For all enrolled subjects, age, gender, blood pressure, body-mass index (BMI), smoking history, and coronary angiography results were documented.

Blood sample collection

Fasting venous blood on admission was collected for biochemical detection; serum total cholesterol (TC), TG, HDL-C, low-density lipoprotein cholesterol (LDL-C), and blood glucose were detected using equipment for chemical analysis (Dimension AR/AVL Clinical Chemistry System) employed by the Clinical Laboratory Department of the First Affiliated Hospital of Xinjiang Medical University.

EL-384A/C genotype detection

A standard phenol-chlorine method was used to extract the genomic DNA; the EL-384A/C genotype was detected using PCR-RFLP. The primers were synthesized according to the literature (Yamakawa-Kobayashi *et al.*, 2003), the upstream primer was 5'-TAG CTC CGC CGG GTTATT GTG C-3' and the downstream primer was 5'-CCA GAT CCT CTC CCC ACT G-3'. Primers were synthesized by the Takara Co. The PCR-RFLP technique was used for genotyping. PCR products were digested by the restriction enzyme *HhaI* (provided by the Takara Co.) at 37.0°C overnight. Digestion products were gel stained in a 3% agarose gel after 100 V electrophoresis for 45 min. Genotyping results were read under UV light. Approximately 10% of the samples were randomly checked a second time.

Statistical analysis

SPSS 17.0 software was used for statistical analysis. Continuous data are expressed as mean $\pm$ standard deviation, the means between the two groups were compared using an independent samples t-test; the χ^2 test was used for categorical data comparison. A variety of ACS-related factors in the elderly were analyzed using logistic regression analysis. A *p* value < 0.05 was considered as statistically significant.

Results

General information in control subjects and ACS patients

Compared with the control group, the smokers were common in the ACS group, and the levels of blood pressure and the concentrations of TG, TC, HDL-C, LDL-C, and glucose were higher than those in the control subjects (all $p < 0.05$). However, we did not find a significant difference in age, gender, and BMI between these two groups (Table 1).

Genotyping of EL-384A/C polymorphism

After PCR amplification, the specific 254 bp fragment was obtained and three genotypes AA (254 bp), AC (254, 142, and

TABLE 1. DEMOGRAPHIC AND RISK PROFILE OF THE STUDY POPULATION

Risk factors	n (%) or mean $\pm$ SD		p-Values
	ACS (n = 341)	Control (n = 380)	
Age (years)	66.47 $\pm$ 5.74	67.44 $\pm$ 6.75	0.08
Female, n (%)	85 (24.9)	79 (20.8)	0.073
Smoking, n (%)	72 (21.1)	55 (14.5)	0.012
BMI (kg/m ²)	24.44 $\pm$ 3.42	24.33 $\pm$ 3.12	0.120
SBP (mmHg)	124.01 $\pm$ 10.13	120.64 $\pm$ 10.07	0.030
DBP (mmHg)	77.44 $\pm$ 8.64	70.42 $\pm$ 7.66	0.014
GLU (mM)	5.14 $\pm$ 0.77	4.44 $\pm$ 0.59	< 0.001
TG (mM)	1.95 $\pm$ 0.59	1.62 $\pm$ 0.38	0.042
TC (mM)	4.56 $\pm$ 0.90	4.19 $\pm$ 0.88	0.018
HDL-C (mM)	0.78 $\pm$ 0.39	1.14 $\pm$ 0.54	0.011
LDL-C (mM)	2.88 $\pm$ 0.91	2.43 $\pm$ 0.81	0.017

ACS, acute coronary syndrome; BMI, body-mass index; DBP, diastolic blood pressure; GLU, glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides.

112 bp), and CC (142 and 112 bp) appeared when the fragment was digested by the *HhaI* restriction endonuclease.

EL-384A/C genotype distribution in the two groups reached the Hardy-Weinberg equilibrium ($p > 0.05$, data not shown). As shown in Table 2, there were significant differences in both genotype distribution ($p = 0.031$) and allele distribution ($p = 0.007$) between these two groups.

Lipids comparison in different genotypes of EL-384A/C

As shown in Table 3, there was a significant difference between the AA and (AC + CC) genotypes in serum TC, TG, and LDL-C concentrations in the ACS group. However, we did not find a similar difference in the control group.

Logistic regression analysis of risk factors in elderly ACS patients

As shown in Table 4, logistic regression analysis showed that after adjustment for gender, age, and other factors, the EL-384A/C gene polymorphism was independently associated with the risk of ACS in the elderly patients.

TABLE 2. DISTRIBUTIONS OF EL-384A/C IN ACUTE CORONARY SYNDROME PATIENTS AND CONTROL SUBJECTS

-384A/C	Groups		p-Value
	ACS, n (%)	Control, n (%)	
Genotypes			
AA	224 (65.7)	282 (71.5)	0.031
AC	102 (29.9)	89 (26.1)	
CC	15 (4.4)	9 (2.4)	
Alleles			
A	550 (80.6)	653 (85.9)	0.007
C	132 (19.4)	107 (14.1)	

EL, endothelial lipase.

TABLE 3. LIPID LEVELS BETWEEN GENOTYPES

Genotype	N	TG (mM)	TC (mM)	HDL-C (mM)	LDL-C (mM)
ACS group					
AC+CC	117	1.95±0.33	4.84±0.38	0.97±0.11	2.91±0.21
AA	224	1.74±0.25	4.15±0.35	1.01±0.12	2.43±0.22
		<i>p</i> =0.021	<i>p</i> =0.033	<i>p</i> =0.122	<i>p</i> =0.015
Control group					
AC+CC	98	1.45±0.21	4.14±0.29	1.14±0.10	2.41±0.22
AA	282	1.35±0.23	4.23±0.35	1.20±0.12	2.13±0.20
		<i>p</i> =0.325	<i>p</i> =0.226	<i>p</i> =0.543	<i>p</i> =0.568

Discussion

EL is located on human chromosome 18q21.1; it consists of 483 amino acids and the molecular weight is about 55 kDa (Boes *et al.*, 2009). *EL* is mainly synthesized by vascular endothelial cells and participates in lipoprotein metabolism with lipase such as hepatic lipase and lipoprotein lipase. *EL* mainly has a stronger phospholipase activity and its substrate is the HDL-C. *EL* can mediate HDL-C and heparan sulfate proteoglycan binding in endothelial cells. It can hydrolyze HDL-C to produce free fatty acids, lysolecithin, and Apo-A 1; later research found that the *EL* also has a weak TG activity.

Since 2002, when deLemos *et al.* (2002) first discovered 17 polymorphic loci of the *EL* gene, a number of new polymorphic loci have been reported. So far, whether the *EL* gene polymorphism has an impact on lipid levels and coronary heart disease pathogenesis remains unclear. In 2003, Yamakawa-Kobayashi *et al.* (2003) reported the relationship between genetic variation of *EL* and HDL-C levels in Japanese schoolchildren; the author reported six gene mutations in *EL* and found that -384A/C in the promoter region and 2237G/A in exon 10 were associated with elevated HDL-C levels. In 2006, Hutter *et al.* also found that the -384A/C gene polymorphism was associated with the level of HDL-C and risk for cardiovascular diseases in a Japanese American population (Hutter *et al.*, 2006). Recently, Huang *et al.* (2008) studied *EL* gene-384A/C polymorphism in 214 normal subjects and 103 hypertriglyceridemia (HTG) patients in Chengdu, China. They discovered that the C allele frequency of -384A/C polymorphism in the *EL* gene was 0.178 in Chinese people; however, they did not find a difference in the C allele frequency between control subjects and HTG patients. Compared with the AA genotype, the serum HDL-C level in C allele carriers (AC and CC genotypes) was not significantly different. In the present study, we found that the C allele frequency in the *EL*-384A/C gene was 19.4%. Our results are consistent with the findings of Yamakawa-Kobayashi *et al.* (2003) and Hutter *et al.* (2006). However, the C allele frequency was moderately higher than that in

Huang *et al.*'s report (Huang *et al.*, 2008). This discrepancy may result from different ethnic groups. In our analysis, the *EL*-384A/C genotype and allele frequency distribution in the elderly ACS group was significantly different from that in the control group. After adjusting for gender, age, diabetes, hypertension, smoking, hyperlipidemia, and other cardiovascular risk factors, the difference remained statistically significant. Meanwhile, we found that compared with the AA genotype, C allele carriers (AC+CC) had significantly higher TC, LDL-C, and TG levels, which were consistent with the Yamakawa-Kobayashi *et al.* (2003) and Hutter's *et al.* (2006) report.

Conclusion

In this study, the association between the *EL* gene-384A/C polymorphism and the risk of ACS in the elderly Xinjiang Uygur population was studied for the first time. The results showed that *EL*-384A/C polymorphism was significantly associated with the risk of ACS in the elderly. Our study suggested that the gene polymorphism may be a predisposing factor of ACS in the elderly Uygur population in Xinjiang. However, our results need to be verified by studying multiple regions, larger sample size, and using functional studies.

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Authors' Contributions

D.H. and X.X. carried out the molecular genetic studies and drafted the manuscript. X.M. and Y.T.M. carried out the genotyping. Y.T.M. and Y.H. participated in the design of the study and performed the statistical analysis. All authors read and approved the final manuscript.

Author Disclosure Statement

The authors declare that no competing financial interests exist.

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TABLE 4. LOGISTIC REGRESSION ANALYSIS

	B	SE	χ^2	p-Value	OR	95% CI
-384A/C	1.505	0.624	5.037	<0.001	2.432	1.021–3.217
Smoking	0.443	0.116	6.433	<0.001	2.232	1.265–2.776
HDL-C	-1.394	0.284	24.050	<0.001	0.248	0.142–0.433
LDL-C	0.876	0.769	5.928	<0.001	1.219	1.002–2.114
Constant	2.099	0.733	8.109	<0.001	4.145	

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