Original Article

The Relation between Metabolic Syndrome Risk Factors and Genetic Variations of Apolipoprotein V in Relation with Serum Triglyceride and HDL-C Level

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Abstract

Introduction: Metabolic syndrome (MetS) is a multi-factorial disorder with five important components. A high triglyceride level combined with low HDL cholesterol has been reported to be associated with Apolipoprotein A5 (APOA5) gene variations. In this study, we aimed to determine the association of single nucleotide polymorphisms including: rs662799, rs3135506 and rs2075291 in the apolipoprotein A-V (APOA5) gene in relation to MetS component like triglyceride and HDL-C level in Tehran Lipid and Glucose Study (TLGS).

Materials and Methods: Metabolic syndrome was defined according to ATPIII and phenotypes were defined by the National Cholesterol Education Program (NCEP) criteria for MetS. Demographic, biochemical parameters and anthropometric variables were measured. Selected APOA5 gene polymorphisms were genotyped using PCR-RFLP method.

Results: From TLGS population, 947 adults, aged 19 – 70 years, were randomly selected and recruited into the study. Mean age, triglyceride and WC were higher and mean HDL was lower in MetS *vs*. non-MetS group. C allele in rs2075291 showed a significant association with MetS (OR: 2.38, 95% CI; 1.11 – 5.08, $P = 1.5 \times 10^{-2}$). The association was shown between higher serum triglyceride and the presence of T allele ($P = 4.5 \times 10^{-4}$) and also lower serum HDL-C and the presence of T allele ($P = 1.6 \times 10^{-3}$) in rs2075291. Also this association showed between raised waist circumference and C allele in rs3135506 ($P = 3.5 \times 10^{-2}$) and raised systolic and diastolic blood pressure level and C allele of rs662799 ($P = 4.5 \times 10^{-2}$).

Conclusion: According to the results, there is a relationship between lipid profile and studied polymorphism in the presence of metabolic syndrome. It seems that APOA5 rs2075291 could play an important role in triglyceride and HDL-C level in metabolic syndrome affected, while the association of APOA5 rs662799 polymorphism is still under debate.

Keywords: Apolipoprotein V, metabolic syndrome, SNP, Tehran

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Introduction

Metabolic syndrome (MetS) is a complex disease characterized by the clustering of several metabolic disorders. Excess body weight, insulin resistance, altered plasma lipid levels and glucose homeostasis, as well as increased blood pressure are the principal components of this cluster. Environmental influences (such as low physical activity and inappropriate diet) play a major role in the development of metabolic syndrome. Furthermore, familial aggregation of metabolic disorders has been reported and suggests a genetic contribution to the etiology of this syndrome. Accordingly, it has been reported that genetic variability at several loci is associated with an increased risk of metabolic syndrome.[1]

Apolipoprotein A-V is an important determinant of plasma triglyceride level and a major risk factor for coronary artery disease.[2] Apolipoprotein A-V is encoded by APOA5 gene and affects lipoprotein metabolism by interacting with LDL-C gene family receptors. APOA5 gene is located proximal to the APOA1/C3/A4 gene cluster on human chromosome 11q23. The human APOA5 gene consists of four exons and three introns and finally 366 amino acids.[3] APOA5 is produced in liver in form of intracellular and associated with the cell membrane. The concentration of APOA5 in human plasma is about 20 – 500 ng/mL and mainly as a monomer on chylomicrons, VLDL and HDL. Asian reports showed a reverse relation between APOA5 protein and plasma triglyceride levels. However, in other studies, particularly in hyper triglyceridemic patients were shown a positive correlation between APOA5 and triglyceride.[4] It has been found that, variations in the APOA5 gene are associated with increased plasma triglyceride levels.[5] This association could be found in mice variations of APOA5.[6] Several single nucleotide polymorphisms (SNP) are associated with triglyceride concentrations. It has been found, single nucleotide polymorphisms in the: -1131T>C (rs662799) in the promoter region of the APOA5, c.56C>G (S19W) (rs3135506) in the coding region of APOA5 gene and polymorphism in c.553G>T (rs2075291), significantly associated with concentrations of triglyceride of plasma.[6] In the present article the association of three common apolipoprotein A5 gene polymorphisms with MetS com-

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ponents especially HDL-C and triglyceride levels in participants of Tehran Lipid and Glucose Study (TLGS) were evaluated.

Materials and Methods

Subjects and biochemical analysis

Subjects were selected from the TLGS that designed to determine the risk factors for major non-communicable disorders in Tehran population.[7] Written informed consent was obtained from each subject and the research council of the Research Institute of Endocrine Sciences of the Shahid Beheshti University of Medical Sciences approved this study. Demographic factors and smoking habits were asked and body weight, height, as well as blood pressure were measured at study entry.[8] Metabolic syndrome was defined based on the third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATPIII)[9] which was modified locally.[10]

Total cholesterol, HDL-C levels and its sub-fractions, triglyceride (TG), fast blood sugar (FBS), systolic blood pressure (SBP), diastolic blood pressure (DBP) and anthropometric variables as waist circumference (WC) were measured as described previously.[11,12] LDL-C concentrations in samples with serum triglyceride levels less than 400 mg/dL were calculated using Friedewald's equation.[13] Coefficients of variation (CV) for total cholesterol, HDL-C and triglyceride measurements were below 5%.

Genetic analysis

DNA was extracted using Proteinase K and salting out methods.[14] Apolipoprotein V polymorphisms (rs3135506, rs662799 and rs2075291) were genotyped in all samples using restriction fragment length polymorphism (RFLP) technique. Primer sequences are described in Table 1.

Statistical analysis

All continuous data were expressed as mean ± standard deviation and categorical data were expressed as frequencies and percentages. Allele frequency and genotype frequency calculated by Power Marker v.3.25,[15] and compared between two groups using Chi-square test. Statistical significance was defined as $P < 0.05$. The association between selected polymorphisms and metabolic syndrome were analyzed by the PLINK program (Ver 1.07) using Fisher's exact test. Before doing the analysis with PLINK, all the variables adjusted for age and sex by SPSS (1.8),[16] and PASW Statistics 20 (IBM Corp, in IBM SPSS Statistics for Windows. 2011, NY: Armonk).

Results

In this cross-sectional study, 947 adults, aged 19 – 70 years, were randomly selected from TLGS population. According to the Iranian modified metabolic syndrome definition,[17] study subjects divided into MetS affected and non-MetS group. Demographic, biochemical parameters and anthropometric variables were compared between these two groups. The result showed that the age, triglyceride concentration and waist circumference were higher; however HDL concentration was lower in MetS *vs*. non-MetS group significantly (Table 2).

The genotype distributions deviated from Hardy-Weinberg equilibrium in rs662799 and rs3135506 ($P < 0.05$). However, rs2075291 did not deviate the genotype distributions ($P > 0.05$). The minor allele frequencies for three SNPs were: rs3135506 (C = 0.0026), rs662799 (T = 0.3384) and rs2075291 (C = 0.0332) (Table 3). Comparing allele frequencies among MetS and non-MetS group showed a significant association between the presence of the risk allele (C) in rs2075291 and the affection status with MetS (OR: 2.38, 95% CI: 1.11 – 5.08, $P = 0.015$). Other polymorphisms did not show any association with MetS.

To address the association and the impact of risk alleles on MetS components (i.e. SBP, DBP, WC, FBS, Triglyceride and HDL-C), multiple association analysis using p-link showed a significant association between higher serum triglyceride and T allele ($P = 0.00045$), as well as a lower serum HDL-C and T allele ($P = 0.0016$) in rs2075291. Moreover, there was an association between raised waist circumference and the presence of the C allele in rs3135506 ($P = 0.035$). This association also found in raised systolic and diastolic blood pressure level in the presence of the C allele of rs662799 ($P = 0.045$).

Discussion

Iranian subjects are susceptible to different metabolic diseases, including metabolic syndrome, obesity, diabetes, and hypertension, which are famous risk factors of cardiovascular diseases. In addition, dyslipidemia that is characterized by the formation of atherosclerotic plaque in the bloodstream, a supplementary risk for cardiovascular disease, is prevalent in Iran. The plasma triglyceride level known to be influenced by a large number of factors, including: sex, age group, obesity, metabolic syndrome, hypertension, diabetes, smoking and alcohol drinking. In the present study, some APOA5 single nucleotide polymorphisms (rs662799, rs3135506 and rs2075291) were associated with lipid variables as components of MetS.

According to some studies in Caucasian or predominantly Caucasian populations,[18–20] and Chinese populations,[21] the rs662799 variant is associated with a significant increase in levels of triglyceride, although the effects were considerably lower in magnitude than seen here with the rs2075291 SNP. Our findings showed that variations related to the rs2075291, could determine the triglyceride level in the presence of the metabolic syndrome, nevertheless our other results shows that, rs662799 variant were no significant differences between case and control group in terms of allele frequencies.

The APOA5 polymorphisms were identified to be implicated in the regulation of blood pressure and development of hypertension in the Japanese population.[22] Also, in a recent study Ouatou, et al. found a significant difference between patients with arterial hypertension (AHT) and controls regarding the frequency of rs3135506 and rs662799 genotypes.[23] According to Ouatou, this regulatory role of APOA5 may contribute to the association between APOA5 and hypertension, in our study this association has been shown by rs662799 ($P = 4.5 \times 10^{-2}$).

Also, a previous study in Caucasians showed a stronger association of rs662799 with plasma triglycerides in men.[2] However, in a recent study in Turkey, the association of the APOA5 genetic variants with plasma triglycerides and the metabolic syndrome was shown to be more significant in women than in men.[24] This may be an example of a sex difference in the contribution of an SNP to a phenotype.[25] However, this sex differences were not highlighted in our result. Indeed, several studies were reported associations between APOA5 SNPs and metabolic syndrome (Table 4).[22,26,27]

Table 1. Genotyping procedure detail

SNP	Common Name		Primers	Annealing Temperature	Incubated Temperature	Restriction Enzyme	Homozygote (bp)	Homozygote (bp)	Homozygote (bp)
rs662799	-1131T>C	Forward	GATTGATTCAAGATGCATTTAGGA	56°C	37°C	*MseI*	CC: 187	TC: 187, 167, 20	TT: 167, 20
		Reverse	CCCCAGGAACTGGAGCGAAATT						
rs3135506	c.56C>G	Forward	CCACCCTGGGGGAGGAGAGCCCAAGCCCTG	67°C	37°C	*Sau96I*	CC:81, 186	CG: 81, 151, 186	GG: 81, 151
		Reverse	AGAGCTAGCACCGCTCCTTT						
rs2075291	c.553G>T	Forward	CACCAAGGCCCAGTTGCTGGG	67°C	37°C	*HaeIII*	GG: 11, 76, 51	GT: 11, 127, 76, 51	TT:11,127
		Reverse	ATGCCGCTCACCAGGCTCTCGGCG						

Table 2. Biochemical characteristic of MetS and non-MetS

Parameters	Non-MetS (n = 490)	MetS (n = 457)	*P*-value
Age (Year)	35 ± 15.6	52 ± 16.5	**0.001**
Fasting Blood Sugar	90.0 ± 7.6	101.8 ± 20.3	0.092
Total Cholesterol (mg/dL)	177.9 ± 35.0	203.7 ± 42.3	0.506
HDL Cholesterol (mg/dL)	51.7 ± 11.7	44.4 ± 10.196	**0.026**
LDL Cholesterol (mg/dL)	106.6 ± 30.8	125.8 ± 38.2	0.613
Triglycerides (mg/dL)	98.0 ± 37.4	169.1 ± 78.9	**0.001**
Systolic Blood Pressure	108.1 ± 11.2	121.8 ± 16.9	0.661
Diastolic Blood Pressure	72.2 ± 8.3	78.5 ± 10.8	**0.087**
Body Mass Index	25.8 ± 4.2	30.0 ± 4.8	0.927
Hip Circumference	98.6 ± 7.6	103.3 ± 8.6	0.946
Waist Circumference	87.9 ± 10.0	100.2 ± 9.4	**0.003**
Weight	68.4 ± 11.9	78.5 ± 14.5	0.443
Wrist Circumference	16.2 ± 1.4	17.2 ± 1.7	0.292
Height	163.0 ± 10.2	161.8 ± 10.7	0.251

Table 3. Genotype and allele frequency in 3 studied SNPs

Polymorphism	Number	Frequency
rs3135506 C/G		
Genotype		
CC	1	0.0017
CG	1	0.0017
GG	570	0.9965
Allele		
C	3	0.0026
G	1141	0.9973
rs2075291 G/T		
Genotype		
GG	886	0.9346
GT	61	0.0643
TT	1	0.0011
Allele		
G	1833	0.9667
T	63	0.0332
rs662799 C/T		
Genotype		
CC	31	0.2385
CT	26	0.2
TT	73	0.5615
Allele		
C	88	0.3384
T	172	0.6615

Table 4. Association study result with Plink by Fisher's exact test

Variables	Case / Control *P*-Value	Chr	SNP	Number
Systolic Blood Pressure (mmHg)	8.3×10^{-1}	11	rs662799	130
Diastolic Blood Pressure (mmHg)	4.5×10^{-2}	11	rs662799	130
Total cholesterol (mg/dL)	4.2×10^{-2}	11	rs3135506	571
Triglycerides (mg/dL)	4.5×10^{-4}	11	rs2075291	947
HDL cholesterol (mg/dL)	1.6×10^{-3}	11	rs2075291	947
LDL cholesterol (mg/dL)	3.6×10^{-2}	11	rs2075291	947
Metabolic syndrome	1.5×10^{-2}	11	rs662799	130

According to the Iranian modified NCEP definition for metabolic syndrome, other lipid dependent variables such as triglyceride and HDL level showed significant differences in individuals with metabolic syndrome compared to healthy individuals. We proposed that APOA5 rs2075291 could have a functional polymorphism, while APOA5 rs662799 function is still under debate. Consequently, due to the rarity of these SNPs, more studies in a larger population are required to reconfirm these results.

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