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Gender-specific associations of the APOA1 −75G>A polymorphism with several metabolic syndrome components in Turkish adults

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ABSTRACT

Background: Variations in the apolipoprotein A-1 (*APOA1*) gene, a determinant of plasma high-density lipoprotein cholesterol (HDL-C) and apoA-I levels, may contribute to cardiovascular diseases. We evaluated the effects of a promoter polymorphism (−75G>A) in the *APOA1* gene on metabolic syndrome (MetS) components in a Turkish population sample.
Methods: Randomly selected 1515 Turkish adults (age 49.9 ± 11.8 years, 785 females) were genotyped for −75G>A polymorphism using hybridization probes in Real-Time PCR LC480 device. MetS and atherogenic dyslipidemia were defined using the criteria of ATP III.
Results: The −75AA genotype prevailed in 3.9% of men and 2.4% of women, and was independently associated with significantly higher HDL-C concentrations. Independent associations with the −75GA genotype existed only in men: higher diastolic and systolic blood pressure (BP) levels ($p < 0.05$) were observed in male −75GA heterozygotes. Logistic regression revealed that the GA genotype confers elevated risk for atherogenic dyslipidemia (OR = 1.57, 95% CI 1.06–2.3) after adjustment for associated risk factors. Independent associations with atherogenic dyslipidemia or elevated BP did not emerge in women.
Conclusion: APOA1 −75G>A polymorphism is independently related to HDL-C concentrations. Independent associations of the −75GA genotype with elevated BP and atherogenic dyslipidemia were confined to men. These gender-modulated associations suggest novel gene–gender–environmental interactions.

1. Introduction

The metabolic syndrome (MetS) is characterized by the clustering of several factors: abdominal obesity, insulin resistance and/or glucose intolerance, atherogenic dyslipidemia, increased systolic and diastolic blood pressure and a pro-inflammatory/pro-thrombotic state [1]. Atherogenic dyslipidemia related to MetS is a common metabolic disorder consisting of elevated plasma triglyceride (TG) and, decreased concentrations of high-density lipoprotein cholesterol (HDL-C) is associated with increased risk of coronary heart disease (CHD). The molar ratio of fasting TG/HDL-C was shown to be a strong independent predictor of myocardial infarction [2], and a nominal ratio in excess of 3.6 has been reported to be strongly associated with MetS [3].

Apolipoprotein (apo)A-I is the major surface protein constituent of HDL-C and plays a vital role in lipid transport and metabolism. Genetic studies have identified polymorphisms and mutations in the several genes associated with HDL-C and apoA-I levels [4]. Genome-wide association studies (GWAS) of HDL-C concentrations have previously identified several loci with genome-wide significant associations near candidate genes [5]. One of these is the *APOA1* locus that maps to the long arm of chromosome 11 [4]. Overexpression of the human *APOA1* gene in mice increased plasma HDL-C concentrations and protected the animals from the development of diet-induced atherosclerosis [6].

The *APOA1* gene polymorphisms have been found to be strongly associated with variation in MetS traits such as serum apoA-I, HDL-C and glucose concentrations [7–10]. A common G-to-A transition located 75 base pairs upstream from the transcription start site of the *APOA1* gene has been studied extensively [7]. Moreover, increased transcription efficiency was observed in −75A allele carriers in comparison with G allele homozygotes [11]. Individuals carrying the −75A allele

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have been shown to have higher concentrations of apoA-I and HDL-C [8–10,12]; however, the results have been inconsistent and inconclusive, with a few studies reporting either no association [13,14] or negative association [15] between *APOA1* −75G>A polymorphism and plasma lipids. In addition, *APOA1* −75AA genotype was reported to be associated with increased risk of type 2 diabetes [16]. Nonetheless, −75GG genotype/G allele has been found to be strongly associated with myocardial infarction [17], hypertension [18] and MetS components [19], but also with decreased blood pressure levels [20].

We, therefore, examined the relation of the −75G>A polymorphism with MetS traits in a randomly selected 1515 sample of the Turkish Adult Risk Factor Study (TARF) cohort, representative of Turkish adults [21].

2. Subjects and methods

2.1. Study population

The design and methodology of the TARF Study have been previously described [21]. It involves a random sample of the Turkish adult population, representatively stratified for sex, age, geographical regions and for rural–urban distribution. Participants were recruited from randomly selected communities using a probability–proportionate-to-size method and they were visited during the 8 follow-up surveys through 2010. Data were obtained for history of the past years via a questionnaire, physical examination of the cardiovascular system and recording of a resting electrocardiogram. Randomly selected 1515 subjects (730 males and 785 females) were examined for their *APOA1* −75G>A genotypes, based on the availability of determinations of fasting concentrations of HDL-C, triglyceride and glucose. Study subjects were unrelated and gave written consent to participate in the study after being informed of its nature. The study protocol was approved by the Ethics Committee of the Istanbul Medical Faculty, Istanbul University.

2.2. Definitions

Obesity was defined as a BMI ≥30 kg/m^2. Individuals with *diabetes* were diagnosed with criteria of the American Diabetes Association [22], namely when plasma fasting glucose was >126 mg/dL (or 2-h postprandial glucose >200 mg/dL) and/or the current use of diabetes medication. Oral glucose tolerance test was not performed. Individuals with MetS were identified when 3 out of the 5 criteria of the National Cholesterol Education Program (ATP III) [1] were met, modified for prediabetes (fasting glucose 100–125 mg/dL) [23] and further for abdominal obesity using as cutpoint ≥95 cm (instead of 102 cm) in men, as assessed in the Turkish Adult Risk Factor study [24]. Atherogenic dyslipidemia was defined by high (>150 mg/dL) fasting triglyceridemia and low concentrations (<40/<50 mg/dL) of HDL-C [1]. Physical activity was graded by the participant himself into four categories of increasing order with the aid of a scheme [21]. Grades I and II were considered in the present study as low physical activity.

2.3. Measurement of risk factors

Current smokers, never and former smokers formed the categories in cigarette smoking. Anyone consuming alcohol once a week or more was considered as an alcohol user. Weight was measured without shoes in light indoor clothes using a scale. Body mass index (BMI) was computed as weight divided by height squared (kg/m^2). Waist circumference was measured with a tape (Roche LI95 63B 00), the subject standing and wearing only underwear, at the level midway between the lower rib margin and the iliac crest.

Blood samples were collected after an 11-hour or longer fasting. Samples were shipped to Istanbul to be stored at −75 °C, until analyzed at a central laboratory. Serum concentrations of total cholesterol (TC), fasting triglycerides, glucose, and HDL-C (directly without precipitation) were determined using enzymatic kits from Roche Diagnostics with a Hitachi 902 autoanalyzer. Concentrations of apoA-I and B were measured by Behring kits and nephelometry (BN Prospec, Behring Diagnostics, Westwood, MA).

2.4. Genetic analysis

2.4.1. Determination of the APOA1 −75G>A genotypes

DNA was extracted from peripheral blood leucocytes using a QIAmp® DNA Maxi KIT (Qiagen, Hilden, Germany). The DNA concentrations have been standardized and stored in an 8 × 12 format at −20 °C. Unselected 1515 subjects (730 male and 785 female) were examined for their *APOA1* −75G>A genotypes. Genotyping was performed using hybridization probes in Real-Time PCR LightCycler® 480 device. DNA amplification was set up in 96 well plates (ABGENE Ltd.) Typical 10 μL PCR reaction consisted of 2 μL LightCycler® 480 genotyping master ready mix (Roche), 0.15 μL probes and 0.2 μL primers, 5.3 μL distilled water. 2.5 ng genomic DNA was added to the PCR mixture. PCR was carried out on LightCycler® 480 using the following conditions: 95 °C for 10 min, 95 °C for 5 s, 56 °C for 5 s, and 72 °C for 7 s (45 cycles). Melting curve analysis was assessed using the LightCycler® 480 genotyping software.

2.5. Statistical analysis

The genotypic distributions were compared using the chi-square test. Hardy–Weinberg equilibrium was computed to the expected genotype distribution. Due to skewed distributions fasting triglyceride was logarithmically transformed for analyses and expressed as geometric means and standard deviation (SD). Multinominal regression analyses were performed for interactions between SNP and subgroups. Interactions between SNP and the studied traits were examined by using univariate analysis. Two-tailed *t* test and analysis of variance (ANOVA) test were used to compare continuous variables and expressed as means and SD, while categorical variables were compared using the chi-square test. ANOVA was employed to compare values among genotypes. One-way analysis of covariance (ANCOVA) was performed with these values as dependent variables and the following factors as independent variables: *APOA1* genotype, age, waist circumference, lipid lowering medication, current smoking and diabetes mellitus in subgroups (men and women). Logistic regression models were used for *APOA1* genotype to derive maximum odds ratio (OR) estimates and associated 95% confidence intervals (CIs), adjusted for several environmental factors as confounders. A *p*-value <0.05 was considered significant, as was a 0.05 level in multiple testing after application of the Bonferroni correction. All statistical analyses were performed using Windows SPSS version 10.0 software.

3. Results

3.1. Study characteristics

The biometric parameters and characteristics of the participants of the study population are shown in Table 1, by gender. The sample comprised of selected 1515 (mean age; 49.9 ± 11.8, 48.2% male) Turkish adults. The prevalence of MetS, CHD, atherogenic dyslipidemia, obesity, hypertension and type 2 diabetes was around 49.0%, 7.3%, 28.9%, 32.8%, 46.0% and 7.9% of the participants, respectively. Of all the subjects, 3.6% were already being treated with lipid-lowering drugs.