Serum apolipoprotein E concentrations among Turks: information additive to genotype relative to dyslipidemia and metabolic syndrome

Halkımızda serum apolipoprotein E konsantrasyonları: Dislipidemi ve metabolik sendroma ilişkin genotipe ek bilgi

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Objectives: We investigated the relationship of serum apolipoprotein E (apoE) levels with dyslipidemia and metabolic syndrome (MS) in the general population and their degree of independence of the apoE genotype.

Study design: This cross-sectional study included a random sample of Turkish adults whose serum apoE concentrations were measured. Metabolic syndrome was defined with the ATP-III criteria with modification for male abdominal obesity.

Results: Of 454 participants (222 men, 232 women; mean age 54.1±9.6 years), the median serum apoE concentration was 3.93 mg/dl with an interquartile range of 1.75 to 5.82 mg/dl. Higher apoE concentrations were found in male carriers of the ε4 allele than homozygous ε3 subjects. Multivariate analysis showed the apoE genotype (grouped into 3) as a determinant of apoE levels, and serum apoB levels as a major covariate. In logistic regression analysis, doubling of the apoE level showed significant associations, independent of the apoE polymorphism, with total cholesterol, elevated apoB (OR 4.54, 95% CI 2.83; 12.3) and triglyceride/HDL-cholesterol dyslipidemia (OR 2.82, 95% CI 1.67; 5.18). Doubling of the apoE level was also associated in both genders with MS (OR 1.72, 95% CI 1.24; 2.38), after adjustment for confounders.

Conclusion: ApoE concentrations in Turkish adults are significantly linked to serum total cholesterol, hyperapoB, and atherogenic dyslipidemia, independent of the apoE polymorphism. They are also significantly and independently associated with MS. Male carriers of the ε4 allele have no lower apoE concentrations than homozygous ε3 individuals, suggesting a close link between apoE and apoB levels.

Key words: Alleles; apolipoproteins B; apolipoproteins E; cholesterol, LDL; dyslipidemias; genotype; metabolic syndrome X.

Amaç: Serum apolipoprotein E (apoE) düzeylerinin genel nüfusta dislipidemi ve metabolik sendromla (MS) ilişkisi ve apoE genotipinden bağımsızlık derecesi araştırıldı.

Çalışma planı: Serumda apoE konsantrasyonları ölçülmüş ve yetişkinlerimizin rastgele bir örneklemini oluşturan bireyler kesitsel biçimde incelendi. Metabolik sendrom tanısında erkekte abdominal obezite için modifikasyonlu ATP-III ölçütlerine uyuldu.

Bulgular: Çalışmaya alınan 454 bireyde (222 erkek, 232 kadın; ort. yaş 54.1±9.6) ortanca (çeyrek dilimlerarası) serum apoE konsantrasyonu 3.93 (dağılım 1.75-5.82) mg/dl idi. ApoE değerleri erkek ε4 allel taşıyıcılarında, homozigot ε3 kişilere göre daha yüksek bulundu. Çokdeğişkenli analizde, üç grupta toplanan apoE genotipi apoE düzeylerinin belirleyicisi; serum apoB düzeyleri de majör bir kovariyat olarak belirlendi. Lojistik regresyon analizinde, apoE düzeyinin ikiye katlanmasının apoE polimorfizminden bağımsız şekilde total kolesterol ile, yüksek apoB (OR 4.54, %95 GA 2.83; 12.3) ve trigliserid/HDL-kolesterol dislipidemisi (OR 2.82, %95 GA 1.67; 5.18) ile ilişkili olduğu görüldü. ApoE düzeyinin ikiye katlanması, her iki cinsiyette uygun ayarlamalardan sonra MS ile ilişkili (OR 1.72, %95 GA 1.24; 2.38) bulundu.

Sonuç: Yetişkinlerimizde apoE konsantrasyonları, apoE polimorfizminden bağımsız şekilde, serum total kolesterol, hiperapoB ve aterojen dislipidemi ile bağlantılıdır. Seviyeler MS ile de anlamlı ve bağımsız biçimde ilişkilidir. Erkek ε4 allel taşıyıcılarının apoE konsantrasyonları ε3 homozigotlardan düşük olmayıp, bunun apoE ve apo B düzeyleri arasında bağlantıya yol açtığı düşünülmektedir.

Anahtar sözcükler: Allel; apolipoprotein B; apolipoprotein E; kolesterol, LDL; dislipidemi; genotip; metabolik sendrom X.

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Apolipoprotein E (apoE) is a structural component of triglyceride-rich particles, their remnants, and high-density lipoproteins. It plays a crucial role in the metabolism of triglyceride-rich lipoproteins and particularly in the hepatic clearance of their remnants through low-density lipoprotein (LDL) receptor and LDL receptor-related protein.[1] The impact on serum lipids and lipoproteins[2-4] and on coronary heart disease (CHD)[5,6] of the six common isoforms of apoE in plasma, encoded by the ε2, ε3 and ε4 alleles on chromosome 19, has been fairly well established in the past two decades. In summary, compared with the most common apoE 3/3 genotype, subjects with 4/3 and 4/4 genotypes are recognized to have higher total and LDL-cholesterol levels and the former also higher triglyceride and lower HDL-cholesterol[3] and are associated with higher CHD risk.[5] However, no effect of the apoE ε4 allele on CHD risk was reported in the Whitehall II study, a cohort with a low smoking prevalence.[7] The impact of apoE 3/2 genotype is more divergent inasmuch as it is associated with lower total and LDL-cholesterol levels but higher triglyceride concentrations[3] and no evidence of excess CHD.[5]

In the last few years, serum apoE concentrations have been studied in various Western population samples involving large samples as a whole, mainly in the ApoEurope Project[8] and the French Stanislas cohort.[9] These studies stratified the odds of genotypes, by age, gender, and geographic region and the corresponding apoE concentrations.[8] Furthermore, reference limits were proposed according to age, gender, and the common apoE polymorphism with the purpose of improving cardiovascular risk assessment.[9] The ethnic variability of the apoE genotype, and a decreasing north-south gradient of the ε4 allele frequency in Europe have been described[10] as well as an increasing north-south gradient in apoE concentrations.[8]

Nonetheless, the clinical relevance of serum apoE levels has not been adequately investigated in regard to their relation to the two types of main dyslipidemias and the metabolic syndrome (MS), particularly in diverse populations. Also the magnitude of the effect of a specific apo ε allele and the corresponding serum apoE concentration may diverge in various populations depending on environmental factors.[11] The apoE phenotype of Turks has been studied in a large population sample in the Turkish Heart Study.[4] Yet, serum apoE levels have not been reported thus far.

In this study based on a general adult population sample, representative of Turks, among whom MS is highly prevalent,[12] we intended to contribute to the relationship of serum apoE levels by describing cross-sectionally: *(i)* the covariates of apoE concentrations, *(ii)* the relation of the main apoE genotypes with apoE concentrations, *(iii)* the independent associations of apoE with elevated LDL-cholesterol, elevated apoB levels (hyperapoB), with dyslipidemia of the insulin resistance type and MS.

PATIENTS AND METHODS

Sample population. The study sample was recruited randomly in one-half of participants of the 2006 follow-up survey of the Turkish Adult Risk Factor Study, a prospective study on the prevalence of cardiac disease and risk factors in a representative sample of adults in Turkey, carried out periodically almost biennially since 1990 in 59 communities throughout all geographical regions of the country.[13] Details of the overall sampling were described previously.[14] The study was approved by the Ethics Committee of the Istanbul University Medical Faculty. Individuals of the cohort were visited in their addresses on the eve of the examination and written informed consent for participation was obtained. Partial logistic support was provided by the Turkish Ministry of Health. Data were obtained by history of the past years via a questionnaire, physical examination of the cardiovascular system, sampling of blood, and recording of a resting 12-lead electrocardiogram. Serum concentrations of apoE were assayed in randomly selected 222 men and 244 women, mean age 54.1 years. Ten women who had been using contraceptive drugs or under hormone replacement therapy were excluded, as were two women with the ε4/ε2 genotype. This left a total of 454 subjects for analysis. Ten men and 24 women who were under lipid lowering medication were adjusted in the multivariate analysis.

Measurement of risk factors. Blood pressure (BP) was measured with an aneroid sphygmomanometer (Erka, Germany) in the sitting position on the right arm, and the mean of two recordings 3 min apart was recorded. 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. In regard to cigarette smoking, nonsmokers, former smokers, and current smokers formed the categories. Anyone consuming alcohol once a week or more was considered an alcohol user. Physical activity was graded by the participant himself into four categories of increasing order with the aid of a scheme.[14]

Blood samples were collected in the fasting state in over four-fifths of the sample, spun at 1000 g for 10 minutes and shipped within a few hours on cooled gel packs at 2-5 °C to Istanbul to be stored in deep-freeze at -75 °C, until analyzed at the Yıldız Technical University in the same city. Serum concentrations of apoE, apoB, apoA-I, and total homocysteine were measured by nephelometry (BN Prospec, Behring Diagnostics, Westwood, MA). 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 triglycerides, glucose, LDL- and HDL-cholesterol (LDL-C, HDL-C plus 2nd generation, direct quantification with no pretreatment) were determined by using enzymatic kits from Roche Diagnostics with a Hitachi 902 autoanalyzer. Insulin concentrations were determined by the chemiluminescent immunometric method using Roche kits and an Elecsys 2010 immunautoanalyzer (Roche Diagnostics, Mannheim, Germany).

Genomic DNA was isolated from leukocytes in EDTA whole blood by the QIAmpR DNA Maxi KIT (Qiagen, Hilden, Germany). APOE Ɛ2/Ɛ3/Ɛ4 alleles (Cys112Arg and Arg158Cys polymorphisms) were analysed by the TaqMan technology (ABI 7900HT, Applied Biosystems, UK). Forward and reverse primer sequences were 5'-GCGGGCACGGCTGT-3' and 5'-GCTTGCGCAGGTGGGA-3' for the position 112 polymorphism, and 5'-TCCGCGATGCCGATGAC-3' and 5'-CCCCGGCCTGGTACAC-3' for the position 158 polymorphism, respectively. The probes were VIC-CATGGAGGACGTGTGC-TAMRA and FAM-ATGGAGGACGTGCGC-TAMRA; VIC-CAGGCGCTTCTGC-TAMRA and FAM-CAGGCACTTCTGC-TAMRA for SNP's at position 112 and 158, respectively. Allelic discrimination was assessed using the TaqMan software.

Definitions. Hypertension was defined as a blood pressure ≥140 mmHg and/or ≥90 mmHg, and/or use of antihypertensive medication. Dyslipidemia with triglycerides ≥150 mg/dl and HDL-cholesterol ≤40/50 mg/dl was designated as atherogenic dyslipidemia, while levels of apoB >120 mg/dl were briefly referred to as hyperapoB. Metabolic syndrome was identified when three out of the five criteria of the National Cholesterol Education Program ATP-III[15] were met, modified for prediabetes (fasting glucose 100-125 mg/dl)[16] and further for abdominal obesity using the cutpoint ≥95 cm in men, as recently assessed in the Turkish Adult Risk Factor study.[17] Missing data on fasting triglycerides did not preclude the identification of MS since availability of no more than three criteria was required, and the MS status of a previous survey was taken into account in 25 individuals presenting with two positive criteria. Diabetes was diagnosed with the criteria of the American Diabetes Association,[18] namely by self report or when plasma fasting glucose was ≥126 mg/dl or when 2-h postprandial glucose was >200 mg/dl. Homeostatic model assessment (HOMA) was calculated with the following formula:[19] insulin (mIU/L) x glucose (in mmol/l)/22.5.

Diagnosis of nonfatal CHD was based on the presence of angina pectoris, of a history of myocardial infarction with or without accompanying Minnesota codes of the ECG,[20] or on a history of myocardial revascularization. Among women, typical angina and age >45 years were prerequisites for a definitive diagnosis when angina was isolated. ECG changes of "ischemic type" of greater than minor degree (Codes 1.1-2, 4.1-2, 5.1-2, 7.1) were considered myocardial infarction sequelae or myocardial ischemia, respectively.

Data analysis. Descriptive parameters were shown as mean±standard deviation or as age-adjusted mean estimate±standard error, and in percentages. Due to skewed distribution, values derived from log-transformed (geometric) means were used for insulin, HOMA and homocysteine. Pearson's correlation test was used for continuous variables and Spearman correlations were used for log-transformed variables. Two-sided t-test and Pearson's chi-square test were used to analyze the differences between means and proportions of groups; pairwise comparisons were made to detect significance between groups of estimated means. Multiple linear regression analyses were performed with continuous parameters. Likelihood estimates (odds ratios, OR) and 95% confidence intervals (CI) were obtained by use of logistic regression analyses in models that were adjusted for sex, age, and other confounders. Likelihood estimates for log-transformed variables were expressed in terms of doubling of the relevant variable. A value of $p<0.05$ on the two-tail test was considered statistically significant. Statistical analyses were performed using SPSS-10 for Windows.

RESULTS

The study sample consisted of 222 men and 232 women. The mean age of the sample was 54.1±9.6 years. All seven geographic regions were sufficiently represented in the study population, though contribution of the Central Anatolia and Marmara regions was

less. Metabolic syndrome was identified in 108 men (48.7%) and in 129 women (55.6%).

Distribution of apoE concentrations. The mean apoE concentration was 4.25±1.93 in men and 4.38±1.6 mg/dl in women (p>0.05). The median (interquartile range) value was 3.93 (range 1.75-5.82) mg/dl. No significant difference in apoE concentrations was observed between fasting and nonfasting sera. Genotyping for apoE was available only in 231 participants. The common ε3/ε3 genotype was observed in 173 individuals (74.9%), while - apart from two women with the ε2/ε4 genotype - the ε3/ε2 genotype was found in 28 (12.1%), the ε4/ε3 in 25, and ε4/ε4 in three individuals (the latter two combined 12.1%). ε2/ ε3/ ε4 allele frequencies were 6.5%, 86.4%, and 7.1%, respectively. To avoid possible misclassification, the two persons with the ε2/ε4 genotype were disregarded in the analyses, alike other studies.[9] The distribution of sex- and age-adjusted estimated marginal means of lipids and other variables of the study sample for the apoE isoforms is presented in Table 1. Subjects with apo ε2 and ε4 alleles displayed significantly higher apoE concentrations (5.24 mg/dl and 5.13 mg/dl, respectively) than the common ε3 homozygotes (4.13 mg/dl). Serum levels of total and LDL-cholesterol and apoB of the ε4 allele were significantly higher than levels in persons with ε2 allele, while ε3 homozygotes lay intermediately between these variables. ApoA-I followed the same trend of distribution though without reaching significance. By contrast, fasting triglycerides, HOMA index, and total homocysteine values were the lowest in homozygous ε3 persons, but not significantly.

Covariates of apoE concentrations. Table 2 depicts associations of covariates of serum apoE levels in a highly significant linear regression model with eight independent variables including apoB. Independent of systolic BP, demographic and lifestyle confounders, apoB in both genders and (inversely) physical activity grade in men were significant covariates. When apoE polymorphism in three genotypes was added to this model, only apoB level and apoE ε2 allele carriers remained independently significant. Carrying the ε2 allele enhanced apoE concentrations with respect to ε3 homozygotes in both genders, whereas female apo ε4 carriers had significantly lower apoE levels. No inhibition, even an insignificant enhancement was observed in male apo ε4 carriers.

In univariate correlations with apoE, waist circumference, triglycerides (p<0.001), and apoB were strongly correlated (with r 0.34 in men, 0.55 in women; both p<0.001); furthermore, LDL-cholesterol was correlated only in women (p=0.026).

Association of apoE concentrations with dyslipidemias. Table 3 demonstrates the association of apoE concentrations with two types of dyslipidemias, adjusted for sex, age, smoking status, alcohol usage, waist circumference, physical activity grade, systolic BP, statin use, and HOMA index. ApoE was significantly associated with elevated LDL-cholesterol (≥130 mg/dl) in men (for a 2-fold apoE, OR=1.81; 95% CI 1.06; 3.08) and both genders combined. For dyslipidemia of high triglyceride/low HDL-cholesterol, analysis revealed apoE levels to be highly significantly associated in each gender: the OR in both sexes combined corresponding to a doubling of apoE was 3.34 (95% CI 2.24; 4.98), independent especially of HOMA, statin usage, and other confounders.

Two types of dyslipidemias were examined for associations concomitantly with levels and isoforms of apoE, adjusted for sex, age, statin use, and HOMA

Table 1. Sex- and age-adjusted estimated marginal means of lipids and other variables for apo E isoforms

		ε3/ε2	ε3/ε3	ε4 carriers†	
	n	Mean±SE	Mean±SE	Mean±SE	*p*
	229	28	173	28†	
Total cholesterol (mg/dl)	229	189.1±7.8	199.4±3.1	**220.0±7.8***	0.016
HDL-cholesterol (mg/dl)	229	41.5±2.1	42.3±0.9	42.8±2.1	NS
LDL-cholesterol (mg/dl)	229	**102.8±6.0***	118.9±2.4	128.8±6.0	0.015
Fasting triglycerides (mg/dl)	209	172.3±20.8	166.4±7.9	196±20	NS
ApoA-I (mg/dl)	226	138.9±4.5	145.6±1.8	149.8±4.5	NS
ApoB (mg/dl)	229	92.6±4.4	**102.9±1.8***	113.4±4.6	0.037
ApoE (mg/dl)	229	5.2±0.3	**4.1±0.1***	5.1±0.3	0.008
Fasting insulin (μU/ml)+	190	10.6±1.2	8.6±1.1	10.6±1.2	NS
HOMA index+	182	2.2±1.2	2.0±1.1	2.5±1.2	NS
Homocysteine (μmol/l)+	159	13.9±1.1	11.8±1.0	12.8±1.1	NS

NS: Not significant; +Log-transformed; *Significantly different from both of the other groups;
†25 apo ε4/ε3 genotype, and 3 ε4 homozygotes.

Table 2. Linear regression analysis for independent covariates of apoE levels+ in two models, with or without apoE polymorphism

	Model 1		Model 2					
	Total (n=365)		Total (n=182)		Men (n=81)		Women (n=101)	
	β	*p*	β	*p*	β	*p*	β	*p*
Gender (Female)	1.047	NS	1.029	NS				
Age	1.000	NS	0.999	NS	1.000	NS	1.000	NS
ApoB	**1.007**	0.000	**1.008**	0.000	**1.008**	0.000	**1.0083**	0.000
Systolic blood pressure	1.000	NS	1.000	NS	1.000	NS	1.000	NS
HOMA+	1.090	0.054	1.015	NS	1.016	NS	0.998	NS
Physical activity grade	**0.96**	0.044	0.973	NS	0.991	.NS	0.955	0.14
Cigarette smoking (0-1-2)	1.026	.NS	0.999	.NS	1.000	.NS	0.951	0.22
Alcohol usage	0.984	NS	0.987	NS	0.955	NS		
ApoE ε2 carriers			**1.396**	0.000	**1.48**	0.000	**1.37**	0.000
ApoE ε4 carriers			0.974	0.70	1.13	0.36	**0.873**	0.048
Explained apoE variance	28%		38%		25%		50%	

Both models significant (p<0.001); NS: Not significant; +Log-transformed values.

index. Since HOMA and, particularly apoE genotyping were missing in the majority of the study sample, regression analyses were carried out in 184 men and women (Table 4). ApoE genotypes, grouped in three isoforms, E2/E3/E4, were significantly associated with hyperapoB (≥120 mg/dl) but not with the atherogenic dyslipidemia. Doubling of apoE concentrations were significantly associated with both dyslipidemias, OR being higher in hyperapoB (OR=4.54, 95% CI 2.83; 12.3 *vs* OR=2.82, 95% CI 1.67; 5.18).

Finally, apoE concentrations were adjusted for apoE genotype for association with total cholesterol levels in a linear regression analysis comprising 229 men and women, along with sex, age, and statin usage as further independent variables. The model explained 15% of total cholesterol variance. Apart from female gender being significant, the apoE genotype grouping was significantly (p<0.005) associated with an increment of 15.3 mg/dl of total cholesterol, and apoE concentrations were independently, significantly and positively associated (p<0.001) whereby doubling of apoE corresponded to an increment of 14.4 mg/dl of total cholesterol.

Association of apoE concentrations with MS and coronary disease. In a logistic regression model for MS, present in 237 persons, serum apoE was associated significantly with MS likelihood in both men and women. The OR corresponding to a doubling of apoE level in

Table 3. Adjusted associations between serum apoE and elevated LDL-cholesterol* and triglyceride/HDL dyslipidemia, respectively

	Total (n=367)		Men (n=169)		Women (n=198)	
	OR	95% CI	OR	95% CI	OR	95% CI
For elevated LDL-cholesterol						
Gender (Female)	**1.88**	1.05; 3.4				
Age	1.025	0.998; 1.05	**1.057**	1.01; 1.106	1.016	NS
Doubling of apoE	**1.50**	1.10; 2.09	**1.81**	1.06; 3.08	1.35	0.89; 2.07
Statin use	0.45	0.18; 1.14	0.25	NS	0.47	NS
HOMA+	0.80	NS	1.13	NS	0.68	NS
For atherogenic dyslipidemia						
Gender (Female)	0.60	NS				
Age	0.97	NS	0.97	NS	0.987	NS
Doubling of apoE	**3.34**	2.24; 4.9	**3.19**	1.76; 5.75	**3.39**	1.91; 6.0
Statin use	**2.68**	1.17; 6.13	**10.6**	1.7; 65.5	1.57	NS
HOMA+	**2.78**	1.25; 6.16	2.52	0.8; 7.91	**3.08**	1.02; 9.34
+Log-transformed			*≥130 mg/dl			

NS: Not significant; Both models comprised also waist circumference, physical activity grade, systolic blood pressure, smoking status, and alcohol usage (all not significant independently). Model 1 comprised 44 men and 72 women with elevated LDL-cholesterol, model 2 included 56 men and 59 women with atherogenic dyslipidemia.

Table 4. Associations of apoE genotype and apoE concentrations with hyperapoB or triglyceride/HDL dyslipidemia (n= 184 men and women)

	HyperapoB*		Triglyceride/HDL dyslipidemia	
	OR	95% CI	OR	95% CI
Gender (Female)	1.76	NS	0.51	NS
Age	1.052	1.006; 1.10	0.98	NS
ApoE3	**5.57**	1.33; 23.3	0.95	NS
ApoE4	**9.83**	1.74; 55.4	1.02	NS
Doubling of apoE conc.+	**4.54**	2.83; 12.3	**2.82**	1.67; 5.18
Statin use	0.79	NS	3.89	1.16; 13
HOMA+	0.75	NS	4.42	1.56; 12.5

+Log-transformed *≥120 mg/dl

NS: Not significant; Models comprised 45 subjects with hyperapoB and 62 with triglyceride/HDL dyslipidemia.

both sexes combined was 1.72 (95% CI 1.24; 2.38), independent of age, waist girth, systolic BP, alcohol usage, statin usage, and other confounders (Table 5).

The likelihood of apoE for CHD in the whole sample adjusted for sex, age, and statin usage was not significant due to limited sample size.

DISCUSSION

The present study in a middle-aged general population sample of the Mediterranean area demonstrated the apoE genotype to be the main determinant of serum apoE concentrations, with apoB being a major independent covariate. Knowledge of apoE concentrations added substantial information to that provided by the apoE genotype, since levels were significantly linked to levels of total cholesterol, apoB, and to atherogenic dyslipidemia, independent of the apoE polymorphism. Male carriers of the ε4 allele had significantly higher apoE concentrations than homozygous ε3 subjects, as distinct from reported European populations. Serum apoE concentrations were significantly associated with elevated LDL-cholesterol in men, and with MS in both genders, when adjusted for multiple confounders.

Overall, serum apoE concentrations in this study measured by nephelometry are comparable to those reported for six European countries: clearly lower than in the sample of Greece but alike that of the Iberian peninsula.[8] Thus, present findings cannot be considered confirmatory of an increasing north-south gradient in apoE concentrations.[8] Whereas the prevalence of the E3/E2 genotype was similar to that in the ApoEurope Project,[8] the 75% prevalence of the homozygous ε3 allele among Turks was substantially higher, that of the ε4 allele carriers being significantly lower. The latter finding is consistent with the decreasing north-south gradient in the frequency of the ε4 allele noted in Europe.[8] The herein reported distribution of the apoE genotype is highly similar to that described by Mahley et al.[4] in the Turkish Heart Study on 8,366 participants.

ε2/ ε3/ ε4 allele frequency distribution was also similar to that found in smaller studies conducted with Turkish participants living in Turkey[21-23] and in Germany.[24]

We confirmed the impact of the apoE genotype on plasma lipid and lipoproteins found in the Turkish Heart Study, which is characterized by virtually no impact on HDL-cholesterol levels, and by low concentrations of total and LDL-cholesterol in individuals with the ε3/ε2 genotype, contrasted with high con-

Table 5. Adjusted associations between serum apoE and metabolic syndrome

	Total (n=454)		Men (n=222)		Women (n=232)	
	OR	95% CI	OR	95% CI	OR	95% CI
Gender (Female)	1.46	NS				
Age	0.995	NS	0.98	NS	1.015	0.985; 1.09
Doubling of apo E*	**1.72**	1.24; 2.38	**1.77**	1.10; 2.84	**1.67**	1.025; 2.71
LDL-cholesterol	0.999	NS	1.004	NS	0.995	NS
Waist circumference	**1.099**	1.07; 1.13	**1.117**	1.007; 1.17	**1.089**	1.05; 1.13
Alcohol usage	**0.34**	0.12; 0.96	**0.27**	0.08; 0.90	0.66	NS
Statin use	**3.15**	1.24; 8.0	**13.8**	1.52; 125	1.65	NS
Systolic blood pressure	**1.044**	1.03; 1.06	**1.057**	1.03; 1.08	**1.038**	1.02; 1.06

+Log-transformed; Model comprised also physical activity grade and smoking status (not significant independently);
Model comprised 108 men and 129 women with metabolic syndrome.

centrations of total and LDL-cholesterol in carriers of the ε4 allele. We demonstrated a significant gradient (reaching 21 mg/dl) of apoB within a range from the ε3/ε2 genotype, through the ε3 homozygous persons to carriers of the ε4 allele. No significant difference was observed in regard to sex- and age-adjusted triglyceride levels between the variants and the common isoform, though the triglyceride levels tended to be higher compared to ε3 homozygous individuals.

A relevant difference from reported European populations is the finding of higher apoE concentrations in male carriers of the ε4 allele than homozygous ε3 subjects. The latter was by 0.2 mg/dl lower, namely, in the ApoEurope Project,[8] and by about 0.7 mg/dl lower in the Stanislas cohort.[9] Since circulating apoE was herein shown to be linked to atherogenic dyslipidemia (and to MS) more strongly than to elevated LDL-cholesterol, this may, despite a lower prevalence of the ε4 allele carriers, partly account for the higher predisposition to atherogenic dyslipidemia[17] and MS[12] among Turks than in European populations.

Having investigated the effect of the apoE polymorphism on plasma levels of apoE and apoB in 394 predominantly male subjects, Boerwinkle and Utermann[25] showed that the correlation between levels of apoE and apoB was slightly negative and that the apoE polymorphism accounted for 20% and 12% of the interindividual variability of plasma concentrations of apoE and apoB, respectively. In the present study, apoE was strongly (in women even more strongly) associated with both hyperapoB and atherogenic dyslipidemia, likely due to the concordance between the serum concentrations in ε4 allele carriers, independent of the apoE genotype and HOMA index. The stated close link between levels of apoE and apoB likely underlies the prediction in Turks of cardiometabolic events, independent of markers of central obesity and inflammation.[26]

In line with numerous studies having shown associations between apoE levels and apoE isoforms or genotypes,[25,27-30] apoE genotype proved to be a major determinant for the apoE levels in our linear regression analysis, reaching 1.4-fold those of ε3 homozygotes in ε2 carriers, being 13% lower in female ε4 carriers and insignificantly higher in male ε4 carriers.

We attempted to quantify the associations between increments in apoE levels and likelihood for elevated LDL-cholesterol, dyslipidemia of high triglyceride/low HDL-cholesterol and MS by logistic regression analyses adjusted for multiple potential confounders, including gender, age, HOMA index, waist circumference, statin usage, smoking status, alcohol usage, physical activity grade, and systolic BP. Present findings indicate that, in this population sample, apoE concentrations are significantly associated with total cholesterol whereby doubling of apoE corresponded to an increment of 14.4 mg/dl serum cholesterol, independent of sex, age, and the two groups of apoE genotype. We present evidence that a doubling of apoE levels (i.e. from 3 to 6 mg/dl) is associated with a 2.8-fold likelihood of atherogenic dyslipidemia, independent of the apoE genotype and a surrogate of insulin resistance, suppressing the association of the apoE genotype, while both genotype and levels of apoE contributed to the association with respect to hyperapoB. Relations with lipid traits of both apoE genotypes and of apoE concentrations differed in two studied Chinese populations,[30] underlining the potential ethnic modification of these relations as well as the additive information to be obtained from apoE levels.

As a consequence of the described close link of serum apoE concentrations with atherogenic dyslipidemia, these levels were significantly associated in both genders also with the MS likelihood even after adjustment for the powerful covariates of waist girth, systolic BP, and other confounders. Hence, recognition of apoE concentrations may be a good indicator of the presence of atherogenic dyslipidemia and/or MS.

Our analysis of apoE levels with respect to prevalent CHD did not yield significant findings in either gender, likely due to limited statistical power of the sample size. However, in men an OR of 1.50 corresponding to a doubling of apoE suggests that this increment might become significant in a larger sample, since, in a meta-analysis of 14 published observational studies, the summary estimate of OR associated with CHD for both sexes combined was 1.26 in ε4 allele carriers compared with the ε3 allele.[5]

Our findings extend previous conclusions that apoE concentration adds to the apoE polymorphism to explain the variability in serum lipids and that it may mask the apoE polymorphism effect,[29] indicating that apoE concentrations may serve as a surrogate of the apoE genotypes in assessment of cardiometabolic risk, having even an independent additive value, at least in associations with serum levels of total cholesterol, hyperapoB, and atherogenic dyslipidemia. In regard to likelihood of MS, apoE levels seem to offer information independent of waist circumference, systolic BP, and other confounders.

The reasons why an excess rather than a deficiency of apoE concentrations serve in the direction of risk are unclear. It has been recognized that, in general, apoE concentrations are higher in hypertriglyceridemia than in hypercholesterolemia, and levels observed in patients with type 2 diabetes are high, and with cardiovascular diseases very variable.[10] Triglyceride-rich lipoproteins selectively enriched with apoE were observed in higher concentrations in patients with coronary artery disease even after controlling for triglyceride levels.[31]

Limitations. ApoE genotyping was available in only half the sample, and the possibility of a chance effect underlying the relation between apoE concentrations and ε4 allele cannot be absolutely ruled out, though this is extremely unlikely, having a p=0.007, an increment of 24%, and in view of the multivariably adjusted high association between levels of apoE and apoB. That the influence of nonfasting apoE determinations in a small part of the sample may not be great on overall evaluation is evidenced by values being not significantly different from those in fasting participants. The study sample, being representative of a general population, the appropriate exclusion of participants and adjustment for confounders including HOMA index, availability of apoB values, log-transformed analysis of apoE values form the strengths of the study.

To conclude, serum total cholesterol levels, hyper-apoB, and atherogenic dyslipidemia are significantly linked to apoE concentrations, independent of the apoE polymorphism. In contradistinction to European populations, Turkish male carriers of the ε4 allele have higher apoE concentrations than homozygous ε3 subjects, possibly resulting in a close link to apoB levels. The observation that serum apoE concentrations, when adjusted for multiple confounders, are significantly associated with MS in both genders may provide information regarding prediction of cardiometabolic disorders which extends beyond that provided by apoE genotypes.

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