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ARTICLE

Apolipoprotein A5 T-1131C Variant Confers Risk for Metabolic Syndrome

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The -1131C is a naturally occurring variant of the apolipoprotein A5 (ApoA5) gene, which has been shown to associate with increased triglyceride levels. This variant has also been shown to confer risk for development of ischemic heart disease and stroke. The gene is in linkage disequilibrium with factors known to correlate with impaired glucose homeostasis. These observations prompted us to study the prevalence of the ApoA5 -1131C allele in patients with metabolic syndrome. A total of 201 metabolic syndrome patients and 210 controls were studied. In both groups the triglyceride levels of patients with -1131C allele were significantly increased compared to the subjects with -1131T allele (3.22±0.43 mmol/l vs. 2.24±0.12 mmol/l, p<0.01 in the metabolic syndrome patients; 2.10±0.19 mmol/l vs. 1.22±0.05 mmol/l, p<0.01 in the controls). In metabolic syndrome patients the prevalence of the ApoA5 -1131C variant was increased compared to the healthy controls (11% vs. 6.20%). Multiplex regression analysis model adjusted for age, gender, serum total cholesterol levels, acute myocardial infarction and stroke events revealed that the examined ApoA5 variant confers risk for the development of metabolic syndrome: the odds ratio at 95% confidence interval was 3.622 (1.200-10.936), p=0.02. Our findings strongly suggest that this variant is a risk factor for the development of hypertriglyceridemia and metabolic syndrome. (Pathology Oncology Research Vol 13, No 3, 243–247)

Key words: metabolic syndrome, glucose intolerance, ApoA5, T-1131C

Introduction

Metabolic syndrome is a common disease which affects the European population at a rate of 7-30%.[4,26] This condition can develop in both sexes at any time of life, however, it is age-dependent. There are more criteria for the diagnosis of the metabolic syndrome, like the classification by the World Health Organization, or by the National Cholesterol Educational Program's Adult Treatment Panel (NCEP, ATPIII).[1,2] In the ATPIII definition hypertension, central obesity, insulin resistance and dyslipidemia are included.[25] Metabolic syndrome is known to mean a major risk for several cardio- and cerebrovascular diseases like coronary heart disease, acute myocardial infarction and stroke, as well. Development of metabolic syndrome depends on environmental factors including the nutrition habits, but can also be attributed to genetic susceptibility.[7,26]

The recently identified apolipoprotein A5 gene (ApoA5) is located approximately 27 kb downstream from the ApoAI-ApoCIII-ApoAIV gene cluster.[11] ApoA5 gene is composed of 4 exons and encodes 366 amino acids.[25] The mature ApoA5 protein is expressed in the liver only and secreted into the plasma as a regulator of the triglyceride levels. In its mechanism of action both the high density- (HDL) and very low density lipoprotein particles (VLDL) are involved.[16] The T-1131C form, as a naturally occurring variant in the promoter region of ApoA5 gene, is known to associate with elevated triglyceride levels and hyperinsulinemia. Therefore, this variation may predispose to cardiovascular diseases, stroke and insulin

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resistance. The goal of the current study was the investigation of the distribution of T-1131C variant in a cohort of metabolic syndrome patients.

Materials and Methods

Study population

The subjects in the present study were unrelated and were randomly selected with sex- and generation-matched technique. A total of 201 patients (95 males and 106 females, mean age: 61.1±0.76 years, range: 25-82 years) with metabolic syndrome were selected for the study. The patients with metabolic syndrome were recruited from the 2nd Department of Medicine and Nephrological Center of the University of Pécs. The sample collection was performed using internationally accepted standards. The blood specimens were centrifuged immediately after the blood collections and the samples were stored at –70°C until the analysis. Under such conditions there is no risk for degradation of metabolites, including cholesterol. The major clinical and laboratory data of the subjects are summarized in *Table 1*. The range of cholesterol levels were as follows: in metabolic syndrome patients it ranged between 3.09-16.4 mmol/l; in controls 2.10-12.6 mmol/l. The principles of modified NCEP, ATPIII were used to diagnose metabolic syndrome. Using these criteria metabolic syndrome was established when patients fulfilled at least three of the following components: waist circumference >102/88 cm (male/female); serum triglycerides ≥1.70 mmol/l; serum HDL-cholesterol <1.04/1.29 mmol/l (male/female); systolic blood pressure >130 mmHg and diastolic blood pressure >85 mmHg; fasting plasma glucose levels ≥5.60 mmol/l.

A total of 210 Caucasian subjects (84 males and 126 females, mean age: 52.9±1.23 years, range: 26-80 years) served as controls. They were healthy and had no clinical, laboratory of history records for any systemic disease, including metabolic syndrome, diabetes or cardiovascular disorders; they were blood donor volunteers, students and staff members of our departments.

Detection of the polymorphism

The molecular studies were performed using DNA extracted from peripheral blood leukocytes with a routine salting out method. The DNA from our patients was stored with their informed consent. The mutation (T-1131C) of the ApoA5 gene promoter region was studied by RFLP after PCR amplification. For PCR the following primers were designed by GenBank reference sequence AY 422949 (http://www.ncbi.nlm.nih.gov/entrez): sense: 5'-CCC CAG GAA CTG GAG CGA AA**T**T-3' and antisense: 5'-TTC AAG CAG AGG GAA GCC TGT A-3'. PCR conditions were as follows: initial denaturation at 96°C followed by 32 cycles of 30 sec at 95°C; 30 sec at 55°C; 30 sec at 72°C and a final extension at 72°C. The amplification was carried out in a final volume of 50 µl containing 200 µM of each dNTP, 1 U of Taq polymerase, 5 µl of reaction buffer (500 mM KCl, 14 mM $MgCl_2$, 10 mM Tris-HCl, pH 9.0), 0.2 mM of each primers and 1 µg DNA extracted.

10 µl of the 396-bp-long PCR product was digested with 1 U of *Tru*I restriction endonuclease and transferred through an ethidium-bromide stained 3% agarose gel. The test was designed to have an obligatory cleavage site of the restriction enzyme to make sure the control of the digestion. A false T base (boldface letter) was incorporated into the forward primer instead of G, which created an artificial *Tru*I recognition site (underlined). In the samples with TT genotype the digestion resulted in 20, 105, 271 bp long fragments. In heterozygous form (TC) 20, 105, 271, 291 bp long products were detected. In the samples carrying the mutation in homozygous form (CC) 105 and 291 bp long fragments were produced.

Table 1. **Major clinical and laboratory data of the patients with metabolic syndrome and control subjects**

	Metabolic syndrome patients n=201	*Controls n=210*
Males/females	95/106	84/126
Age (years)	61.1 ± 0.76	52.9 ± 1.23
BMI (kg/m^2)	32.3 ± 0.41	23.1 ± 0.23
Serum triglycerides (mmol/l)	2.43 ± 0.13*	1.33 ± 0.05
Serum total cholesterol (mmol/l)	5.40 ± 0.11*	4.98 ± 0.11

Values are given as mean ± SEM; * p<0.01 vs. controls

Statistical analysis

All clinical data were expressed as mean ± SEM. The distributions of the variables were examined using Kolmogorov-Smirnov test. All the discreet and continuous variables showed skewed distribution, thus we applied nonparametric tests to compare the differences between groups: Mann-Whitney U test for continuous variables and Chi-square test for discreet variables. Odds ratios derived from multiple logistic regression analysis, which was carried out to evaluate the effect of the ApoA5 genotype on the development of metabolic syndrome. All statistical analyses were performed applying SPSS 11.0 package for Windows (SPSS Inc., Chicago, IL).

Results

The most relevant clinical features and laboratory parameters of our 201 patients and 210 control subjects are summarized in *Table 1*. The major risk factors for meta-

Table 2. **Serum triglyceride and total cholesterol levels in patients with metabolic syndrome and control subjects according to the ApoA5 -1131 genotypes**

	Metabolic syndrome patients		*Controls*	
	Non-carrier (TT) n=163	*Carrier (TC+CC) n=38*	*Non-carrier (TT) n=185*	*Carrier (TC+CC) n=25*
Serum triglycerides, (mmol/l)	2.24 ± 0.12	3.22 ± 0.43*	1.22 ±0.05	2.10 ± 0.19*
Serum total cholesterol, (mmol/l)	5.30 ± 0.12	5.84 ± 0.24	5.02 ± 0.11	4.71 ± 0.39

Values are given as mean ± SEM;
* p<0.01 vs. TT

Table 3. **Multiple logistic regression analysis for the association between carrying ApoA5 -1131C allele and risk for metabolic syndrome**

Genotypes	*Metabolic syndrome patients n=201*	*Controls n=210*	*Unadjusted model Odds ratio (95% CI)*	*Adjusted model *Odds ratio (95% CI)*
Non-carrier (TT)	163 (81.1%) #	185 (88.1%)	1.725 (0.998-2.981) p=0.05	3.622 (1.200-10.936) p=0.02
Carrier (TC+CC)	32+6 (18.9%) #	24+1 (11.9%)		
C allele frequency	11%#	6.20%		

* Adjusted for age, gender, BMI, serum total cholesterol, acute myocardial infarction, stroke
# p≤0.05 vs. controls

bolic syndrome, like serum total cholesterol- and triglyceride levels were significantly higher in the patients than in the controls (p<0.01).

Table 2 shows the serum triglyceride and total cholesterol levels in the groups with different genotypes (non-carrier: TT, carrier: TC+CC). The triglyceride levels were significantly higher in carriers than in non-carriers both in the patients with metabolic syndrome and in the controls as well (p<0.01). Total serum cholesterol levels showed no significant difference in these genotype groups.

The genotypes and ApoA5 -1131C allele frequencies in the two groups are shown in *Table 3*. As it is seen, there was accumulation of -1131C allele frequency in the group with metabolic syndrome (11% vs. control group 6.20%). The genotypes were in Hardy-Weinberg equilibrium both in the patients with metabolic syndrome and also in the control subjects.

Results of analysis of multiple regression model used to determine the significance of ApoA5 -1131C allele as a probable independent risk factor for metabolic syndrome are shown in *Table 3*. The results of the unadjusted model showed that the ApoA5 -1131C carriers had an increased risk for metabolic syndrome (OR=1.725, 95% CI: 0.998-2.981, p=0.05). After adjustment for differences in age, gender, serum total cholesterol levels, acute myocardial infarction and stroke events, the association became two-fold stronger (OR=3.622, 95% CI: 1.200-10.936, p=0.02). Inclusion of triglyceride levels into the adjustment parameters resulted in loss of the association (OR=1.875, 95% CI: 0.576-6.108, p=0.297).

Discussion

Metabolic syndrome usually associates with elevated triglyceride levels. The serum triglyceride concentrations are influenced by environmental conditions, like nutrition, drinking and smoking habits, however, differences in triglyceride levels can also be attributed to genetic factors.[6] Nevertheless, increasing number of observations show that elevated serum triglyceride levels develop due to mutations in different apolipoprotein genes like ApoAI, ApoCIII, ApoAIV, and ApoA5.[30] Amongst them ApoCIII and the recently identified ApoA5 genes have regulatory role in the triglyceride metabolism.[29,31] ApoA5 influences the triglyceride metabolism likely by two mechanisms. It may enhance the intravascular triglyceride hydrolysis by activating lipoprotein lipase, or can decrease the serum concentration of triglycerides through the inhibition of the hepatic VLDL production.[17] Expression of ApoA5 is under the control of the peroxisome proliferator-activated receptor-α (PPAR-α), retinoic acid receptor-related orphan receptor-α (ROR-α)-1, 4 and liver x receptor ligands (LXR).[10,13,24]

Polymorphisms of the ApoA5 gene might influence the function of the protein transcript, which can affect the

lipoprotein lipase resulting in increased triglyceride levels.[20,22] In the current study we examined the correlation between -1131C allele and serum triglyceride levels in metabolic syndrome patients as well as in control subjects. This allele was associated with increased triglyceride levels both in the metabolic syndrome patients and in controls. Beside the effect of the -1131C allele on the triglyceride levels reduced serum cholesterol levels were also reported in several study populations in association with ApoA5 -1131C allele, however, we could not find such association in the current study.[3,8,9,14]

Amongst ApoA5 variants, -1131C is known to confer risk for ischemic heart disease and stroke. The presence of the -1131C allele was approximately twofold higher in metabolic syndrome patients than in controls. Furthermore, using multivariate logistic regression analysis a strong association was found between the presence of ApoA5 -1131C allele and the development of metabolic syndrome. After adjustment for differences in age, gender, serum total cholesterol level, acute myocardial infarction and stroke, the association became even stronger. However, if the triglyceride levels were also included, the association lost, suggesting that carrying C allele in combination with elevated triglyceride level confers risk for the development of metabolic syndrome.

The mechanism of this effect of the -1131C allele might be related to the obligatory association of the allele with increased triglyceride levels. The -1131C allele has been found to associate with increased triglycerides in several ethnic populations and in numerous disease conditions, like coronary heart disease and stroke.[12,15,23] Again, we also found increase in triglycerides in the metabolic syndrome group; the elevated triglyceride levels are common in metabolic syndrome, and both the myocardial infarction and stroke are very frequent manifestations of the disease.

Another explanation comes from the relationship between the T-1131C variant and other genes. The -1131C allele is a determinant of the so-called ApoA5*2 haplotype.[21] This haplotype is in strong linkage disequilibrium with C-482T or T-455C polymorphisms of the ApoCIII gene, which is located nearby the ApoA5 gene.[19] There are indications that the ApoCIII C-482T variant can play a role in conditions corresponding to insulin resistance, the latter circumstance being known to associate with metabolic syndrome.

In a recent study, using extensive genome-wide search for susceptibility genes, Yamada has also found the T-1131C as a susceptibility locus in a huge Japanese population.[33] It is known that certain mutations can be regarded as universal risk factors, while others do not confer obligatory vulnerability and their importance in one population does not necessarily mean susceptibility in others.[5,23] As it has been discussed earlier, the -1131C allele was found to be a risk factor in the current study, which was conducted on Hungarian subjects. The Hungarians are historically different from the surrounding nations in the Carpathian basin due to the Asian origin of the founder tribes. However, recent studies provided support for a language dominance, so the vast majority of the Hungarian population do not differ from other European populations in respect of their genetic origin.[12,28] In conclusion our observations suggest that the -1131C allele can be a universal risk factor which may affect the general European populations. Further studies will be required to establish this conclusion.

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