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Apolipoprotein A5 IVS3+476A Allelic Variant Associates With Increased Triglyceride Levels and Confers Risk for Development of Metabolic Syndrome in Hungarians

Péter Kisfali; Márton Mohás, MD*; Anita Maasz; Ferenc Hadarits, MD**; Lajos Markó, MD*; Katalin Horvatovich; Tamás Oroszlán, MD†; Zoltán Bagosi, MD†; Zoltán Bujtor, MD†; Beáta Gasztonyi, MD, PhD†; István Wittmann, MD, PhD*; Béla Melegh, MD, PhD

Background Metabolic syndrome consists of multiple risk factors that are increasing the cardiovascular mortality. The T-1131C variant of the apolipoprotein A5 gene, associated with increased triglycerides, has been found to confer risk for cardiovascular diseases and metabolic syndrome. Because other naturally occurring variants of the gene also correlate with elevated triglycerides, the possible role of 2 common variants, the IVS3+G476A and T1259C, with metabolic syndrome was investigated.

Methods and Results A total of 213 metabolic syndrome patients and 142 healthy controls were genotyped by polymerase chain reaction-restriction fragment length polymorphism. Serum triglycerides were increased in carriers compared with non-carriers in both groups ($p<0.001$); serum cholesterol levels were similar in all genotypes. The IVS3+476A allele frequency was increased in metabolic syndrome patients compared with controls (8.05 vs 2.47%; $p<0.05$), whereas the 1259C allele frequency did not differ between the groups. Multiple logistic regression analyses adjusted for age, gender, serum total cholesterol, acute myocardial infarction and stroke revealed that the IVS3+476A variant confers risk for development of metabolic syndrome (odds ratio=3.529, 95% confidence interval 1.308–9.029, $p=0.009$), but the 1259C allele had no such an effect.

Conclusions Carrying the IVS3+473A allele is associated with elevated triglycerides and confers risk for development of metabolic syndrome, a combination that represents increased risk for development of atherogenic vascular diseases. (*Circ J* 2008; **72:** 40–43)

Key Words: *APOA5*; IVS3+G476A; Metabolic syndrome; Single nucleotide polymorphism; T1259C

Abdominal obesity, increased level of serum triglycerides and decrease of serum high-density lipoprotein-cholesterol (HDL-C), elevated blood pressure, and glucose intolerance (impaired fasting glucose, impaired glucose tolerance, or the presence of diabetes mellitus) are the major components of the metabolic syndrome[1]. The prevalence of this syndrome is approximately 25% in adult males and 18% in adult females in the industrialized countries[2]. The etiology of the syndrome is complex, and includes several environmental and genetic factors as well, such as lifestyle, physical inactivity, smoking, diet, ageing, and race[3–5]. This syndrome is very often associated with developing type 2 diabetes mellitus, or fatal cardio- and cerebrovascular events[1,6].

The recently identified apolipoprotein A5 (APOA5) gene is located on chromosome 11q23, approximately 27 kb downstream from the *APOA1–CIII–AIV* gene cluster and is involved in lipid metabolism, influencing the level of high-density lipoprotein and very low-density lipoprotein (VLDL) particles[7]. *APOA5* is composed of 4 exons and encodes 366 amino acids[8]. Several single nucleotide polymorphisms (SNPs) have been described for *APOA5*, among which T-1131C, IVS3+G476A, T1259C and C56G are known to be associated with elevated triglyceride levels in several European[4,9–11] and Japanese populations;[4,12] in addition, some of them are known to correlate with diseases as susceptibility genes for ischemic heart disease and stroke[7,8,13]. The T-1131C variant has been demonstrated also as a risk factor for metabolic syndrome[4,14]. As a natural extension, the aim of the current study was to test the possible role of the IVS3+G476A and T1259C variants in the development of metabolic syndrome.

Methods

Study Population

The study population comprised 355 individuals from the Hungarian population. A total of 213 metabolic syndrome patients (99 males, 114 females, mean age: 61.09±1.01 years, range: 25–82 years) were selected according to the criteria of the modified Adult Treatment Panel III of National Cholesterol Education Program whereby meta-

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*Department of Medical Genetics and Child Development, *2nd Department of Medicine and Nephrological Center, University of Pécs, Pécs, **Central Laboratory, Markusovszky County Hospital, Szombathely and †2nd Department of Medicine, Zala County Hospital, Zalaegerszeg, Hungary*

Mailing address: Béla Melegh, MD, PhD, Department of Medical Genetics and Child Development, University of Pécs, Pécs, Szigeti 12, H-7624, Hungary. E-mail: bela.melegh@aok.pte.hu

Table 1 Major Clinical Parameters of the Patients With Metabolic Syndrome and the Control Subjects

	Metabolic syndrome patients (n=213)	*Controls (n=142)*
Gender (M/F)	*99/114*	*66/76*
Age (years)	*61.09±1.01*	*59.53±1.43*
BMI (kg/m²)	*31.99±0.52*	*24.04±0.18**
Serum TG (mmol/L)	*2.65±0.20*	*1.45±0.04**
Serum TC (mmol/L)	*5.49±0.12*	*5.40±0.08*
Serum HDL-C (mmol/L)	*1.22±0.02*	
FPG (mmol/L)	*12.49±2.11*	
SBP (mmHg)	*140±1.27*	
DBP (mmHg)	*84.4±1.12*	

BMI, body mass index; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; FPG, fasting plasma glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure.
*Data are means±SEM. *p<0.001.*

Table 2 Serum TGs and Serum TC in Patients With Metabolic Syndrome and in the Control Subjects According to the IVS3+G476A and T1259C Variants of *APOA5*

	IVS3+G476A				*T1259C*			
	Metabolic syndrome patients		*Controls*		*Metabolic syndrome patients*		*Controls*	
	Non-carrier (GG) (n=180)	*Carrier (GA+AA) (n=33)*	*Non-carrier (GG) (n=135)*	*Carrier (GA+AA) (n=7)*	*Non-carrier (TT) (n=179)*	*Carrier (TC+CC) (n=34)*	*Non-carrier (TT) (n=135)*	*Carrier (TC+CC) (n=7)*
Serum TG (mmol/L)	*2.31±0.11*	*3.21±0.48**	*1.40±0.03*	*1.90±0.20***	*2.89±0.11*	*3.28±0.47#*	*1.34±0.03*	*1.67±0.16##*
Serum TC (mmol/L)	*5.30±0.08*	*5.78±0.27*	*5.43±0.09*	*4.86±0.30*	*5.30±0.08*	*5.80±0.26*	*5.38±0.10*	*5.38±0.26*
Serum HDL-C (mmol/L)	*1.22±0.02*	*1.23±0.05*			*1.21±0.02*	*1.23±0.11*		
FPG (mmol/L)	*12.49±2.11*	*10.275±1.02*			*12.89±2.52*	*10.09±0.92*		
SBP (mmHg)	*140±2.05*	*141±3.33*			*140±2.38*	*141±2.97*		
DBP (mmHg)	*84.4±1.12*	*81.2±3.98*			*84.9±1.10*	*82.2±3.64*		

APOA5, apolipoprotein A5. Other abbreviations see in Table 1.
*Data are means±SEM. *p=0.035; **p=0.016; #p=0.016; ##p=0.023.*

bolic syndrome was defined as the presence of at least 3 of the criteria listed: body mass index (BMI) >25 instead of waist circumference, serum triglycerides ≥1.70 mmol/L; serum HDL-C <0.9/1.1 mmol/L (M/F); systolic blood pressure ≥130 mmHg and diastolic blood pressure ≥85 mmHg; fasting plasma glucose levels >5.60 mmol/L.[2,15] A total of 142 apparently healthy subjects (66 males, 76 females, mean age: 59.53±1.43 years, range: 26–93 years) without any single clinical or laboratory mark of metabolic syndrome served as controls. Serum HDL-C, fasting plasma glucose levels and systolic/diastolic blood pressures were not available for the controls. Neither the subjects with metabolic syndrome nor the controls were taking any medication at the time of investigation.

All participants gave their informed consent and the study was approved by the local Ethics Committee.

Genetic Analysis

Genomic DNA was isolated from peripheral blood leukocytes by a standard salting out procedure. The T1259C and IVS3+G476A SNPs of the *APOA5* were genotyped by polymerase chain reaction-restriction fragment length polymorphism. To test the IVS3+G476A alteration the following oligonucleotides were used for amplification: 5'-CTC AAG GCT GTC TTC AG-3' and 5'-CCT TTG ATT CTG GGG ACTG G-3' (antisense) (Metabion, Martinsried, Germany). The PCR product (15• l) was digested with 1 U of *MnlI* restriction endonuclease (Fermentas, Burlington, ON, Canada) at 37°C overnight. The restriction fragments were analysed by 3% agarose gel stained with ethidium bromide (Fluka Chemie Gmbh, Buchs, Switzerland) and visualized on a UV transilluminator (Uvitec, Cambridge, UK). In the samples with GG genotype, the digestion resulted in 25 bp, 114 bp, 141 bp and in the homozygous samples 25 bp, 41 bp, 73 bp and 141 bp long products were detected.

The T1259C polymorphism were detected using the primers 5'-TCA GTC CTT GAA AGT GGC CT-3' and 5'-ATG TAG TGG CAC AGG CTT CC-3' (antisense) (Metabion). The PCR product was digested with 1 U of *BseGI* restriction endonuclease (Fermentas) at 55°C overnight. After restriction enzyme digestion the normal (TT) genotype gave fragments of 122 bp and 165 bp, whereas the homozygous form (CC) was 35 bp, 87 bp, 165 bp.

PCR conditions (PTC-200, Bio-Rad, Hercules, CA, USA) were similar for both methods: 2 min initial denaturation at 96°C, 35 cycles of 20 s at 96°C; 20 s at 60°C; 20 s at 72°C and the final extension at 72°C for 5 min. The amplification was carried out in a final volume of 50• l•containing 5• l•reaction buffer (500 mmol/L KCl, 14 mmol/L $MgCl_2$, 10 mmol/L Tris-HCl, pH 9.0), 1• l• 50 mmol/L $MgCl_2$, 0.2 mmol/L of each dNTP, 1 U of Taq polymerase, 0.2 mmol/L of each reaction specific primer and 1• g DNA.

Statistical Analysis

Results are expressed as mean±SEM. Statistical significance was assessed by the Mann-Whitney U test to compare the differences between groups. Chi-square tests were used to compare qualitative data. Multiple logistic regression analysis was carried out to evaluate the effect of the *APOA5* genotype on the development of metabolic syndrome. A value of $p<0.05$ was considered statistically significant. All statistical analyses were performed by using the SPSS 11.0 software (SPSS Inc, Chicago, IL, USA).

Table 3 Multiple Logistic Regression Analysis for the Association Between Carrying IVS3+476A and 1259C Allelic Variants and Risk for Metabolic Syndrome

Genotypes	*Metabolic syndrome patients (n=213)*	*Controls (n=142)*	*Unadjusted model OR (95%CI)*	*Adjusted model OR (95%CI)**
IVS3+G476A				
Non-carrier (GG)	*84.5%*	*95.1%#*	*3.503 (1.503–8.162)*	*3.529 (1.308–9.029)*
Carrier (GA+AA)	*15.5%*	*4.9%#*	*p=0.004*	*p=0.009*
T1259C				
Non-carrier (TT)	*84.0%*	*84.5%*	*1.064 (0.583–1.939)*	*0.971 (0.509–1.854)*
Carrier (TC+CC)	*16.0%*	*15.5%*	*p=0.840*	*p=0.929*

OR, odds ratio; CI, confidence interval.
**Adjusted for age, gender, total serum cholesterol, acute myocardial infarction, and stroke.*
#p<0.001.

Results

Major clinical parameters of the patients with metabolic syndrome and controls are summarized in Table 1. Serum triglycerides and BMI were significantly elevated in patients with metabolic syndrome compared with controls ($p<0.001$). The serum total cholesterol did not differ between the 2 groups.

The genotype distributions with their association with triglycerides are shown in Table 2. The genotypes were in Hardy-Weinberg distribution in both groups. The IVS3+476A allelic variant was associated with increased serum triglycerides compared with non-carriers in both metabolic syndrome patients and the controls; similarly, the 1259C variant was also associated with elevated triglyceride levels. The serum total cholesterol of the patients was similar for all genotypes.

The IVS3+476A allele frequency was 8.05% in the metabolic syndrome patients and 2.47% in the controls ($p<0.001$, data not shown), and there was no difference between the 2 groups in distribution of 1259C allele frequency (8.29 vs 7.52%; data not shown).

Multiple regression analysis revealed that carrying the IVS3+476A allelic variant confers an increased risk for developing metabolic syndrome (Table 3). The model adjusted for age, gender, total serum cholesterol, acute myocardial infarction and stroke confirmed the association. Analyzing the 1259C allelic variant showed no association of the allele with metabolic syndrome.

Discussion

APOA5 regulates lipid metabolism through at least 2 major mechanisms. First, it can inhibit the hepatic production of VLDL, and 2nd, it can increase the activity of lipoprotein lipase, resulting in decreased serum triglyceride levels[16,17] Different naturally occurring polymorphisms of *APOA5*, such as T-1131C, T1259C and C56G, have been previously shown to influence the genetic expression of lipoproteins, resulting ultimately in elevation of the serum triglyceride levels[10,18–20]

Several studies have provided evidence that naturally occurring variants of *APOA5* are associated with elevated triglycerides (ie, T-1131C, IVS3+G476A, T1259C, C56G). In addition, some of these were found to confer risk for the development of coronary artery disease in different populations. Several studies revealed that the -1131C, 56G and 1259C variants of *APOA5* are susceptibility SNPs for the development of cardiovascular and cerebrovascular diseases in Chinese, European Whites, Indians and Afro-Carribeans[7,13,22–26] Previous studies described 2 SNPs of *APOA5* (-1131C and 56G) associated with hypertriglyceridemia, cardiovascular disease and risk for atherosclerosis in American populations[7] and in European Caucasian populations[4,7,8,27,28] Studies of Europeans and Middle-Americans have found relationships between serum triglycerides and the abovementioned *APOA5* variants, but they did not support that SNPs of *APOA5* independently confer risk for coronary artery diseases[9,29,30] Moreover, the T-1131C allelic variant of *APOA5* was recently reported to be associated with the metabolic syndrome in Japanese and Romanian populations[4,11] The aim of the current study was to investigate the effect of the IVS3+476A and 1259C variants on triglyceride metabolism, and to extend the observations as a case–control association study for these SNPs to investigate their relationship with development of metabolic syndrome.

Metabolic syndrome is a complex disorder composed of obesity, elevated triglyceride levels, decreased HDL-C, raised systolic and diastolic blood pressures and impaired glucose metabolism[1,31] Elevated serum triglyceride levels, which are 1 of the major hallmarks of metabolic syndrome, are influenced by several exogenous factors, such as diet, smoking or alcohol consumption. In the present study, the serum triglyceride level and the BMI were significantly higher in the metabolic syndrome patients compared with the controls. We found elevated triglycerides in carriers of the IVS3+476A and 1259C variants in both the metabolic syndrome and control groups, although these genotypes did not affect the total serum cholesterol levels. In metabolic syndrome patients there was an approximately 3.3-fold accumulation in the IVS3+476A allelic variant, whereas no difference was found in the distribution of the 1259C variants between the patients and controls. By logistic regression analysis we found a strong association between the IVS3+476A variant, but not for the 1259C variant, and the development of metabolic syndrome; thus, the present results revealed that carrying the IVS3+476A allele, but not the 1259C variant, confers independent risk for the metabolic syndrome. This means that elevated triglyceride levels cannot be the only determinant of the risk effect, because both of them were associated with elevated triglycerides. Therefore, another mechanism of action cannot be ruled out.

Recent observations have revealed that certain SNPs (-1131C, 56G) of *APOA5* are in linkage disequilibrium with *APOC3* variants, which play a major role in the development of insulin resistance, because in its promoter region an insulin-responsive element was identified[8,29,32,33] Two SNPs (T-455C and C-482T) in the distal promoter region

of *APOC3* were found to be associated with reduced affinity for the nuclear transcription factors mediating the downregulating response to insulin[9,10,33,34] The 2 SNPs of *APOA5* are in different linkage with other SNPs of the *APOAI–CIII–AIV* gene cluster. It is possible that the IVS3+476A allele is in stronger linkage with the SNPs of this cluster influencing serum triglycerides and carbohydrate metabolism than the 1259C; however, this assumption has to be confirmed.

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