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Original Article

Association between single nucleotide polymorphisms rs72525532, rs45596738, rs148759216, rs188133936, and rs114627122 of *APOA5* gene in children and adolescents with metabolic syndrome

Samaneh Salehi[1], Modjtaba Emadi-Baygi[1,2*], Parvaneh Nikpour[3,4], Roya Kelishadi[4]

[1]Department of Genetics, Faculty of Basic Sciences, Shahrekord University, Shahrekord, Iran
[2]Research Institute of Biotechnology, Shahrekord University, Shahrekord, Iran
[3]Department of Genetics and Molecular Biology, Faculty of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
[4]Child Growth and Development Research Center, Research Institute for Primordial Prevention of Non-communicable Disease, Isfahan University of Medical Sciences, Isfahan, Iran

*__Corresponding Author:__ Modjtaba Emadi-Baygi, Department of Genetics, Faculty of Basic Sciences, Shahrekord University, Shahrekord, Iran. Tel: +98 38 32324419, Fax: +98 38 32324419, Email: emadi-m@sci.sku.ac.ir

Abstract

Background and aims: The *APOA5* gene is one of the genes involved in metabolic syndrome (MetS), as a constellation of several cardiovascular disease (CVD) risk factors. The present study evaluated the possible associations between five single nucleotide polymorphisms (SNPs) in the microRNA target site (miR-TS-SNPs) of the *APOA5* gene with MetS.

Methods: This case-control study included 57 MetS cases, along with 59 normal children and adolescents aged 9-18 years. All miR-TS-SNPs rs188133936, rs72525532, rs45596738, rs148759216, and rs114627122 were genotyped by polymerase chain reaction-sequencing. Independent t-test, as well as the chi-square test and logistic regression analysis was used to determine the association of SNPs with MetS risk and its clinical components.

Results: The mean (SD) age of MetS participants and controls was 12.35 (0.25) and 13.39 (0.38) years, respectively. Although no nucleotide changes were present in rs188133936, rs45596738, rs148759216, and rs114627122, a greater frequency of A insertion was detected in rs72525532 in MetS cases compared with the control group ($P=0.012$). This variant showed a significant difference in triglycerides (TG) and high-density lipoprotein cholesterol (HDL) levels between different genotype groups ($P<0.0001$ and $P=0.05$, respectively) in controls. Furthermore, AA insertion genotype was correlated with an increased risk of MetS (Odds ratio [95% CI] = 8.12 [0.966-68.27], $P=0.05$).

Conclusion: This study was the first to investigate the association between rs188133936, rs45596738, rs148759216, rs76463524, and rs72525532 variants of the *APOA5* gene and MetS. Our findings reveal that rs72525532 might have an impact on TG, HDL levels, and the risk of MetS.

Keywords: Metabolic syndrome, *APOA5*, Single nucleotide polymorphism, miRNA

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Introduction

Metabolic syndrome (MetS), or the insulin resistance syndrome (syndrome X), is considered as a major public health problem that is the clustering of clinical features including elevated blood pressure, abdominal obesity, insulin resistance, and dyslipidemia. In addition, MetS is recognized as an independent risk factor for type 2 diabetes, cardiovascular diseases (CVD), and CVD mortality (1-3). Based on the result of some studies, this syndrome is prevalent among the Iranian population (4,5). Similarly, MetS has an intricate etiology and represents the results from the interactions of genetics and environmental factors (6). Hyperlipidemia refers to any disease resulting in high levels of plasma triglyceride (TG), cholesterol and low-density lipoprotein, and low levels of high-density lipoprotein cholesterol (HDL-C) concentrations. As the risk factor for CVD, various genes, including apolipoprotein A5 (*APOA5*) located on chromosome 11q23 in the APOA1-C3-A4 gene cluster (7,8), are involved in the pathology of hyperlipidemia. This gene expresses the APOAV protein, which is synthesized in the liver. Further, APOAV protein is believed to increase the levels of plasma TG while reducing the HDL-C levels and interacting with lipoprotein lipase (9,10). MicroRNAs (miRNAs) are a group of non-protein-coding RNAs which are demonstrated to play important regulatory roles in diverse biological processes like adiposity, insulin resistance, and appetite regulation (11), as well as having

a critical role in metabolism (12). MiRNAs also create a perfect Watson-Crick match by binding to nucleotides 2-8 from the 5' end of the miRNA (the seed sequence) to their target sequences in the 3' untranslated region of mRNAs. By binding to the target site, miRNAs inhibit translation and induce cleavage/decay of their target mRNAs (12-14). Single nucleotide polymorphisms (SNPs) in miRNA gene (miR-SNPs) or miRNAs target site (miR-TS-SNP) probably affect the gene expression levels (13-15).

Likewise, miR-TS-SNP can disturb miRNA target sites or create a new miRNA binding site and have a tremendous effect on diverse biological functions, including disease susceptibilities, cancers, Parkinson's disease, osteoporosis, diabetes, hypertension, and MetS (16,17). Several variants (miR-TS-SNPs) have been identified in the *APOA5* gene, including rs72525532 (c.*285_*286insGA), rs188133936 (c.*191 C>T), rs114627122 (c.*172 C>T), rs45596738 (c.*288-289 insGA), and rs148759216 (c.*289-290 insAG) (18). These polymorphisms occur in miRNA binding site on mRNA that leads to the gain or loss of the binding site for these miRNAs. So far, no study has examined the association of these five miR-TS-SNPs of *APOA5* gene, along with the risk of MetS and its major components in children and adolescents. Therefore, the current study aimed to evaluate the genotype and allele distributions of these variants in a cohort of MetS children and adolescent to assess if there is an association between such variants and the risk of MetS.

Materials and Methods

This report is a case-control study which contained 116 children and adolescents from Isfahan, a central province in Iran. The case group included 57 MetS children diagnosed with modified ATP III (Adult Treatment panel III) criteria as follows:

- Waist circumference > 75th percentile for the age and gender in the studied population;
- Fasting TG ≥ 100 mg/dL;
- Serum HDL-C<50 mg/dL;
- Systolic blood pressure/diastolic blood pressure >90th percentile for gender, age, and height fasting blood sugar ≥100 mg/dL.

Further, MetS was defined as the presence of at least three of the above-mentioned metabolic traits (19). Fifty-nine control subjects had no clinical and laboratory marker of MetS, diabetes, or cardiovascular disorders.

MiRNAs targeting variations within apolipoprotein A5 (*APOA5*) target gene, which lead to the gain or loss of the binding site for these miRNAs, were identified by using miRNA-related SNP (20), PolymiRTS (21), and MirSNP databases (22). Furthermore, TargetScan (23), Mirwalk (24), and the miRanda (25) databases were utilized to predict miRNA target sites within the 3' untranslated regions of the *APOA5* gene (Table 1).

Then, DNA was extracted from blood leukocytes by using the Diatome kit (Isogen Laboratory, Russia). The amplification and genotyping of all SNPs at the *APOA5* gene were performed by applying polymerase chain reaction (PCR) and Sanger sequencing methods.

Similarly, primers A5 forward (5'-AGGCACTGGGACTGAGGAAG -3') and A5 reverse (5'-GGCAGCCAGAAGTGACTAGAG-3') were designed for the amplification of the 708 bp regions containing these SNPs. Moreover, to amplify the desired segment, the PCR conditions were followed as initial denaturation at 95°C for 2.5 minutes, 35 cycles of denaturation at 95°C for 50 seconds, annealing at 62°C for 70 seconds, extension at 72°C for 70 seconds, and the final extension at 72°C for 15 minutes. PCR reagent mixture included 0.25 µL of Taq DNA polymerase (5 U/µL, KBC, Tehran, Iran), 10X buffer (2.5 µL), 0.75 µL $MgCl_2$ (50 mM), 0.5 µL deoxynucleoside triphosphate (40 mM), 2 µL DNA (200 ng/µL), 0.5 µL appropriate specific primer pairs (10 pmol/µL), and water in the final volume of 25 µL. The genotypic distribution between the cases and controls were compared by the Chi-square test, followed by an independent *t* test to compare the clinical conditions of MetS between all the genotypes of rs72525532. The association between the genotypes and MetS risk was estimated by simple and multivariate logistic regressions through calculating the odds ratios (ORs) and 95% confidence intervals (CIs). Finally, statistical analyses were performed using SPSS software, version 16.0 (SPSS, Inc., Chicago, IL). All data were expressed as mean ± standard error of mean and *P* values lower than 0.05 were considered statistically significant.

Results

This study included 57 MetS participants (children and adolescents) with the mean (SD) age of 12.35 (0.25) years and 59 controls with the mean (SD) age of 13.39 (0.38) years. Table 2 represents the clinical characteristics of MetS individual and control subjects, including plasma TG, total cholesterol (TC), low-density lipoprotein-cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C) levels, and body mass index.

As expected, the patient subjects had significantly higher TG, TC, LDL-C levels, and body mass index ($P<0.0001$), and lower levels of HDL-C ($P<0.0001$). The result of genotyping of four SNPss at apolipoprotein A5 (*APOA5*) gene, including rs188133936 (c.*191 C>T), rs114627122 (c.*172 C>T), rs45596738 (c.*288-289 insGA), and rs148759216 (c.*289-290 insAG) represented no changes, while an insertion A was found at the 3′ untranslated region (UTR) position 285-286 in rs72525532 (c.*285-286 insGA).

Based on the data obtained from the NCBI, the rs72525532 was reported as the insertion GA in position 285-286 at 3′ UTR of the *APOA5* gene. The genotype distributions of this variant between 116 selected

Table 1. The list of miR-TS-SNPsof *APOA5* gene

Polymorphism	Allele	microRNA	Algorithm
rs114627122	T	hsa-miR-513a-5p	miRNASNP, PolymiRTS Database, MirSNP, and TargetScan
	C	hsa-miR-4722-5p	
rs72525532	-	hsa-mir-7113-3p	PolymiRTS Database, MirSNP, TargetScan, and miRanda
		hsa-mir-2682-3p	
		hsa-mir-4287	
		hsa-mir-4469	
		hsa-mir-4685-3p	
		hsa-mir-6781-3p	
		hsa-mir-6867-3p	
		hsa-miR-3667-3p	
		hsa-mir-4297	
	GA	hsa-mir-4691-5p	
		hsa-mir-6749-3p	
		hsa-mir-6792-3p	
rs188133936	C	hsa-mir-324-5p	TargetScan, miRanda, PolymiRTS Database, and MirSNP
		hsa-miR-5700	
		hsa-miR-2054	
	T	hsa-miR-374c-5p	
		hsa-mir-655-3p	
rs45596738/rs397897431	-	hsa-miR-1470	PolymiRTS Database, MirSNP, TargetScan, and miRanda
		hsa-mir-4287	
		hsa-mir-4469	
		hsa-mir-4667-3p	
		hsa-mir-4685-3p	
		hsa-mir-6867-3p	
		hsa-mir-7113-3p	
	GA	hsa-miR-6845-3p	
		hsa-mir-7110-3p	
rs148759216	-	hsa-mir-2682-3p	PolymiRTS Database, MirSNP, TargetScan, and miRanda
		hsa-mir-6781-3p	
		hsa-mir-3183	
		hsa-mir-4723-3p	
	AG	hsa-mir-6769b-3p	
		hsa-mir-6845-3p	
		hsa-mir-7110-3p	

Table 2. Basic characteristics of participants in the case-control study

	Case Group (n = 57)		Control Group (n = 63)		*P* Value
	Mean	SEM	Mean	SEM	
Age (y)	12.35	0.25	13.39	0.38	0.03
Boys/girls	25/32		26/33		0.49
BMI (kg/m^2)	26.68	0.52	18.31	1.06	<0.001
TG (mg/dL)	110.98	6.73	73.46	3.22	<0.001
TC (mg/dL)	161.86	4.02	141.61	4.27	0.005
HDL-C (mg/dL)	43.25	0.69	49.03	1.23	<0.001
LDL-C (mg/dL)	89.68	2.77	76.14	1.99	<0.001

BMI: body mass index; TG: triglyceride; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; SEM: standard error of mean. Values are expressed as mean ± SEM.

individuals are shown in Table 3. The differences in the distribution of *APOA5* genotypes in rs72525532 were statistically significant between case and control subjects. In addition, the frequency of AA insertion genotype was significantly higher in the MetS group as compared to the control group ($P=0.012$). The data demonstrated that the frequencies of AA insertion genotype were 1.7% and 12.28% in control and case groups, and the frequencies of no insertion genotype were 98.3% and 87.72% in control and case groups, respectively. The comparison of the laboratory parameters of the case and control subjects according to different *APOA5* genotypes is summarized in Table 4. These data showed a significant difference between different genotype groups regarding TG and HDL-C levels, whereas no significant associations were observed between this variant and the other components of MetS.

On the other hand, TG levels were significantly higher

Table 3. Genotype Frequencies of rs72525532 in Case and Control Groups

Group	Total N	No. of Insertion		A/A		*P* Value
		No.	%	No.	%	
Case	57	50	87.72	7	12.28	0.012
Control	59	58	98.3	1	1.7	

among the control samples in AA insertion genotype group compared to no insertion genotype groups ($P<0.0001$), whereas there was a borderline significant difference between the two genotype groups ($P=0.07$) in MetS participants. Furthermore, HDL-C levels were lower among subjects with no insertion genotype in the control groups compared to AA insertion genotype groups ($P=0.05$). The results of logistic regression analysis (Table 5) revealed that the AA insertion genotype was significantly associated with MetS risk [OR (95% CI) = 8.12 (0.966-68.27), $P=0.05$], while this association was insignificant after the adjustment for age [OR (95% CI) = 5.66 (0.629-46.357), $P=0.124$].

Discussion

The current study, to the best of our knowledge, was the first research that explored the associations of five polymorphisms of apolipoprotein A5 (*APOA5*) gene with MetS and its various components in children and adolescents. MetS is associated with the incidence of CVDs and mortality (26-28). Generally, SNPs of *APOA5* are correlated with dyslipidemia, including the increased plasma TG levels (29-31).

Our previous study evaluated the association of some *APOA5* the 3' untranslated region (3'UTR) SNPs with MetS among Iranian population (32). According to one study, miR-TS-SNPs are associated with MetS risk (16), but no study has yet investigated five polymorphisms located in the *APOA5* gene, including rs188133936, rs114627122, rs45596738, rs148759216, and rs72525532 regarding their association with MetS. The result of the sequencing indicated no variations in four *APOA5* gene variants (i.e., rs188133936, rs114627122, rs45596738, and rs148759216); however, an insertion A was found at 3' UTR position 285-286 in rs72525532. In the dbSNP database, the rs72525532 variant was shown as insertion GA at 3' UTR positions 285-286. Further, the genotype distributions of rs72525532 variant were statistically different between the case and control groups. MetS participants showed higher frequencies of A insertion compared to control groups. As regards TG levels, variant rs72525532 demonstrated a significant difference between both genotype groups ($P<0.001$) among the control subjects, while participants with MetS showed a borderline significant difference ($P=0.06$). Therefore, based on the data, participants with AA insertion genotype in the control group had significantly higher levels of TG as compared to those with no insertion genotypes. Conversely, the subject with no insertion AA genotypes in the control group had a lower level of HDL-C compared with the subject with insertion AA genotypes. Meanwhile, there was no significant association between rs72525532 polymorphism and other MetS components, which included low-density lipoprotein-C and body mass index. Our results revealed an association between insertion AA genotypes and a higher risk of MetS although this association was insignificant after adjustment for the age. In a study conducted on the Han Chinese population, Ye et al evaluated the correlation between eight miR-TS-SNPs and the MetS risk and concluded that rs5999924 and rs5750146 in 3' UTR at APOL5 gene were related to MetS. They also found significant relationships between rs11724758 in 3' UTR at the FABP2 gene with MetS. The HDL-C level in the carriers of the AA genotype of

Table 4. Associations of clinical parameters with rs72525532 genotype according to case-control status

	Case			Control		
	No of Insertion	AA	*P* Value	No of Insertion	AA	*P* Value
Age (y)	12.56±0.28	11.29±0.29	0.05	13.27±0.40	13	0.43
BMI (kg/m^2)	26.91±0.57	24.60±0.61	0.09	-	-	-
TG (mg/dL)	114.76±7.35	84±12.35	0.07	71.76±2.78	172	0.001
TC (mg/dL)	163.18±4.25	152.43±12.57	0.19	141.10±4.29	171	0.06
HDL-C (mg/dL)	43.08±0.77	44.43±0.99	0.14	48.88±1.24	58	0.05
LDL-C (mg/dL)	90.10±2.91	86.71±9.37	0.35	76.10±2.03	78	0.186

BMI: body mass index; TG: triglyceride; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

Table 5. The OR at 95% CI calculated by multiple logistic regression analysis models

Genotype	Crude OR (95% CI)	*P* Value	Adjusted OR (95% CI)	*P* Value
AA vs. No insertion	8.12 (0.966-68.27)	0.054	5.66 (0.629-46.357)	0.124

Adjusted for age; OR: odds ratio; CI: Confidence intervals.

rs11724758 variant was significantly higher than that of the non-carrier subjects while the level of TG and fasting blood sugar were lower in the carriers of AA genotype (16).

The current study only tested the associations of the SNPs among the Isfahan population, which is considered as the limitation of the study. However, we explored the association of the SNPs with the MetS for the first time among children and adolescents, which is the strong point of the present study.

Conclusion

This study was the first to evaluate the association between five SNPss located at apolipoprotein A5 3`UTR and MetS and its major components risk. Our results suggest that rs72525532 might be correlated with TG and HDL-C levels. Further studies including more diverse participants of the Iranian population are valuable to strengthen our results in the future.

Conflict of interests

None.

Ethical considerations

The Ethics Committee of Isfahan University of Medical Sciences approved the current study (under the ethics code of 293284). Oral assent and informed written consent were taken from the participants and their parents, respectively.

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