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Polymorphisms of apolipoproteins A-IV and E in a Turkish population living in Germany

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Abstract Human apolipoproteins (apo) E and apo A-IV are polymorphic with significantly different allele frequencies among different ethnic groups. Whereas the variation at the apo E gene locus affects plasma cholesterol levels in all populations studied so far and is associated with longevity in Caucasians, the influence of the common apo A-IV polymorphism on plasma lipoproteins has not been unanimously accepted. We have therefore determined the common apo E and apo A-IV polymorphisms by isoelectric focusing, calculated the respective allele frequencies and studied their effects on plasma lipoproteins in a random sample of 240 nonrelated Turkish subjects (141 males, 99 females) living in Germany and originating from central and eastern Anatolia. When compared with the German population and other Caucasians in Europe a prominence of the apo ε3 allele frequency (0.885) was accompanied by a decrease in the frequencies of both the apo ε2 allele (0.048) and the apo ε4 allele (0.067). Thus, the Turkish population studied here clustered with populations mainly from southern Europe and Japan, which have low ε2 and ε4 allele frequencies. Also, the frequency of the A-IV-1 allele was higher (0.967) and that of the A-IV-2 allele lower (0.033) in the Turkish subjects studied than in other populations. At an average level of total cholesterol of 194.5 ± 45 mg/dl, no significant influence of the A-IV alleles on plasma lipoproteins was seen. However, apo E and apo B differed significantly between apo E phenotypes, with high levels of apo E and low levels of cholesterol and apo B in carriers of the ε2 allele, and vice versa for the ε4 allele. The average cholesterol excess for the ε2 allele was –7.95 mg/dl, for the ε3 allele, –1.34, and for the ε4 allele, +14.15 mg/dl. Thus, despite the unusual frequency distribution of the apo E alleles, their effects on plasma lipoproteins are within the range reported for other populations in Europe.

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Introduction

Apolipoprotein E (apo E) plays an important role in the regulation of plasma lipoprotein metabolism (Davignon et al. 1988). In most human populations three common alleles (ε2, ε3, ε4) at the apo E structural locus on chromosome 19 determine three isoforms, apo E2, E3, and E4, giving rise to six apo E phenotypes (three homozygotes, apo E2/2, E3/3, and E4/4, and three heterozygotes, apo E3/2, E4/3, and E4/2). The isoforms differ by single amino acid substitutions at positions 112 and 158 of the primary apo E sequence, where apo E2 shows two cysteine residues, apo E4 two arginine residues, and apo E3 a cysteine residue at position 112 and an arginine residue at position 158 (Mahley 1988). The apo E2 isoform shows defective binding to the low density lipoprotein (LDL or apo B/E) receptor (Schneider et al. 1981), and most apo E2 homozygotes (about 1% of Caucasian populations) have a form of hypobetalipoproteinemia, called primary dysbetalipoproteinemia (Utermann et al. 1977). When challenged by additional exogenous or endogenous factors leading to hyperlipidemia, these homozygotes may develop type III hyperlipoproteinemia, a condition predisposing to peripheral and coronary atherosclerosis (Utermann 1987). On the other hand, heterozygotes and homozygotes for apo E4, on average, have increased cholesterol concentrations (Davignon et al. 1988; Hallman et al.

1991), accompanied by an increased risk of atherosclerosis (Cumming and Robertson 1984; Davignon et al. 1988). Also the European Atherosclerosis Research Study revealed that the ε4 allele distribution differed between populations with a gradient for the frequency of this allele decreasing from 0.179 (Finland) to 0.105 (mixed population in southern Europe) (Tiret et al. 1994), which seems to follow the gradient of coronary heart disease mortality. Population studies have shown in addition that a significant proportion of the normal interindividual variability in plasma levels of total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C), and concentrations of apo E and apo B can be explained by the common allelic variation in the apo E gene (Boerwinkle and Utermann 1988; Davignon et al. 1988). The apo E allele frequencies are heterogeneous in different ethnic groups in Europe ranging from 0.039 to 0.141 for apo ε2, from 0.675 to 0.857 for apo ε3, and from 0.098 to 0.227 for apo ε4 (for a review, see Davignon et al. 1988; Hallman et al. 1991; Gerdes et al. 1992; Tiret et al. 1994).

The influence of the allelic variation at the apo A-IV locus on plasma lipoprotein parameters in Caucasian populations is not as clearly established as it is for apo E. The apo A-IV-1 wild-type allele is by far the most common in all populations studied, and A-IV-2 (Gln_{360}-His) the most frequent variant in Caucasians (Lohse et al. 1990; Tenkanen et al. 1991; for a review, see Lohse and Brewer 1991; Tenkanen and Ehnholm 1993). In three different populations a significant triglyceride (TG) lowering or high density lipoprotein-cholesterol (HDL-C) elevating effect of the apo A-IV-2 allele could be demonstrated (Menzel et al. 1988, 1990; Eichner et al. 1989). However, other studies could not confirm these results (De Knijff et al. 1988; Hanis et al. 1991; Tenkanen et al. 1991; von Eckardstein et al. 1992; Zaiou et al. 1994; Ehnholm et al. 1994).

In the course of ongoing studies in Turkish populations living in Germany, we determined apo E and apo A-IV allele frequencies in this population and related them to plasma lipid and apolipoprotein levels.

Materials and methods

EDTA-blood was collected from a random sample of 240 unrelated apparently healthy Turkish subjects (141 men and 99 women) participating in a study of Turkish people living in Germany close to Giessen in the state of Hessen. All of the Turks originated from eastern and central Anatolia. The age range was 13–76 years (mean ± SD, 44.3 ± 13.5 years; males, 15–76 years, mean ± SD, 45.8 ± 12.1 years; females, 13–72 years, mean ± SD, 42.1 ± 14.4 years). Plasma was separated within 3 h of collection and immediately frozen at –70°C. Plasma TC and TG concentrations were determined enzymatically, using commercially available test kits (Boehringer Mannheim, Mannheim, Germany). Plasma concentrations of apo A-I and apo B were measured by nephelometry (Beckman Array Protein System). Plasma apo E concentrations were measured by electroimmunoassay as described previously (Steinmetz et al. 1990). Very low density lipoprotein (VLDL) samples were isolated by ultracentrifugation in a Beckman 50 Ti rotor at 39 000 rpm for 18 h at 4°C, at d = 1.006 g/ml, and lipids were extracted in $CHCl_3/CH_3OH$ (3:1, v/v). Apo E phenotypes were determined by isoelectric focusing on rehydrated Immobiline Dry Plates (Pharmacia, Uppsala, Sweden) of delipidated VLDL samples as described by Baumstark et al. (1989). Apo A-IV polymorphism was analyzed by isoelectric focusing of whole plasma and subsequent western blotting (Steinmetz et al. 1988). Data were entered into a database designed for this study and transferred to the SPSS statistical program. All values are expressed as means ± SD. The significance of the differences in the means of plasma lipid and lipoproteins between different groups was determined, respectively, by ANOVA, or Student's *t*-test, or in the case of deviations from a normal distribution, the Kruskal-Wallis test/Wilcoxon test.

Apo E allele frequencies were determined by gene counting. Hardy-Weinberg equilibrium was tested with a χ^2 goodness-of-fit test of the genotype frequencies. The average excess of cholesterol concentration for a given apo E allele was determined as described by Hallman et al. (1991).

Results and discussion

The distribution of the apo A-IV phenotypes as determined by isoelectric focusing and subsequent western blotting in the 240 unrelated Turkish subjects and the calculated allele frequencies are given in Table 1. The A-IV allele frequencies (Hardy-Weinberg equilibrium, $\chi^2 = 2.2182$; $df = 2$; $P > 0.5$, n.s.) calculated for the Turkish study population show a very low apo A-IV-2 allele frequency (apo A-IV-1, 0.967; apo A-IV-2, 0.033). They are very similar to those of a Hungarian population (n = 202) published recently also showing a high frequency of the A-IV-1 and a low frequency of the A-IV-2 allele (Menzel et al. 1995). The A-IV-1 frequency in the Turkish population studied here is higher than reported for other Caucasian populations (Menzel et al. 1988, 1990; Ehnholm et al. 1994); the apo A-IV-1-1 phenotype accounted for 93.8% in the Turkish individuals while the apo A-IV-1-1 phenotype in individuals from five different European regions accounted for 85.1% (ranging from 80.7% to 89.7%) (Ehnholm et al. 1994). Although we did not confirm the A-IV-2 allele to be apo A-IV (Gln_{360}-His) by sequencing, we can extrapolate from published studies that we are most likely dealing with this specific mutation. Unlike the recent study in Hungarians analyzing a similar sample size (Menzel et al.

Table 1 Apolipoprotein (apo) A-IV and E phenotype distribution and calculated allele frequencies in the Turkish study population (n number, m male, f female)

Apo A-IV isoforms			Apo E isoforms		
	m/f	m+f (%)		m/f	m+f (%)
1/1	130/95	93.75	2/3	11/ 9	8.3
2/1	10/ 4	5.83	2/4	2/ 1	1.3
2/2	1/ 0	0.42	3/3	111/77	78.3
			3/4	17/12	12.1
Apo A-IV allele frequencies			**Apo E allele frequencies**		
	m+f			m+f	
A-IV-1	0.967		ε2	0.048	
A-IV-2	0.033		ε3	0.885	
			ε4	0.067	

Table 2 Lipid and apolipoprotein levels in the Turkish study population (n = 240) and the two major apo A-IV phenotype groups. Values are mean ± SD and are in milligrams per deciliter for lipids and apolipoproteins. The A-IV-1 allele includes apo A-IV-1-1 homozygotes, and the A-IV-2 allele represents apo A-IV-1-2 heterozygotes. One carrier (m) was homozygous for the apo A-IV-2 allele and was excluded. The Mann-Whitney U-Wilcoxon Rank Sum W test was employed to examine differences in mean values between males and females. (*TC* total cholesterol, *TG* triglycerides)

	Total (n = 240)	Male (n = 141)	Female (n = 99)	Apo A-IV-1 allele Total (n = 225)	Apo A-IV-2 allele Total (n = 14)
TC	194.5 ± 45.0	198.1 ± 43.7	189.4 ± 46.5	193.5 ± 44.4	207.1 ± 53.3
TG	168.4 ± 132.8	180.1 ± 140.2	151.8 ± 120.2	167.0 ± 133.3	192.6 ± 131.4
Apo A-I	150.3 ± 34.1	145.6 ± 35.0	157.1 ± 31.7	150.4 ± 34.4	149.6 ± 31.7
Apo B	97.6 ± 33.6	102.6 ± 34.2	90.7 ± 31.8	97.0 ± 33.3	108.0 ± 39.5
Apo E	8.6 ± 4.1	8.5 ± 3.8	8.9 ± 4.5	8.59 ± 4.17	9.29 ± 3.2

1995), we did not detect additional less frequently occurring apo A-IV isoforms in the present study population (n = 240). The detection system employed would, however, only detect mutations that affect protein charge or apparent isoelectric point. A larger sample size would thus be more appropriate to search for these mutants. On the other hand, using molecular biology techniques, additional polymorphisms have been identified that may escape detection by the one presently applied (Tenkanen and Ehnholm 1993).

The observed apo E phenotype distribution and corresponding apo E allele frequencies in the Turkish population are presented in Table 1. No homozygous carriers of plasma apo E isoforms E2 and E4 were detected. As expected, no significant gender differences in ε2, ε3, and ε4 allele frequencies were found in the present study. Apo E allele frequencies in the Turkish population studied were in Hardy-Weinberg equilibrium (χ^2 = 3.0322; df = 5; P > 0.7, n.s.) and were different to allele frequencies reported for other European countries (Hallman et al. 1991; Gerdes et al. 1992). The apo ε3 allele frequency is thus the highest reported of all at the expense of both apo ε2 and apo ε4, and exceeding known high frequencies in a Japanese population or in Indians and Chinese from Singapore (Hallman et al. 1991). In addition, the Turkish population also shows one of the most pronounced underrepresentations of the ε4 allele reported so far and the frequency is lower than those calculated for different populations from Italy, France, and Spain where the apo ε4 allele frequencies are the lowest within Europe (James et al. 1993; Tiret et al. 1994). Furthermore, the very low frequency of the apo ε2 allele resembles frequencies reported from a population in Finland (Ehnholm et al. 1986) or for an Indian population in Singapore (Hallman et al. 1991).

In addition, selected lipid (TC, TGs) and apolipoprotein parameters (apo A-I, B, and E) were measured; these values are shown in Table 2, separately for males and females. Plasma TC and TG concentrations in Turkish subjects studied here (n = 240) are somewhat higher than recently reported from Turkey by Örem et al. (1994) where 248 subjects were investigated, or by Onat and Senocak (1994) and Onat et al. (1992), in the study on the prevalence of cardiac disease and risk factors in Turkish adults (n = 3689). The latter data for TC and TG were, however, obtained by refloton measurement. In the present study mean values of plasma lipid and apolipoprotein levels were different between males and females and are in accordance with Örem et al. (1994). Both, TC, TG, and apo B concentrations were significantly (P < 0.05) higher in males than in females (Table 2). No gender differences were observed for plasma apo E concentrations (males, 8.5 ± 3.8 mg/dl; females, 8.9 ± 4.5 mg/dl). Overall mean plasma levels of apo A-I were comparably low, but the known gender difference with significantly (P < 0.05) higher concentrations in females (157.1 ± 31.7 mg/dl) than in males (145.6 ± 35.0 mg/dl) was sustained (Table 2). In a recent study HDL-C levels in both males and females of Turkish origin living in Germany were reported to be lower than those of a German control population (Lüttmann et al. 1994). These findings are in agreement with the relatively low apo A-I levels, the major protein component of HDL, found in our population. Knowledge of lipids and apolipoproteins in Turkish people living in Germany is sparse; however, it became apparent that, compared with Germans, Turks generally exhibit significantly lower plasma concentrations of TC, LDL-C, and especially HDL-C (Lüttmann et al. 1994). Lüttmann et al. (1994) reported similar TC levels in 396 Turkish men (193.2 ± 41.7 mg/dl) as compared with somewhat lower TC levels (174.2 ± 35.6 mg/dl) for 155 women (subset of study population from the PROCAM study, Assmann and Schulte 1986). In that study mean levels of TGs, although at lower values, were also higher in males (135.5 mg/dl) than in females (87.5 mg/dl); apolipoproteins were not reported (Lüttmann et al. 1994). The reasons for the higher TG levels found in the present study are not obvious but these levels are in line with those found in ongoing studies in Turkish people living in Germany (Oezcueruemez and Klör, unpublished observations). All participants of our study originated mainly from eastern and central Anatolia; the origin of the study population reported by Lüttmann et al. (1994) was, however, not specified.

The reports on the effects of the common genetic polymorphism at the apo A-IV locus on plasma lipid and apolipoprotein levels have been controversial. The present study did not reveal a significant influence of the apo A-IV-2 allele on lipid and apolipoprotein parameters. Mean values of both TC (193.5 mg/dl), TG (167.0 mg/dl), and apo B (97.0 mg/dl) were lower in carriers of the A-IV-1

Table 3 Lipid and apolipoprotein levels as a function of apo E alleles. The apo E2 allele includes E 2/3; the apo E3 allele represents E 3/3, and the apo E4 allele represents E 3/4. Values are mean ± SD and are in milligrams per deciliter for lipids and apolipoproteins. Modified LSD (Bonferroni) test with significant level of $P < 0.05$ (* = E2 vs E3 and * = E2 vs E4) was used for comparison of the different apo E allele carriers

	ε2 allele m+f (n = 20)	ε3 allele m+f (n = 188)	ε4 allele m+f (n = 29)
TC	186.8 ± 45.3	193.4 ± 44.9	208.9 ± 44.9
TG	191.7 ± 163.9	164.3 ± 135.5	173.4 ± 88.9
Apo A-I	150.6 ± 34.1	148.3 ± 33.9	161.6 ± 35.9
Apo B	75.2 ± 24.2*	99.5 ± 33.9	101.6 ± 33.1
Apo E	11.5 ± 4.8*	8.4 ± 4.18	8.0 ± 2.7

allele when compared with carriers of the A-IV-2 allele (TC, 207.1 mg/dl; TG, 192.6 mg/dl; apo B, 108.0 mg/dl) but the difference is not statistically significant (Table 2). This adds to the array of studies that failed to establish a significant association between plasma lipid and apolipoprotein concentrations and the different A-IV alleles (De Knijff et al. 1988; Hanis et al. 1991; Tenkanen et al. 1991; von Eckardstein et al. 1992; Ehnholm et al. 1994; Zaiou et al. 1994). The variability of TGs, considering in addition the relatively high levels seen in the present study, might demand larger sample numbers to solve finally the question of the influence of this polymorphism on lipoprotein metabolism. In screening studies, apo A-IV-1-2 heterozygotes were found to exhibit significantly higher levels of HDL-C (Menzel et al. 1988, 1990). Although HDL-C levels could not be determined in the present study, levels of apo A-I, the main protein component of HDL, were not different between the two groups (Table 2).

As the genetic polymorphism of apo E influences circulating lipoprotein levels, we subdivided the study group according to apo E phenotype distribution. The lipid and apolipoprotein concentrations of the three major apo E allele groups (ε2, E 2/3; ε3, 3/3; ε4: 4/3) were compared (Table 3). The three apo E2/4 heterozygotes were not included in this analysis. Although mean values of TC increased from the ε2 to ε4 groups, the differences were not statistically significant. Also no significant difference was found in plasma concentrations of TG and apo A-I between carriers expressing different apo E isoforms. However, the known inverse relation between apo E and apo B, with levels of apo E decreasing from apo ε2 to apo ε4 and showing the inverse behavior for apo B, was clearly seen. Also levels of apo E and B differed significantly ($P < 0.05$) between the three apo E phenotypic groups. Thus, our data confirm previous findings obtained in a population of different ethnic origin (Hallman et al. 1991). The average excess for the ε2 allele on TC in the Turkish population studied was –7.95 mg/dl, for the ε3 allele, –1.34, and for the ε4 allele + 14.15 mg/dl. These observations are in line with findings obtained in different populations; the overall average excess of the ε2 allele was –14.12 mg/dl (range –31.63 to –8.882 mg/dl); for the ε3 allele, 0.04 mg/dl (range –1.87 to 1.58 mg/dl); and for the ε4 allele, 8.14 mg/dl (range –1.71 to 13.31 mg/dl) (Hallman et al. 1991). The influence of the apo E phenotype on cholesterol variability is estimated in a white population at 14% for adults (Davignon et al. 1988). In a west German population the apo E polymorphism was calculated to account for 20% of the variability in plasma apo E levels, 12% of the variability of plasma apo B concentrations, and 4% of the variability of TC levels (Boerwinkle and Utermann 1988). It may be interesting to investigate the influence of migration from Turkey to Germany on the variability of lipids and lipoproteins for a given apo E allele in this population.

Besides other factors the very low frequencies of ε2 should decrease the probability of developing type III hyperlipoproteinemia in Turkish people. The recently reported large pedigrees with heterozygous familial hypercholesterolemia genetically linked to the LDL receptor locus, showed a strong interaction between the presence of familial hypercholesterolemia and a single apo E2 allele, resulting in a markedly increased prevalence of type III hyperlipoproteinemia (Hopkins et al. 1991). The higher concentrations of plasma cholesterol in carriers of the ε4 allele and the relative loss of apo E4 phenotype in nonagenarians (Kervinen et al. 1994) and centenarians (Louhija et al. 1994) are consistent with the hypothesis that the ε4 allele may affect life expectancy adversely, as the apo E polymorphism is associated with longevity (Schächter et al. 1994). Although this hypothesis remains to be proven in the Turkish population, the low ε4 allele frequency observed should be considered beneficial. Also, as the ε4 allele is associated with late-onset and familial Alzheimer's disease (Corder et al. 1993) the low frequency of ε4 in Turks might exert additional protective effects. This, however, needs to be demonstrated by further studies.

Summarizing, we showed that the apo A-IV allele frequency distribution in the Turkish study population living in Germany is similar to a Hungarian population and thus different from other Caucasian populations. Although in some studies, apo A-IV-1-2 heterozygotes had higher plasma HDL-C and lower TG levels than those who were homozygous for the apo A-IV-1 allele, we could not reveal any significant correlation between the apo A-IV polymorphism and plasma lipid and apolipoprotein concentrations. In addition, we could show that the decreased ε2 (0.048) and ε4 (0.067) alleles in the Turkish study population are among the lowest reported worldwide. The overall allele frequencies thus most closely resemble those reported for Japanese and may argue for a central Asian origin of the Turkish population. This hypothesis is additionally supported by the low ε2 (0.054) and ε4 (0.070) allele frequencies in a Greek-Cypriot population of Cyprus with an approximate ethnic composition of 80% Greek Cypriots including Armenians, Maronites, and Latins, and 18% Turkish Cypriots, and 2% foreigners (Cariolou et al. 1995).

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Note After the submission of this paper, Mahley et al. (1995) published an extensive study on lipids, lipoproteins, and apolipoproteins in the Turkish population living in Turkey. In a subset of 8366 participants, apo E allele frequencies were determined to be 0.86 for apo ε3, 0.061 for apo ε2, and 0.079 for apo ε4, in close accordance with our data on a very high frequency of apo ε3 at the expense of apo ε2 and apo ε4. The authors found a very similar apo E phenotype distribution among the different study centers in Turkey. The city of Kayseri and the surrounding region, one of the centers of the Turkish Heart Study, would reflect the origin of the Turkish people of our study.

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