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The *APOE* −219G/T and +113G/C polymorphisms affect insulin resistance among Turks

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Abstract

The −219G/T (rs405509) and +113G/C (rs440446) polymorphisms within the regulatory region of the apolipoprotein E (*APOE*) gene have been related to the transcriptional activity of the gene. We examined the effect of the stated polymorphisms and their construct haplotypes with the *APOE* ε2/ε3/ε4 polymorphism on lipid levels and insulin resistance in the Turkish Adult Risk Factor Study. Randomly selected 1774 adults (mean age, 55.0 ± 11.7 years; 51.2% women) participating in the population-based Turkish Adult Risk Factor Study were cross-sectionally analyzed for the −219G/T, +113G/C, and ε2/ε3/ε4 polymorphisms as well as their haplotypes. Insulin resistance was defined as the 70th percentile in the sample (>2.51) of the homeostatic model assessment (HOMA). The frequencies of the −219T and +113C alleles were 0.477 and 0.423, respectively; and those of haplotype 1 (GGε3) and haplotype 2 (TCε3) were 44.1% and 41.9%, respectively. The −219G/T and +113G/C genotypes (both $P < .04$) and diplotypes of haplotype 2 (TCε3) ($P < .014$) were inversely related to serum fasting insulin and the HOMA index, even after controlling for 8 relevant covariates, but not to serum lipids. Within the *APOE3* group, haplotype 2 (TC−/TC+) heterozygotes had an odds ratio of 0.66 (95% confidence interval, 0.42-0.99) for HOMA of insulin resistance after adjusting for 8 covariates. *APOE* promoter polymorphisms and their diplotypes are independently related with serum fasting insulin levels and HOMA index among Turks.

1. Background

The apolipoprotein E (*APOE*) gene located at 19q13.2 [1] encodes the polypeptide of 299 amino acids [2]. Three alleles (ε2, ε3, and ε4) of the gene encode 3 major apoE isoforms: E2, E3, and E4 [3]. The *APOE* ε2/ε3/ε4 polymorphism is one of the most thoroughly studied polymorphisms, and it has been found relevant to apoE serum levels [4,5], receptor binding affinity of apoE, plasma lipid and lipoprotein concentrations, coronary heart disease (CHD) [6,7], and atherosclerosis [8]. Several single nucleotide polymorphisms (SNPs) have also been defined in the regulatory region of the *APOE* gene [9]. In the present study, we concentrated on 2 of the functional promoter SNPs, that is, −219G/T (rs405509) and +113G/C (rs440446) [9,10]. Of these 2 SNPs, −219G/T is located in the promoter region and related to the transcriptional

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.

EKB wrote the manuscript, carried out the experimental work, and performed the statistical analyses; AO initiated the study and participated in its design as well as the coordination and collection of clinical data, in addition to editing the manuscript; BY and NM assisted in planning the study and participated in the experimental work; RL and MK assisted in planning the study and drafting the manuscript; GH carried out the biochemical experimental work and edited the manuscript; TL and NEU were responsible for the molecular genetic studies and participated in planning the study and drafting the manuscript.

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activity of the *APOE* gene [9]. It has been shown to associate with plasma apoE concentrations [11,12], insulin resistance (IR) [13], insulin sensitivity, as well as serum low-density lipoprotein cholesterol (LDL-C) levels and LDL susceptibility to oxidation in response to a diet rich in saturated fats [14,15]. In addition, an association has been established with postprandial lipidemic response [12], myocardial infarction [11], premature CHD [13], and the severity of coronary artery disease (CAD), independently of the *APOE* ε2/ε3/ε4 polymorphism [16]. Our previous functional studies on Finns revealed differences in the transcriptional activity of various *APOE* +113G/C (rs440446) alleles [10], demonstrating an association with serum lipid concentrations in men [17].

Insulin resistance has been associated with type 2 diabetes mellitus (T2DM), obesity, hypertension, CHD, and a dyslipidemic profile characterized by high plasma triacylglycerol concentrations and low high-density lipoprotein cholesterol (HDL-C) [18]. Diabetic dyslipidemia is also characterized by small dense LDL as well as high triglyceride (TG) and low HDL-C [19]. Insulin resistance has a complex and heterogeneous genetic background. Some studies have suggested that the *APOE* ε2/ε3/ε4 polymorphism may modify the effect of insulin on CHD or some CHD risk factors, including obesity and lipid profile levels [20-22], whereas the Framingham Offspring Study found that this polymorphism was not associated with IR [23], suggesting the possible involvement of various genetic or environmental factors in the link between the *APOE* gene and IR. However, no population-based study has yet evaluated the possible association of the *APOE* −219G/T and +113G/C polymorphisms and their haplotypes with IR among adults. We therefore examined the effect of these 2 polymorphisms and the *APOE* haplotypes involving the ε2/ε3/ε4 polymorphism with serum lipids and IR in a sample of the Turkish Adult Risk Factor (TARF) Study, representative of Turkish adults.

2. Methods

2.1. Study sample

The design and methodology of the TARF Study have been described previously [24]. In brief, participants were randomly selected among the residents of 7 regions in Turkey; and they attended the 6 surveys carried out between 1998 and 2008. Individual data were obtained with a questionnaire, a physical examination of the cardiovascular system, and a resting electrocardiogram. Approximately 2200 participants agreed to the genetic analysis, constituting more than 75% of the surveyed cohort. Study subjects were unrelated and gave written consent to participate in the study after being informed of its nature. Unselected 1774 persons (908 female and 866 male) were analyzed for their *APOE* genotypes and haplotypes. The study protocol was approved by the Ethics Committee of the Istanbul Medical Faculty, Istanbul University.

2.2. Definitions

Type 2 diabetes mellitus was diagnosed with the criteria of the American Diabetes Association/World Health Organization [25], namely, by means of self-reporting, or when the plasma fasting glucose level was at least 126 mg/dL or the 2-hour postprandial glucose level was at least 200 mg/dL. Insulin-resistant individuals were identified by a homeostatic model assessment (HOMA) index exceeding the 70th percentile value of the study sample, excluding subjects with diabetes (n = 140).

2.3. Measurement of risk factors

Waist circumference was measured—with the subject standing and wearing only underwear—at the level midway between the lower rib margin and the iliac crest. Body mass index was calculated as weight in kilograms divided by height in meters squared. Smoking status was determined by classifying the subjects as never smokers, former smokers, and current smokers. Anyone consuming alcohol once a week or more was considered an alcohol user. Physical activity was graded by the participant himself into 4 categories of increasing order with the aid of the following scheme: grade 1: white collar worker, sewing or knitting, walking not more than 1 km daily; grade 2: repair worker, housework, walking 1 to 2 km daily; grade 3: mason, carpenter, truck driver, cleaning floors and windows, walking 4 km daily; and grade 4: heavy labor, farming, regular sports activity. Participants engaging in daily sports activities during their leisure time or in heavy occupational or housework were classified into the maximum grade. Low physical activity was classified as grades 1 + 2; high, as grades 3 + 4.

Blood samples were collected after an at least 11-hour fast and stored at −75°C until analysis at the Yildiz Technical University. Serum concentrations of apoE, apoB, and apoA-I were measured by means of nephelometry (BN Prospec; Behring Diagnostics, Westwood, MA). The within-run and day-to-day coefficients of variation for apoE assays were 1.74% and 3.06%, respectively. Serum concentrations of total cholesterol, fasting TG, fasting glucose, as well as LDL and HDL cholesterol (LDL-C, HDL-C plus second generation, direct quantification with no pretreatment) were determined by using enzymatic kits by Roche Diagnostics (Mannheim, Germany) with a Hitachi 902 autoanalyzer. Concentrations of fasting insulin were determined by the chemiluminescent immunometric method using Roche kits and the Elecsys 2010 immunoautoanalyzer (Roche Diagnostics). Homeostatic model assessment was calculated with the following formula [26]: insulin (in micro–international units per liter) ∗ glucose (in millimoles per liter)/22.5.

2.4. Genotyping

From EDTA whole blood, genomic DNA was isolated from leukocytes by means of the QIAmpR DNA Maxi KIT (Qiagen, Hilden, Germany). The genotyping of the *APOE* gene SNPs −219G/T (rs405509), +113G/C (rs440446), +334T/C (rs429358, Cys112Arg), and +472C/T (rs7412, Arg158Cys) was performed using the ABI prism 7900HT Sequence Detection System for both polymerase chain reaction and allelic discrimination with TaqMan technology (Applied Biosystems, Foster City, CA). For SNP −219G/T genotyping, an Assay On Demand (C_905013_10APOE) kit by Applied Biosystems was used. For +113G/C, the forward and reverse primer sequences were 5′-CCAGGAGCCGGT-GAGAAG-3′ and 5′-CCGAGTAGCTCTCCTGAGACTA-3′. The probes were VIC-CCTGGGAAGCCCTG-TAMRA and FAM-CTGGGAACCCCTG-TAMRA. The forward and reverse primer sequences for +334T/C were 5′-GCGGG-CACGGCTGT-3′ and 5′-GCTTGCGCAGGTGGGA-3′. The probes were VIC-CATGGAGGACGTGTGC-TAMRA and FAM-ATGGAGGACGTGCGC-TAMRA. For +472C/T, the forward and reverse primer sequences were 5′-TCCGCGATGCCGATGAC-3′ and 5′-CCCCGGCCTG-GTACAC-3′; and the probes were VIC-CAGGCGCTTC-TGC-TAMRA and FAM-CAGGCACTTCTGC-TAMRA.

2.5. Statistical analyses

The genotype and haplotype frequencies of the *APOE* SNPs were determined for 1774 participants of the TARF study population. The genotyping success rate was 100% for all SNPs. The Hardy-Weinberg equilibrium was computed for the expected genotype distribution. Two-tailed *t* tests were used to compare continuous variables and expressed as means and SD, whereas categorical variables were compared using the χ^2 test. Analysis of variance (ANOVA) was used to compare biochemical variables (lipids, glucose, insulin, and HOMA index) among genotypes or diplotypes. One-way analysis of covariance (ANCOVA) was performed with biochemical values (lipids, glucose, insulin, and HOMA index) as dependent variables and the following factors as independent variables: age, sex, waist circumference, hypertension, T2DM, current smoking, physical activity, and alcohol use. Likelihood estimates (odds ratios [ORs]) and 95% confidence intervals were obtained by means of logistic regression analyses in models that were adjusted for age, sex, physical activity, alcohol use, smoking status, waist circumference, and *APOE* genotypes or diplotypes. Because of skewed distributions, values derived from log-transformed (geometric) means were used for TG, apoE, insulin, and HOMA. Subjects with genotype ε2ε4 (n = 21) and those using statins (n = 129) were excluded from the statistical analysis (leaving a total of 1624 individuals) because of the potential confounding effects of these situations on the lipid profile. In addition, subjects with T2DM (n = 153) were excluded in the IR analyses. The *APOE* ε2/ε3/ε4 genotypes were grouped for statistical analyses as the apoE2 group (ε2ε3), the apoE4 group (ε4ε4 and ε3ε4), and the apoE3 group (ε3ε3). A P value of $< .05$ was considered statistically significant. All statistical analyses were performed using Windows SPSS version 14.0 software (SPSS, Chicago, IL).

Haplotypes were estimated from the 4 SNPs using the PHASE v2.0.2 program (Department of Statistics, University of Washington, Seattle, WA), which uses a Bayesian statistical method for reconstructing haplotypes from population genotype data and lists the most probable haplotype pairs for each individual. Individuals with genotyping results for all 4 SNPs were included in the haplotype estimation procedure (n = 1774). Haplotypes were coded as haplotype numbers 1 to 8 according to the overall frequency, and the results are shown in the order −219G/T, +113G/C, +334T/C, and +472C/T; for example, haplotype 1 is G-G-T-C (GGε3). The ε2/ε3/ε4 polymorphism comprises 2 SNPs (+334T/C and +472C/T) in complete linkage disequilibrium: 334T-472T (ε2), 334T-472C (ε3), and 334C-472C (ε4). Linkage disequilibrium between sites was estimated using Haploview version 4.0 (www.broad.mit.edu/mpg/haploview), with the data presented as D'. Power calculations were performed using PowerSampleSize software for power and sample size calculations (http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize).

3. Results

3.1. Linkage disequilibrium and frequencies of the APOE SNPs and haplotypes

The genotype and haplotype frequencies of the *APOE* SNPs were determined for 1774 participants of the TARF study population. The frequencies of the ε2/ε3 (+334TT/+472TC), ε2/ε4 (+334TC/+472TC), ε3/ε3 (+334TT/+472CC), ε3/ε4 (+334TC/+472CC), and ε4/ε4 (+334CC/+472CC) genotypes of the ε2/ε3/ε4 polymorphism were 9.8%, 1.2%, 75.9%, 12.5%, and 0.6%, respectively. The frequencies of the GG, GT, and TT genotypes of −219G/T were 27.1%, 50.2%, and 22.7%, respectively. The respective frequencies of the GG, GC, and CC genotypes of +113G/C were 33.5%, 48.5%, and 18%. For all SNPs, the genotypic distributions of alleles were in Hardy-Weinberg equilibrium. The ε2, ε3, ε4, −219T, and +113 C allele frequencies in the adult Turkish population were 0.055, 0.871, 0.074, 0.477, and 0.423, respectively.

In our study population, the −219G/T and +113G/C SNPs were strongly linked with the D' value of 0.984. The +113G/C, +334T/C, and +472C/T SNPs showed a negative allelic association with the D' value of −1.0 ($P < .05$), with the rare alleles never seen unambiguously in a haplotype with the rare allele of the other SNP. Otherwise, the −219G/T and the +334T/C SNP demonstrated an allelic association with the D' value of 0.395.

Of the 8 haplotypes revealed by haplotype analyses, the frequencies of haplotype 1 (GGε3), haplotype 2 (TCε3),

haplotype 3 (GGε2), haplotype 4 (TGε4), haplotype 5 (GGε4), and haplotype 6 (TGε3) were 44.1%, 41.9%, 5.5%, 5%, 2.4%, and 0.8%, respectively. Haplotype 7 (GCε3) and haplotype 8 (TGε2) were very rare, with a frequency of less than 0.3%.

3.2. Association of APOE SNPs with serum levels of biochemical variables

We examined the relationship between the levels of biochemical variables and the SNPs of the *APOE* gene in a total of 1624 individuals after the exclusion of subjects with the ε2ε4 (n = 21) genotype and those using lipid-lowering drugs (n = 129). The associations of the genotypes of the *APOE* −219G/T, +113G/C, and ε2/ε3/ε4 polymorphisms with serum levels of biochemical variables for a total of 1624 individuals are shown in Table 1. Because of missing values, the n values of the variables may vary. In addition, Table 1 shows the ANCOVA results of serum levels of the biochemical variables after adjustment for age, sex, waist circumference, hypertension, T2DM, current smoking, physical activity, and alcohol use. The ε2/ε3/ε4 polymorphism demonstrated strong associations with serum concentrations of total cholesterol, HDL-C, LDL-C, apoB, and apoE in ANOVA and ANCOVA ($P < .001$ for all traits). In ANCOVA, this polymorphism was also associated significantly with fasting glucose levels ($P = .023$). The −219G allele homozygotes had significantly higher serum apoE levels ($P = .031$); but in homozygous +113 G allele carriers, the association was of borderline significance ($P = .058$). After adjusting for the confounders in ANCOVA, these SNPs were not significantly associated with apoE levels. There were no significant associations between the −219G/T and +113G/C genotypes and other lipid parameters. In addition, the SNPs −219G/T and +113G/C were in association with fasting insulin levels and HOMA index in ANOVA ($P < .02$) and ANCOVA ($P < .035$). The heterozygous individuals had lower levels of insulin and a lower HOMA index when compared with the homozygotes of these SNPs.

Power analyses of apoE levels, fasting insulin levels, and the HOMA index were performed by assuming an additive model. With the current sample size, the power of our study as regards the *APOE* promoter SNPs was calculated as being more than 95%.

The associations of the −219G/T and the +113G/C polymorphism with fasting insulin levels and the HOMA index in the *APOE* ε2/ε3/ε4 genotype groups without T2DM are shown in Fig. 1. The significant associations with fasting insulin levels and the HOMA index were found only in the apoE3 group (ε3ε3). There were no significant associations between the 2 polymorphisms and the HOMA index or insulin levels in subjects with T2DM (data not shown).

3.3. Association of APOE diplotypes with serum levels of the biochemical variables

Table 2 indicates the serum levels of the biochemical variables according to the diplotypes of haplotype 1 (GGε3) and haplotype 2 (TCε3). The diplotypes of these common haplotypes did not associate with any lipid levels ($P > .05$), with the exception of LDL-C ($P = .042$) in ANOVA. The diplotypes were found to have significant associations with

Table 1
Association of *APOE* genotypes with serum biochemical variables (mean ± SD)

Trait (n)	TC (n = 1621)	HDL-C (n = 1615)	LDL-C (n = 1574)	TG (n = 1341)	ApoA-I (n = 940)	ApoB (n = 951)	ApoE (n = 546)	Glucose (n = 1588)	Insulin (n = 1083)	HOMA (n = 1069)
Genotype	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL	mIU/L	
ε2/ε3/ε4										
APOE2	182.0 ± 38.8	43 ± 12.4	104.2 ± 29.9	147.9 ± 1.7	136.6 ± 24.9	90.5 ± 23.6	4.9 ± 1.31	97.4 ± 35.2	7.94 ± 1.91	1.82 ± 2.09
APOE3	197.2 ± 40.3	44.01 ± 11.8	121.6 ± 33.8	138.0 ± 1.7	140.2 ± 26.9	101.5 ± 26.3	3.8 ± 1.41	100.4 ± 38.9	8.32 ± 2.09	1.95 ± 2.34
APOE4	199.1 ± 41.2	40.7 ± 12.4	124.2 ± 37.3	144.5 ± 1.7	134.6 ± 28.4	107.8 ± 25.6	3.8 ± 1.48	100.5 ± 41.2	8.71 ± 1.86	1.99 ± 1.99
P^a	**.0001**	**.001**	**.0001**	.28	.062	**.0001**	**.0001**	.65	.71	.6
P^b	**.0001**	**.004**	**.0001**	.68	.19	**.0001**	**.0001**	**.023**	.76	.32
−219G/T										
GG	196.5 ± 41.3	43.9 ± 12.0	119.9 ± 34.8	144.5 ± 1.7	138.4 ± 26.3	101.8 ± 26.3	4.2 ± 01.38	98.8 ± 35.5	8.51 ± 2.04	1.99 ± 2.24
GT	195.7 ± 40.0	43.5 ± 12.0	120.4 ± 33.9	138.0 ± 1.7	140.4 ± 26.8	101.2 ± 25.8	3.8 ± 1.38	100.1 ± 38.9	7.94 ± 2.04	1.82 ± 2.29
TT	195.7 ± 40.8	42.9 ± 12.0	119.7 ± 34.7	141.3 ± 1.7	136.9 ± 28.0	101.3 ± 26.9	3.8 ± 1.48	101.9 ± 42.6	9.12 ± 1.99	2.19 ± 2.19
P^a	.94	.51	.93	.4	.26	.96	**.031**	.52	**.02**	**.02**
P^b	.94	.76	.88	.3	.47	.92	.065	.41	**.03**	**.035**
+113G/C										
GG	196.4 ± 40.9	43.6 ± 12.2	120.1 ± 34.6	144.5 ± 1.7	138.2 ± 26.2	101.6 ± 25.6	4.1 ± 1.38	100.3 ± 38.6	8.51 ± 1.99	1.99 ± 2.19
GC	196.3 ± 40.4	43.3 ± 11.9	120.7 ± 34.6	138.0 ± 1.7	140.0 ± 27.3	102.2 ± 26.8	3.8 ± 1.41	99.0 ± 36.8	7.76 ± 2.09	1.82 ± 2.29
CC	194.0 ± 40.1	43.7 ± 11.9	118.6 ± 33.0	138.0 ± 1.7	138.3 ± 27.9	98.5 ± 25.3	3.7 ± 1.45	102.9 ± 44.2	9.33 ± 1.99	2.24 ± 2.24
P^a	.66	.83	.69	.32	.63	.28	.058	.34	**.01**	**.004**
P^b	.72	.84	.56	.26	.62	.14	.13	.72	**.017**	**.007**

Because of missing values, n values may vary. $P < .05$ as indicated in boldface. TC indicates total cholesterol.

[a] ANOVA/[b]ANCOVA, adjusted for age, sex, waist circumference, hypertension, T2DM, current smoking, physical activity, and alcohol use.

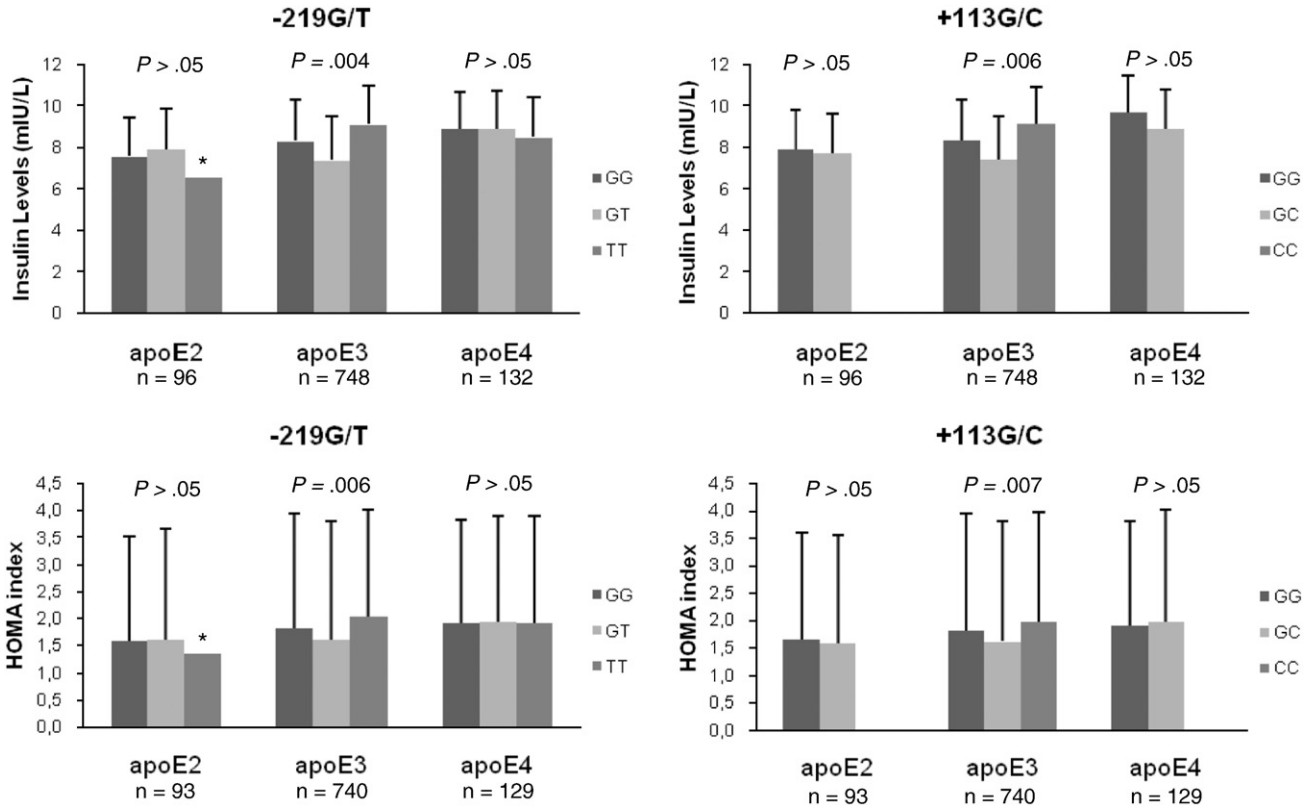

Fig. 1. Estimated mean (±SD) levels of serum fasting insulin and HOMA index across the genotypes of the −219G/T (rs405509) and +113G/C (rs440446) SNPs for *APOE* ε2/ε3/ε4 genotype groups. Significant associations were found only among ε3 homozygotes (apoE3 group). One hundred fifty-three subjects with T2DM were excluded from analysis. *No SD for n = 1.

insulin levels and the HOMA index; however, after adjusting for age, sex, waist circumference, hypertension, T2DM, current smoking, physical activity, and alcohol use in ANCOVA, haplotype 1 was not associated with LDL-C or insulin levels or the HOMA index ($P > .05$), whereas haplotype 2 showed an association with insulin levels and the HOMA index ($P = .014$ and $P = .006$, respectively). Heterozygous carriers of haplotype 2 (TCε3+/TCε3−) had lower values than carriers of the other diplotypes of haplotype 2 in ANOVA and ANCOVA analyses.

Power analyses of apoE and fasting insulin levels as well as the HOMA index were performed by assuming an additive model for the diplotypes of haplotype 2. With the current sample size, the power of the present study with

Table 2
Association of *APOE* diplotypes with serum biochemical variables (mean ± SD)

Trait (n)	TC (n = 1621)	HDL-C (n = 1615)	LDL-C (n = 1574)	TG (n = 1341)	ApoA-I (n = 940)	ApoB (n = 951)	ApoE (n = 546)	Glucose (n = 1588)	Insulin (n = 1083)	HOMA (n = 1069)
Diplotypes	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL	mg/dL	mIU/L	
Haplotype 1										
GGε3−/GGε3−	193.3 ± 40.7	42.7 ± 12.2	117.0 ± 34.2	144.5 ± 1.7	137.1 ± 28.0	100.3 ± 25.9	3.9 ± 1.48	101.7 ± 43.0	8.91 ± 1.99	2.09 ± 2.19
GGε3+/GGε3−	196.1 ± 39.8	43.6 ± 11.8	120.9 ± 34.2	134.9 ± 1.6	140.1 ± 26.3	101.0 ± 26.5	3.9 ± 1.38	99.0 ± 36.3	7.94 ± 2.04	1.82 ± 2.29
GGε3+/GGε3+	199.4 ± 41.9	44.2 ± 12.0	122.9 ± 34.6	147.9 ± 1.7	139.6 ± 27.2	103.9 ± 25.6	4.0 ± 1.38	100.6 ± 38.3	8.71 ± 2.09	2.04 ± 0.37
P^a	.11	.18	**.042**	.082	.32	.32	.72	.47	**.05**	**.03**
P^b	.20	.60	.12	.14	.88	.25	.46	.97	.18	.12
Haplotype 2										
TCε3−/TCε3−	196.6 ± 40.7	43.5 ± 12.1	120.2 ± 34.6	144.5 ± 1.7	138.5 ± 26.2	101.9 ± 25.8	4.1 ± 1.38	100.2 ± 38.4	8.51 ± 1.99	1.99 ± 2.19
TCε3+/TCε3−	196.2 ± 40.5	43.4 ± 12.0	120.7 ± 34.6	138.0 ± 1.7	139.8 ± 27.2	102.0 ± 26.6	3.8 ± 1.41	99.0 ± 36.9	7.76 ± 2.09	1.82 ± 2.29
TCε3+/TCε3+	193.7 ± 40.3	43.6 ± 11.7	118.5 ± 33.2	138.0 ± 1.7	138.5 ± 28.0	98.5 ± 25.5	3.7 ± 1.45	103.0 ± 44.5	9.33 ± 1.99	2.24 ± 2.24
P^a	.61	.94	.66	.25	.77	.31	.064	.33	**.009**	**.003**
P^b	.68	.93	.55	.16	.75	.15	.13	.72	**.014**	**.006**

SNP order is as follows: −219, +113, and ε (+334/+472). Because of missing values, n values may vary. $P < .05$ as indicated in boldface.

[a] ANOVA/[b]ANCOVA, adjusted for age, sex, waist circumference, hypertension, T2DM, current smoking, physical activity, and alcohol use.

Table 3
Baseline characteristics of subjects in the apoE3 group with HOMA determination, by presence or absence of IR

	Non-IR	IR	*P*
Sex: male/female	243/278	93/126	NS
−219, GG/GT/TT genotypes	126/277/118	57/101/61	NS
+113, GG/GC/CC genotypes	129/275/117	60/100/59	NS
H1 (GG) diplotypes, −−/−+/++	120/276/125	61/101/57	NS
H2 (TC) diplotypes, −−/−+/++	130/276/115	60/100/59	NS
Age, y	54.3 ± 11.5 (521)	56.0 ± 11.2 (219)	NS
Waist, cm	90.9 ± 10.6 (519)	100.4 ± 11.5 (217)	.0001
TC, mg/dL	196.8 ± 39.5 (521)	199.7 ± 37.5 (219)	NS
HDL-C, mg/dL	45.0 ± 11.8 (521)	42.8 ± 11.2 (219)	.02
LDL-C, mg/dL	123 ± 33.6 (511)	120.8 ± 32.2 (218)	NS
TG[a], mg/dL	134.9 ± 1.7 (454)	154.9 ± 1.6 (190)	.002
Fasting glucose, mg/dL	86.2 ± 11.7 (521)	98.8 ± 13.7 (219)	.0001
ApoA-I, mg/dL	142.4 ± 26.1 (345)	141.1 ± 26.1 (149)	NS
ApoB, mg/dL	99. ± 25 (346)	101.5 ± 25.0 (150)	NS
ApoE[a], mg/dL	3.72 ± 1.4 (199)	3.7 ± 1.4 (98)	NS
Fasting insulin[a], mIU/L	5.9 ± 1.7 (521)	17 ± 1.5 (219)	.0001
HOMA index[a]	1.3 ± 1.8 (521)	4.1 ± 1.6 (219)	.0001
Smoking: non/former/current	279/86/149	129/48/37	.003
Alcohol use: no/yes	450/63	192/25	NS
Physical activity: low/high	220/290	84/134	NS
Hypertension: no/yes	342/179	112/107	.0001
Metabolic syndrome: no/yes	368/150	77/141	.0001

Continuous variables are presented as mean ± SD (number); categorical variables, as numbers. Two-tailed *t* test for comparison of means; χ^2 test for comparison of categorical variables. Subjects with T2DM and those with statin use were excluded from analysis. NS indicates not significant.

[a] Geometric mean values.

regard to the *APOE* diplotypes was calculated to be more than 85%.

3.4. Association of APOE SNPs and diplotypes with IR

Table 3 demonstrates the baseline characteristics of subjects with IR and no IR in the *APOE3* group. Although no significant relationship was found in the *APOE3* group for the genotypes −219G/T and +113G/C or the diplotypes with IR (Table 3), logistic regression analysis did reveal associations for heterozygous carriers of haplotype 2 (TC−/TC+) when compared with homozygous haplotype 2 (TC+/TC+) carriers (Table 4). After adjusting for age, sex, physical activity, alcohol use, smoking status, and waist circumference, the TC−/TC+ diplotype was associated with a protective effect against IR in the *APOE3* group. However, no associations were observed with the TC−/TC− diplotype in the same logistic regression analysis. With the sample size (n = 713) of the present study, we obtained a 60% power to detect the observed ORs of 0.75 and 0.65 for the TC−/TC− and the TC−/TC+ diplotype, respectively. Moreover, there were borderline significant associations with lower occurrence of IR for heterozygotes of the −219G/T and the +113G/C SNP after adjusting for the mentioned covariates ($P = .055$ and $P = .054$, respectively).

4. Discussion

In this cross-sectional study, we analyzed the impact of the *APOE* SNPs on lipid, apolipoprotein, glucose, and insulin serum concentrations in the TARF cohort, a representative of Turkish adults. Our results demonstrated that the G allele carriers of the −219G/T and the +113G/C genotype within the regulatory region of the *APOE* gene, as well as their diplotypes, had an inverse relation with fasting insulin, the HOMA index, and IR independently of the *APOE* ε2/ε3/ε4 polymorphism.

The *APOE* ε2/ε3/ε4 polymorphism was one of the first polymorphisms associated with cardiovascular disease to be studied thoroughly in both health and disease, and the serum concentration of apoE has been found to be related to the *APOE* ε2/ε3/ε4 genotype [4,5]. Furthermore, the apoE levels are associated with total cholesterol levels [27] and linked with cardiovascular disease and cardiovascular mortality [28]. This common *APOE* variation influences the lipoprotein metabolism and plasma concentrations of total cholesterol, LDL-C, apoB, and apoE, incurring a risk for CHD [7]. A previously published study on Turks found a sex-specific impact for *APOE* genotype on HDL-C and LpA-I levels [29]. The apoE concentrations in Turkish adults have been found to be significantly linked with hyperapoB and atherogenic dyslipidemia independently of this polymorphism, the HOMA index, or other confounders [30].

In the present study, we found a significant association between the *APOE* ε2/ε3/ε4 polymorphism and serum levels of total cholesterol, HDL-C, LDL-C, apoB, and apoE. In the large unselected population-based Framingham Offspring Study, no association was observed between the

Table 4
Association of the −219G/T (rs405509) and +113G/C (rs440446) SNP genotypes and their diplotypes with IR in the apoE3 group by logistic regression analysis

Genotype/Diplotype	*P* value	OR	95% CI
−219, GG	.22	0.73	0.45-1.2
GT	.055	0.66	0.43-1.008
+113, GG	.26	0.76	0.46-1.23
GC	.054	0.65	0.42-1.008
H1, GG−/GG−	.24	1.35	0.82-2.2
GG−/GG+	.61	0.89	0.58-1.37
H2, TC−/TC−	.24	0.75	0.46-1.22
TC−/TC+	**.048**	0.65	0.42-0.99

Subjects with T2DM and those on statin therapy were excluded from analysis. The SNP order is as follows: −219G/T and +113 G/C for haplotype 1 (H1, GG) and 2 (H2, TC). Models comprised 713 adults with IR (n = 213) and without IR (n = 500) after adjusting for age, sex, physical activity, alcohol use, smoking status, and waist circumference. Referents have −219TT, +113CC, GG+/GG+ for H1 and TC+/TC+ for H2. $P < .05$ as indicated in boldface. CI indicates confidence interval.

APOE ε2/ε3/ε4 polymorphism and IR, a finding that is in line with the present results [23]. However, another study has shown that obesity modulates the association between this polymorphism and levels of fasting insulin and glucose in men [22]. Furthermore, numerous other studies have shed light on the interactions between *APOE* ε2/ε3/ε4 genotype and the possible effect of modifiers, such as diet, age, sex, and lipid-lowering drugs use [7]. These factors may play important roles in modulating the relationships.

In line with our current study, previous reports [11,14] have not shown any association for the −219G/T polymorphism with lipid and other apolipoprotein levels. Our previous study with middle-aged Finnish men, however, demonstrated that the promoter polymorphisms −219G/T and +113G/C, as well as their haplotype, modulate very low-density lipoprotein cholesterol, HDL-C, TG, and apoB levels, especially within the subgroup of the most common *APOE* ε3/ε3 genotype [17]. The discrepant results between the current and the Finnish results might be due to differences in the study populations and design. Another large population-based family study conducted in Finland and Sweden on families with T2DM found a relationship for the polymorphisms of the *APOE* gene and the nearby muscle glycogen synthase (*GYS1*) gene on chromosome 19 with cardiovascular mortality independently of each other [31]. Our focus on the effect of *APOE* polymorphisms, without assessing other functional polymorphisms in the *GYS1* gene or other probably linked genes, may constitute a further limitation to the present study.

In the current population, we found a significant association between the −219G/T polymorphism and apoE serum concentrations; but the significance was borderline with regard to the +113G/C polymorphism. Our findings concerning the association between the −219G/T polymorphism and apoE levels are in partial agreement with those reported by Lambert et al [11] in the ECTIM (étude cas-temoin de l'infarctus myocarde) study and by Moreno et al [12]. Moreno et al reported that the −219TT subjects had the lowest postprandial levels of serum apoE [12]. In the ECTIM study, the −219T allele was associated with lower plasma levels of apoE and the risk of myocardial infarction [11]. In this study, the impact of the −219T allele on myocardial infarction was explained by more subtle and local mechanisms, for instance, in the course of atherosclerotic plaque formation [11]. In a large cohort of patients with angiographically documented CAD, Ye et al [16] indicated that the −219G/T polymorphism influenced CAD severity, which was independent of and possibly additive to the effect of the ε2/ε3/ε4 polymorphism. According to our current results, IR may play a role in these associations. Indeed, Viitanen et al [13] reported that, in addition to the ε4 allele, the −219G/T promoter polymorphism of the *APOE* gene was associated with early-onset CHD. The authors indicated that the effect of these polymorphisms on cardiovascular risk factor clustering with IR might be of importance [13]. However, another study found no associations for the −219G/T and +113G/C polymorphism with the incidence of death and myocardial infarction or with stenosis after stenting in coronary arteries [32]. A further investigation with Finnish young adults also reported no association between the said polymorphisms and measurements of carotid artery wall intima-media thickness), brachial artery flow-mediated dilatation, and carotid artery compliance as related to subclinical atherosclerosis [33]. Our findings suggest that the −219G/T and +113G/C polymorphisms significantly affect the levels of serum fasting insulin and the HOMA index in Turkish adults with the *APOE* ε3ε3 genotype. However, these relationships with the development of atherosclerosis and myocardial infarction also require further evaluation within the Turkish population.

Apolipoprotein E is primarily involved in plasma lipid homeostasis. However, a number of studies with experimental mouse models have shown that apoE also has an important role in the development of obesity and IR [34,35]. In addition, recent investigations on reduced adiposity and improved glucose homeostasis in apoE-deficient mice suggest that apoE may also play a key role in the energy metabolism of peripheral organs, including adipose tissue [36]. Apolipoprotein E is involved in excess fat accumulation and energy metabolism, including the regulation of food intake and energy expenditure. Therefore, excess fat accumulation via an apoE-dependent pathway might play a role in the development of IR [34]. Functional genetic variants in the *APOE* gene may lead to the development of IR because of different lipolytic responses in adipose tissue.

In a previous study, the −219G/T polymorphism was associated with insulin sensitivity in healthy young adults with the *APOE* ε3/ε3 genotype, independently of the diet consumed [12]. Although no significant association between the −219G/T polymorphism and insulin levels was found in 43 subjects, heterozygous carriage did have an effect on insulin levels, which is in line with our current results. In the present study, heterozygotes (−219G/T or +113G/C) and haplotype 2 (TCε3) carriers had lower fasting insulin and HOMA levels when compared with homozygous −219T or +113C allele carriers or carriers of the diplotype of haplotype 2, especially within the *APOE* ε3/ε3 genotype group. One earlier study investigated the association of these polymorphisms with longitudinal change in LDL and total cholesterol concentrations, and found a relationship with heterozygotes as compared with homozygotes [33]. The *APOE* −219G/T polymorphism was also previously shown to affect *APOE* transcription, the −219G allele associating with higher *APOE* promoter activity [9]. The heterozygous genotypes or diplotypes for these SNPs may lead to differences in the binding of nuclear proteins on regulatory regions.

A second limitation of the present study is that serum apoE and insulin level measurements were unavailable for a sizeable part of the sample, which hindered the examination of the associations with the studied SNPs. To avoid misinterpretation, further investigations with a

larger sample for accurate independent epidemiologic studies are required to analyze the genetic association with IR and/or its related parameters in the Turkish population or other populations.

In summary, this study is the first to report the allelic and haplotypic frequency of the SNPs −219G/T and +113G/C in the *APOE* gene in a large sample of Turkish adults. We conclude that heterozygous carriers of haplotype 2 have lower fasting insulin and HOMA levels and a reduced likelihood of developing IR than homozygous carriers of the diplotype of haplotype 2 within the *APOE* ε3/ε3 genotype group.

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