# Identification of a New Single-nucleotide Polymorphism within the Apolipoprotein A5 Gene, Which is Associated with Metabolic Syndrome

## Abstract

**Background:** Metabolic syndrome (MetS) is a common disorder which is a constellation of clinical features including abdominal obesity, increased level of serum triglycerides (TGs) and decrease of serum high-density lipoprotein-cholesterol (HDL-C), elevated blood pressure, and glucose intolerance. The apolipoprotein A5 (APOA5) is involved in lipid metabolism, influencing the level of plasma TG and HDL-C. In the present study, we aimed to investigate the associations between four INDEL variants of APOA5 gene and the MetS risk. **Materials and Methods:** In this case–control study, we genotyped 116 Iranian children and adolescents with/without MetS by using Sanger sequencing method for these INDELs. Then, we explored the association of INDELs with MetS risk and their clinical components by logistic regression and one-way analysis of variance analyses. **Results:** We identified a novel insertion polymorphism, c. *282–283 insAG/c. *282–283 insG variant, which appears among case and control groups. rs72525532 showed a significant difference for TG levels between various genotype groups. In addition, there were significant associations between newly identified single-nucleotide polymorphism (SNP) and rs72525532 with MetS risk. **Conclusions:** These results show that rs72525532 and the newly identified SNP may influence the susceptibility of the individuals to MetS.

**Keywords:** *Apolipoprotein A5, INDELs, metabolic syndrome*

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## Introduction

Metabolic syndrome (MetS) is a common disorder recognized as an independent risk factor for cardiovascular diseases and type 2 diabetes,[1] and it is a constellation of clinical features including abdominal obesity, increased level of serum triglycerides (TGs) 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).[2] MetS seems to result from a collision between genetics and environmental factors.[3] Among Iranian children and adolescent populations, low HDL-C and high TG are the most important components of the MetS.[4] The apolipoprotein A5 (APOA5) gene is located on chromosome 11q23 on the APOA1/C3/A4/A5 gene cluster, and consists of four exons encoding 366 amino acids.[5] The APOA5 protein is synthesized in the liver and is involved in lipid metabolism, influencing the level of plasma TG and HDL-C.[6-8]

According to the previous studies, APOA5 gene polymorphisms are associated with the criteria of MetS in Iranian population.[9,10]

MicroRNAs, which are 17–25 bases long,[11] create a perfect Watson–Crick match between the 5'-end or seed region of the mature form of the miRNA and a target site in the 3' untranslated region (3'UTR) of the mRNA and have been shown to play critical regulatory roles in a wide range of biological and pathological processes such as development, differentiation, proliferation, apoptosis, metabolism, and cancer.[12-16] Because of this complementarity in miRNA binding, genetic variants, such as single-nucleotide polymorphisms (SNPs) that affect miRNA targeting (miR-TS-SNPs) can disrupt miRNA target sites or create a new miRNA site and have been shown to impact gene expression and are associated with a wide range of diseases,[17,18] including cancers,[19-21] Parkinson disease,[22] osteoporosis,[23] diabetes,[24] and hypertension.[25] Several

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INDEL variations have been described in the APOA5 gene locus including rs72525532 (c. *285_*286insGA), rs45596738 (c. *288_*289insGA, c. *288_*289insAG), rs148759216 (c. *289_*290insAG), and rs397897431 (c. *284_*285insGA). Thus far, no study has examined the relationship of these four variants of APOA5 gene and the risk of MetS and its components. In this study, we aimed to determine the allelic and genotypic distributions of these polymorphisms as well as a novel insertion (ins) polymorphism c. *282_*283insG, c. *282_*283insGA, identified by our group.

## Materials and Methods

### Study population

The study population comprised 116 (2–19 years of age) children and adolescents from Isfahan. A total of 57 MetS patients (25 males and 32 females, mean age: $12.40 \pm 0.25$ years) were recruited according to the modified ATPIII definition,[4] whereby MetS is defined as the presence of at least three of the following criteria: Waist circumference >75th percentile for age and gender in the studied population, fasting TGs ≥100 mg/dl, serum HDL-C < 50 mg/dl), systolic blood pressure/diastolic blood pressure >90th percentile for gender, age, and height,[26] and fasting blood sugar ≥100 mg/dl. A total of 59 control subjects (26 males and 33 females, mean age: $13.26 \pm 0.392$ years) were selected from healthy subjects included those without any clinical and laboratorial mark of MetS. Informed consent was given by all participants and the study was approved by the Ethics Committee of Isfahan University of Medical Sciences.

### DNA genotyping

Genomic DNA was extracted from blood leukocytes using the Diatome kit (Isogen Laboratory, Russia) according to the vendor's recommended protocol. Genotyping of all SNPs was carried out using polymerase chain reaction (PCR) and direct sequencing methods. The SNP-containing regions were amplified using primers such as A5 forward (5'-AGGCACTGGGACTGAGGAAG-3') and A5 reverse (5'-GGCAGCCAGAAGTGACTAGAG-3'). The PCR conditions were the following for all polymorphisms: 2.5 min initial denaturation at 95°C, forty cycles of 50 s at 95°C; 70 s at 62°C; 70 s at 72°C, and the final extension at 72°C for 1.5 min.

### miRNA targeting single-nucleotide polymorphisms selection

miRNASNP (http://www.bioguo.org/miRNASNP/), PolymiRTS Database (http://compbio.uthsc.edu/miRSNP/),[27] and MirSNP (http://202.38.126.151/hmdd/mirsnp/search/) were used to identify miRNAs targeting variations within APOA5 target gene. Furthermore, we obtained insertion and deletion polymorphisms located on APOA5 3'UTRs with SNP IDs from the National Center for Biotechnology Information (NCBI) dbSNP database (http://www.ncbi.nlm.nih.gov/) and predicted miRNA target sites from TargetScan[28] (http://www.targetscan.org/) and the miRanda (http://www.microrna.org/microrna/getMirnaForm.do).[29] SNPs in predicted miRNA target sites are shown in Table 1.

### Statistical analysis

Chi-square test was performed to compare the genotypic distribution between cases and controls. Differences in the mean values of quantitative traits among case and control groups were evaluated using Student's *t*-test. All the genotypes of the 282–283 ins AG in the APOA5 gene were categorized as AAGG insertion, GG insertion and no insertion genotypes, and in 285–286 ins A as AA insertion

**Table 1: List of INDELs in miRNA target sites inside APOA5 gene**

| dbSNP ID | Allele | miR ID | Algorithms |
|---|---|---|---|
| rs148759216 | - | hsa-mir-2682-3p | PolymiRTS database and MirSNP and TargetScan and miRanda |
| | | hsa-mir-6781-3p | |
| | AG | hsa-mir-3183 | |
| | | hsa-mir-4723-3p | |
| | | hsa-mir-6769b-3p | |
| | | hsa-mir-6845-3p | |
| | | hsa-mir-7110-3p | |
| rs72525532 | - | hsa-mir-2682-3p | PolymiRTS database and MirSNP and TargetScan and miRanda |
| | | hsa-mir-4287 | |
| | | hsa-mir-4469 | |
| | | hsa-mir-4685-3p | |
| | | hsa-mir-6781-3p | |
| | | hsa-mir-6867-3p | |
| | | hsa-mir-7113-3p | |
| | GA | hsa-miR-3667-3p | |
| | | hsa-mir-4297 | |
| | | hsa-mir-4691-5p | |
| | | hsa-mir-6749-3p | |
| | | hsa-mir-6792-3p | |
| rs45596738/rs397897431 | - | hsa-miR-1470 | PolymiRTS database and MirSNP and 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 | |
| Newly identified polymorphism (c*282-283 insAG/c*282-283 insG) | - | hsa-miR-4469 | TargetScan and miRanda |
| | | hsa-miR-642 | |
| | | hsa-miR-1979 | |
| | | hsa-miR-1470 | |
| | | hsa-miR-4667 | |

SNP: Single-nucleotide polymorphism

and no insertion. One-way analysis of variance was used to compare the features of MetS between all genotypes of two variants. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by simple and multivariate logistic regression analyses, respectively, to estimate the associations between the genotypes and MetS risk. The multivariate regression analysis models were adjusted for age. Statistical analyses were conducted with the SPSS 16.0 statistical package (SPSS, Inc., Chicago, IL, USA). Values were expressed as mean ± standard error of mean and $P < 0.05$ was considered statistically significant.

## Results

Clinical characteristics of MetS patients and control subjects are shown in Table 2. There were significant age differences between the groups, but not in gender. TG, total cholesterol (TC), low-density lipoprotein-C (LDL-C) levels, and body mass index (BMI) were significantly higher in patient subjects ($P < 0.001$), but HDL-C was lower in MetS subjects ($P < 0.001$). While no changes were present in the rs148759216, rs454596738, and rs397897431 polymorphisms, we found an insertion A at 3` UTR position 285–286 in rs72525532. This variation has been previously reported as insertion GA, which was based on the data obtained from the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov). Furthermore, we identified a novel insertion polymorphism, designated as c. *282–283 insAG/c. *282–283 insG variant [Figure 1], which appears among both case and control groups. The genotype distributions of the two APOA5 variants, which showed a change between different individuals, are shown in Tables 3 and 4. The differences in the distribution of APOA5 genotypes in two variants were statistically significant comparing cases with control subjects. The basic characteristics of the patients and control subjects stratified according to the different APOA5 genotypes are shown in Tables 5 and 6. Variant rs72525532 showed a significant difference for TG levels between different genotype groups ($P < 0.001$) in control samples and a borderline significant difference ($P = 0.06$) in patient subjects. There were no significant associations between this variant and TC, LDL-C, and HDL-C among the two groups. There was a borderline association between the newly identified SNP and BMI levels in control group ($P = 0.08$). In addition, in the case group, a borderline association was seen between newly identified SNP and HDL-C levels ($P = 0.07$). In two groups, HDL-C levels and BMI were elevated in subjects with AAGG insertion genotype. Results of the logistic regression analysis are shown in Table 7. In both variants, the association between APOA5 genotypes and MetS was statistically significant. In the newly identified SNP, when genotypes were grouped in no insertion, AAGG insertion, and GG insertion, the presence of the AAGG insertion genotype was associated with higher MetS risk (OR [95%

### Table 2: Major clinical parameters of the study populations

| Clinical parameters | Case group (*n*=57) | | Control group (*n*=59) | | *t* | *P* |
|---|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | | |
| Age (years) | 12.40 | 0.25 | 13.26 | 0.392 | 1.89 | 0.03 |
| Boys/girls | 25/32 | | 26/33 | | 0.001 ($\chi^2$) | 0.49 |
| BMI (kg/m²) | 26.68 | 0.52 | 18.31 | 1.06 | 7.32 | <0.001 |
| TG (mg/dl) | 110.98 | 6.73 | 73.46 | 3.22 | 5.08 | <0.001 |
| TC (mg/dl) | 161.86 | 4.02 | 141.61 | 4.27 | 3.45 | 0.005 |
| HDL-C (mg/dl) | 43.25 | 0.69 | 49.03 | 1.23 | 8.24 | <0.001 |
| LDL-C (mg/dl) | 89.68 | 2.77 | 76.14 | 1.99 | 3.98 | <0.001 |

Values are expressed as mean±SEM. SEM: Standard error of mean, BMI: Body mass index, TG: Triglyceride, TC: Total cholesterol, HDL-C: High-density lipoprotein-cholesterol, LDL-C: Low-density lipoprotein-cholesterol

### Table 3: Genotype frequencies of the APOA5 c*285-286 ins A genotype variant

| Group | Total (*n*) | No insertion *n* (%) | A/A *n* (%) | $\chi^2$ | *P* |
|---|---|---|---|---|---|
| Case | 57 | 50 (87.72) | 7 (12.28) | 5.06 | 0.012 |
| Control | 59 | 58 (98.3) | 1 (1.7) | | |

### Table 4: Genotype frequencies of the c*282-283 insAG variant

| Group | | Genotype frequency *n* (%) | | | | |
|---|---|---|---|---|---|---|
| | Total (*n*) | No insertion | G/G | AG/AG | $\chi^2$ | *P* |
| Case | 57 | 38 (66.67) | 1 (1.75) | 18 (31.57) | 15.183 | 0.0005 |
| Control | 59 | 18 (30.5) | 2 (3.4) | 39 (66.1) | | |

### Table 5: Clinical measurements by APOA5 c*285-286 insA genotype according to case-control status

| Clinical parameters | No insertion | | AA | | *t* | *P* |
|---|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | | |
| Case | | | | | | |
| Age (year) | 12.56 | 0.28 | 11.29 | 0.29 | 1.70 | 0.04 |
| BMI (kg/m²) | 26.91 | 0.57 | 24.60 | 0.61 | 1.34 | 0.09 |
| TG (mg/dl) | 114.76 | 7.35 | 84 | 12.35 | 1.52 | 0.06 |
| TC (mg/dl) | 163.18 | 4.25 | 152.43 | 12.57 | 0.86 | 0.19 |
| HDL-C (mg/dl) | 43.08 | 0.77 | 44.43 | 0.99 | 0.64 | 0.26 |
| LDL-C (mg/dl) | 90.10 | 2.91 | 86.71 | 9.37 | 0.39 | 0.35 |
| Control | | | | | | |
| Age (year) | 13.27 | 0.40 | 13 | - | 0.10 | 0.46 |
| BMI (kg/m²) | - | - | - | - | - | - |
| TG (mg/dl) | 71.76 | 2.78 | 172 | - | 4.69 | 0.000 |
| TC (mg/dl) | 141.10 | 4.29 | 171 | - | 0.91 | 0.18 |
| HDL-C (mg/dl) | 48.88 | 1.24 | 58 | - | 0.95 | 0.17 |
| LDL-C (mg/dl) | 76.10 | 2.03 | 78 | - | 0.12 | 0.45 |

SEM: Standard error of mean, BMI: Body mass index, TG: Triglyceride, TC: Total cholesterol, HDL-C: High-density lipoprotein-cholesterol, LDL-C: Low-density lipoprotein-cholesterol
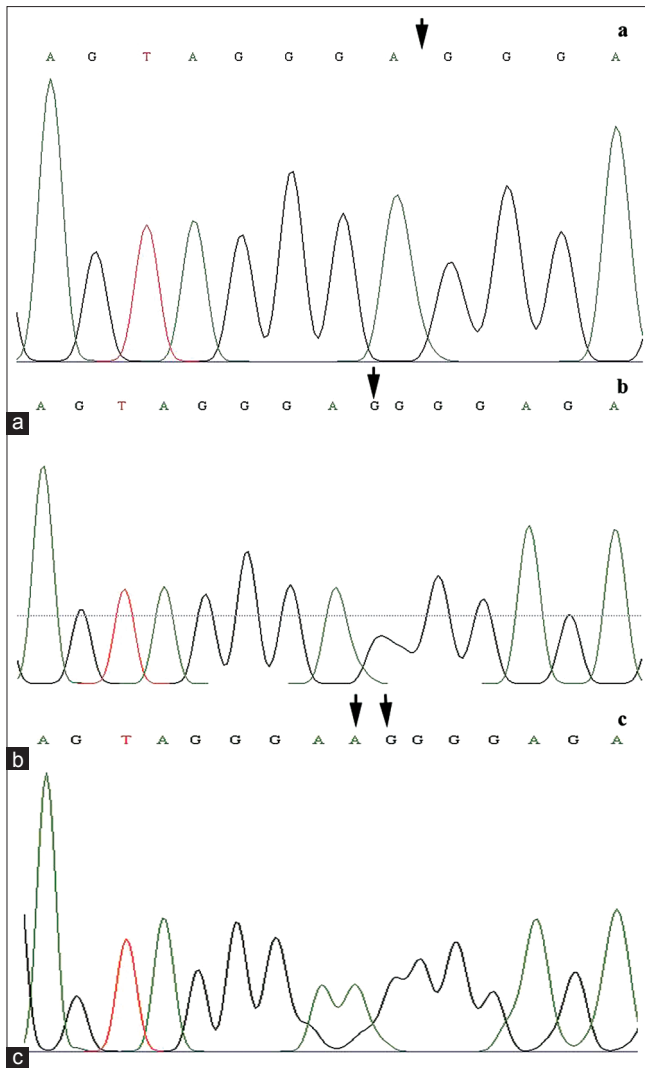
Figure 1: Electropherogram of the polymerase chain reaction product amplified from 3` UTR of APOA5 gene. A sample showing no insertion in nucleotide 282 (a), a sample having a G nucleotide insertion (b), and a representative sample with an insertion of AG (c)

CI] =4.574 [2.073–10.093], *P* < 0.001 [crude model], OR [95% CI] =3.510 [1.510–8.156], *P* = 0.004 [model adjusted to age]). In rs72525532, the multiple logistic regression analysis revealed the following associations for AA insertion genotypes: (OR [95% CI] =8.12 [0.966–68.27], *P* = 0.05 [crude model], OR [95% CI] =5.66 [0.653–49.112], *P* = 0.116 [model adjusted to age]).

## Discussion

Previous studies suggest that variation in the APOA5 gene influences plasma TG levels[6,7] and are associated with MetS risk.[30] In the present study, we investigated four insertion polymorphisms locating in the APOA5 3` UTR inside microRNA target sites for association with MetS and its various components. Previous studies demonstrate that miR-TS-SNPs correlate with MetS risk as well.[31] No mutations were found in some of the APOA5 gene variants (rs148759216, rs454596738, and rs397897431), but in rs72525532 variant, we found an insertion A at 3` UTR positions 285-286. The APOA5 rs72525532 variant has been previously reported as insertion GA at 3' UTR positions 285–286, which was based on the data obtained from the NCBI dbSNP database (http://www.ncbi.nlm.nih. gov/). Data emerging from the APOA5 3'UTRs sequencing reveal a novel insertion polymorphism, designated as c. *282–283 insAG/c. *282–283 insG variant. In this study, which included 57 MetS patients and 59 controls, the genotype distributions of APOA5 gene variants (rs72525532 and newly identified SNP) were statistically different between the patients and controls. In the newly identified insertion polymorphism, control subjects show higher frequencies of AG insertion than patients. While in the APOA5 rs72525532 variants, we found a higher frequency of A insertion in patients compared with control groups. In this case–control study including children and adolescent populations, variant rs72525532 showed

Table 6: Clinical measurements by APOA5 c*282-283 insAG genotype according to case-control status

| Clinical parameters | No insertion | | GG | | AAGG | | *t* | *P* |
|---|---|---|---|---|---|---|---|---|
| | Mean | SEM | Mean | SEM | Mean | SEM | | |
| Case | | | | | | | | |
| Age (year) | 12.25 | 0.31 | 12 | - | 12.72 | 0.48 | 0.63 | 0.33 |
| BMI (kg/m²) | 26.68 | 0.65 | 27.2 | - | 26.61 | 0.98 | 0.12 | 0.49 |
| TG (mg/dl) | 103.03 | 7.53 | 121 | - | 127.22 | 13.72 | 1.19 | 0.12 |
| TC (mg/dl) | 160.42 | 4.98 | 123 | - | 167.06 | 6.93 | 1.06 | 0.16 |
| HDL-C (mg/dl) | 42.32 | 0.75 | 44 | - | 45.17 | 1.43 | 1.38 | 0.07 |
| LDL-C (mg/dl) | 89.68 | 3.75 | 60 | - | 91.33 | 3.52 | 1.03 | 0.18 |
| Control | | | | | | | | |
| Age (year) | 13.31 | 0.64 | 16 | - | 13.14 | 0.51 | 0.74 | 0.29 |
| BMI (kg/m²) | 15.9 | 0.79 | - | - | 19.21 | 1.31 | 1.46 | 0.08 |
| TG (mg/dl) | 75.17 | 6.74 | 77.5 | 21.5 | 72.46 | 3.72 | 0.31 | 0.45 |
| TC (mg/dl) | 143.44 | 7.25 | 152.5 | 26.5 | 140.21 | 5.43 | 0.41 | 0.42 |
| HDL-C (mg/dl) | 49.61 | 1.46 | 48 | 12 | 48.82 | 1.69 | 0.23 | 0.47 |
| LDL-C (mg/dl) | 76.33 | 3.86 | 74 | 0 | 76.15 | 2.47 | 0.14 | 0.49 |

SEM: Standard error of mean, BMI: Body mass index, TG: Triglyceride, TC: Total cholesterol, HDL-C: High-density lipoprotein-cholesterol, LDL-C: Low-density lipoprotein-cholesterol

**Table 7: The odds ratios at 95% confidence intervals calculated by multiple logistic regression analysis models**

| Polymorphism | Allele/genotype | Crude OR (95%CI) | *P* | Adjusted[a] OR (95%CI) | *P* |
|---|---|---|---|---|---|
| c*285-286 insA | AA versus no insertion | 8.12 (0.966-68.27) | 0.054* | 5.66 (0.629-46.357) | 0.124 |
| c*282-283 insAG | GG versus no insertion | 4.22 (0.36-49.67) | 0.25 | 1.61 (0.08-30.98) | 0.75 |
| | AAGG versus no insertion | 4.57 (2.07-10.09) | <0.001* | 3.51 (1.51-8.16) | 0.004* |
| | AAGG versus GG | 1.08 (0.09-12.74) | 0.95 | 1.79 (0.10-31.39) | 0.69 |

[a]Adjusted for age. CI: Confidence interval, OR: Odds ratio, *Indicates significant *P* values

a significant difference for TG levels between genotype groups (*P* < 0.001) among participants without MetS and a borderline significant difference (*P* = 0.06) in patient subjects. In the control group, subjects with AA insertion genotype had significantly higher levels of TG than those with no insertion mutation.

Other MetS components, which include HDL-C, LDL-C, and BMI, were not associated with rs72525532 polymorphism. In addition, this study did not find any significant associations between newly identified SNP and various MetS components. Variant rs72525532 was associated with a higher risk of MetS. Although association of this variant with MetS was convincingly statistically significant, this association was not significant after adjustment for age. The newly identified APOA5 variant, c. *282–283 insAG/c. *282–283 insG, was associated with the risk of MetS before and after adjustment.

## Conclusions

The present study is the first to investigate the association between 3` UTR insertion variants of the APOA5 gene and MetS and its various components. Our findings show that rs72525532 might have an effect on TG levels and it is recognized as a risk factor for MetS. In addition, the newly identified SNP, c. *282–283 insAG/c. *282–283 insG, may influence the susceptibility of the individual to MetS.

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### Conflicts of interest

There are no conflicts of interest.

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