Apolipoprotein A-V: a potential modulator of plasma triglyceride levels in Turks[S]

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Abstract **The apolipoprotein A-V gene (*APOA5*) plays an important role in determining plasma triglyceride levels. We studied the effects of *APOA5* polymorphisms on plasma triglyceride levels in Turks, a population with low levels of HDL cholesterol and a high prevalence of coronary artery disease. We found 15 polymorphisms, three of which were novel. Seven haplotype-tagging single nucleotide polymorphisms (SNPs) were chosen and genotyped in ~3,000 subjects. The rare alleles of the –1464T>C, –1131T>C, S19W, and 1259T>C SNPs were significantly associated with increased triglyceride levels (19–86 mg/dl; $P < 0.05$) and had clear gene-dose effects. Haplotype analysis of the nine common *APOA5* haplotypes revealed significant effects on triglyceride levels ($P < 0.001$). Detailed analysis of haplotypes clearly showed that the –1464T>C polymorphism had no effect by itself but was a marker for the –1131T>C, S19W, and 1259T>C polymorphisms. The –1131T>C and 1259T>C polymorphisms were in a strong but incomplete linkage disequilibrium and appeared to have independent effects. Thus, the *APOA5* –1131T>C, S19W, and 1259T>C rare alleles were associated with significant increases in plasma triglyceride levels. At least one of these alleles was present in ~40% of the Turks. Similar associations were observed for –1131T>C and S19W in white Americans living in San Francisco, California.**—Hodoğlugil, U., S. Tanyolaç, D. W. Williamson, Y. Huang, and R. W. Mahley. **Apolipoprotein A-V: a potential modulator of plasma triglyceride levels in Turks. *J. Lipid Res.* 2006.** 47: **144–153.**

Supplementary key words Turkish population • polymorphism • haplotype • high density lipoprotein cholesterol

Atherogenic dyslipidemia, including hypertriglyceridemia, is a risk factor for coronary artery disease (CAD) (1, 2). Family and twin studies have shown that triglyceride levels are controlled by genetic factors, although heritability estimates vary widely (3–5). Recently, the multinational Genetic Epidemiology of Metabolic Syndrome project (6) conducted a genome scan for atherogenic dyslipidemia and found significant evidence for linkage to triglyceride levels near the apolipoprotein A-V gene (*APOA5*), on chromosome 11q22, only in Turkish families (7). ApoA-V is an important regulator of plasma triglyceride levels (8, 9). Triglyceride levels are 4-fold higher in *Apoa5* knockout mice and significantly lower in transgenic mice (8) or in adenovirus-treated mice expressing human *APOA5* (9) than in wild-type mice. ApoA5 may decrease plasma triglyceride levels by increasing lipoprotein lipase activity (10, 11) and reducing hepatic levels of very low density lipoprotein triglyceride (11).

Several single nucleotide polymorphisms (SNPs) within the *APOA5* locus (–1131T>C, –3A>G, S19W, IVS3+476G>A, 1259T>C, and G185C) have been identified, and their rare alleles are associated with increased plasma triglyceride levels in different populations (8, 12–22). The –1131T>C, –3A>G, IVS3+476G>A, and 1259T>C SNPs (haplotype *APOA5**2) were in almost complete linkage disequilibrium (LD) in European populations (17, 20). Therefore, any one of these polymorphisms might serve as a marker for the others in these populations. The frequencies of the rare alleles of –1131T>C and S19W vary greatly among populations (8, 12–22). The plasma triglyceride increase associated with these rare alleles also varies, ranging from no association (20, 22) to 69% higher triglyceride levels in CC than in TT subjects with the –1131T>C polymorphism (16) and from no association (23, 24) to 20–30% higher triglyceride levels in SW than in SS subjects with the S19W polymorphism (20).

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Abbreviations: *APOA5*, apolipoprotein A-V gene; BMI, body mass index; CAD, coronary artery disease; HDL-C, high density lipoprotein cholesterol; htSNP, haplotype-tagging single nucleotide polymorphism; LD, linkage disequilibrium; SNP, single nucleotide polymorphism; THS, Turkish Heart Study; UTR, untranslated region.

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[S] The online version of this article (available at http://www.jlr.org) contains three additional tables.

Haplotype analysis in European populations identified three common haplotypes, two of which, uniquely described by the rare alleles –1131T>C and S19W, are associated with higher triglyceride levels than the most common haplotype (18, 20, 21). Although haplotype structure and distributions were different in Chinese (15), African-Americans (22), and three different Singaporean populations (16), significant haplotype-triglyceride associations were identified.

APOA5 SNPs have also been associated with reduced high density lipoprotein cholesterol (HDL-C; –1131T>C, –3A>G, and IVS3+476A>G) (16), decreased LDL cholesterol size (1259T>C and –3A>G) (13), and increased numbers of remnant-like particles (–1131T>C and S19W) (17). The –1131T>C SNP was more frequent in CAD patients (25). Both the –1131T>C and S19W SNPs were associated with cardiovascular events (17) but not with coronary artery diameter (23).

In this study, we explored the association between *APOA5* sequence variations and plasma triglyceride levels in >3,000 participants in the Turkish Heart Study (THS), a large, cross-sectional epidemiological survey of the Turkish population (26). The *APOA5* gene was sequenced to detect polymorphisms, haplotype-tagging single nucleotide polymorphisms (htSNPs) were genotyped, and these SNPs and haplotypes were associated with significantly increased levels of triglycerides.

MATERIALS AND METHODS

Study population and biochemical analyses

The primary study population consisted of 3,020 subjects randomly selected from the THS (26). A second cohort of 802 self-reported white American bank employees from a broad range of socioeconomic levels was used for some assays (27). Detailed biodata and blood samples obtained after an overnight fast were collected for each subject. Plasma lipids were measured as described (26). The protocols were approved by the Committee on Human Research of the University of California, San Francisco, and were in accordance with the Helsinki Declaration. Subjects who were taking lipid-lowering medication, had a history of diabetes mellitus, or had a plasma triglyceride level > 800 mg/dl were excluded.

Detection of *APOA5* polymorphisms

Primers were designed to amplify across the *APOA5* promoter, the 5′ untranslated region (UTR), and all exons, including intron/exon splicing boundaries when possible. DNA from 23 subjects (13 THS participants and 10 white Americans) was sequenced to identify polymorphisms in *APOA5*. DNA sequences were aligned and analyzed with Sequencher DNA analysis software (Gene Codes, Ann Arbor, MI).

Genotyping

After amplification by polymerase chain reaction, each polymorphism was genotyped by restriction fragment length polymorphism, digesting the primary amplification with restriction endonucleases and separating the resulting fragments with 1–3% agarose gels. The conditions of all assays are described in supplementary Table 1.

Statistics and data analysis

Data were analyzed with SPSS 10.0, Microsoft Access, and Excel. Associations between genotypes, lipids, and other parameters were analyzed separately for males and females. Lipid levels are expressed in mg/dl, and all values are reported as means ± SD. Mean values were compared with the *t*-test according to genotype or haplotype; $P < 0.05$ (two-tailed) was considered significant. Because triglyceride levels were not normally distributed, log-transformed values were used for statistical comparison; untransformed mean values are reported here. Analysis of covariance was used to construct a model to explain the variation in triglyceride levels and the overall effect of haplotype on plasma triglyceride levels. Body mass index (BMI), age, smoking, and alcohol consumption were included as covariates, and genotype score was included as a fixed factor in the model (GLM Univariate, SPSS 10.0). The proportion of variation in plasma triglyceride level from each SNP or haplotype was estimated from partial regression coefficients (28). Chi-square analysis was used to test differences between the observed and expected frequencies of alleles (assuming a Hardy-Weinberg equilibrium) and to compare genotype, allele, or haplotype frequencies after stratification by age- and gender-adjusted triglyceride percentiles ($\leqslant 20^{th}$ and $\geqslant 80^{th}$).

The expectation-maximization algorithm was used to estimate the maximum-likelihood haplotype frequencies from multilocus genotypic data without known gametic phase (Arlequin software, version 2.00) (29). All subjects with missing genotype data were excluded during haplotype prediction. Haplotypes that could be unambiguously attributed to individuals were further analyzed for associations with lipid and demographic data. The LD between polymorphisms was similarly calculated with Arlequin (29) and expressed in terms of $D' = D/D_{max}$ or D/D_{min} (30).

RESULTS

Population characteristics

Demographic and biochemical characteristics of 3,020 THS participants are presented in **Table 1**. Both males and females had low plasma HDL-C levels and high total cholesterol/HDL-C ratios. Detailed analyses of the THS data

TABLE 1. Demographic and biochemical characteristics of Turkish Heart Study participants (n = 3,020)

Variable	Males (n = 1,661)	Females (n = 1,359)	*P*
Age (years)	42 ± 13	42 ± 15	NS
Body mass index (kg/m^2)	26.1 ± 3.9	26.6 ± 5.4	<0.05
HDL cholesterol (mg/dl)	35.8 ± 7.5	41.2 ± 9	<0.001
Total cholesterol (mg/dl)	184 ± 45	183 ± 42	NS
LDL cholesterol (mg/dl)	126 ± 41	116 ± 39	<0.05
Triglycerides (mg/dl)	153 ± 107	110 ± 70	<0.001
Total cholesterol/HDL cholesterol ratio	5.8 ± 2.9	4.5 ± 1.4	<0.01
Systolic blood pressure (mm Hg)	125 ± 23	122 ± 21	NS
Diastolic blood pressure (mm Hg)	82 ± 14	81 ± 13	NS
Consumption of alcohol (%)[a]	29.9	5.5	<0.001
Cigarette smoking (%)[b]	56.7	24.1	<0.001

Values are means ± SD or percentages. Means were compared by *t*-test, and percentages were analyzed by chi-square test.
[a] One or more drinks per week.
[b] One or more cigarettes per day.

TABLE 2. Description and frequency of *APOA5* polymorphisms in Turks

Polymorphic Site[a]	Nucleotide Change	Location in the Gene	Location on Chromosome 11[b]	Rare Allele	Number[c]	SNP Identifier	Reference[d]
				%			
−1464T>C	T/C	Promoter	−1,456	29.0	1,574/1,304	rs10750097	—
−1275G>A	G/A	Promoter	−1,267	9.4	129/106	rs17120035	—
−1131T>C	T/C	Promoter	−1,123	12.8	1,601/1,302	rs662799	8
−1099C>T	C/T	Promoter	−1,091	10.3	1,505/1,181	rs1729411	16
−1021G>A	G/A	Promoter	−1,012	5.8	1,596/1,288		New
−3A>G	A/G	5′ UTR	6	13.9	230/188	rs651821	8
C56G (S19W)	C/G	Exon 3	178	5.6	1,633/1,334	rs3135506	20
C132A (I44I)	C/A	Exon 3	254	6.1	130/107	rs12287066	20
IVS3 + 476G>A	G/A	Intron 3	759	13.6	176/144	rs2072560	8
G457A (V153M)	G/A	Exon 4	1,097	4.6	1,502/1,162	rs3135507	15, 16
G553T (G185C)	G/T	Exon 4	1,193	0.6	201/288	rs2075291	15, 16
1177C>T	C/T	3′ UTR	1,817	4.5	333/272		15
1259T>C	T/C	3′ UTR	1,899	14.6	1,634/1,327	rs2266788	8
1387–1388delAG	(AG)	3′ UTR	2,027–2,028	~4.6	13[e]		New
1495T>C	T/C	3′ UTR	2,135	4.8	136/111		New

APOA5, apolipoprotein A-V gene; SNP, single nucleotide polymorphism; UTR, untranslated region.
[a] Relative to ATG start, reference sequence AAS68229.1. Synonymous and nonsynonymous changes and their locations are shown in parentheses.
[b] (+) strand ENSEMBLE, NCBI build 35, Ch11, 116165297:116167794:1.
[c] Number of males/females genotyped by restriction fragment length polymorphism.
[d] First publication of the particular polymorphism.
[e] Variant frequency determined by direct sequencing.

have been reported (26, 31, 32). It is noteworthy that low plasma HDL-C levels were found to increase the relative risk for CAD, and the plasma total cholesterol/HDL-C ratio was found to be an independent predictor of coronary events in Turks (33, 34).

APOA5 polymorphisms

Fifteen SNPs with rare allelic frequencies from <1% to 29% were identified (**Table 2**). Five SNPs were in the promoter region, including the novel –1021G>A, and one in the 5′ UTR (–3A>G). Four SNPs were in the coding sequence: three were nonsynonymous (S19W, V153M, and G185C) and one was synonymous (I44I). Four SNPs were in the 3′ UTR: two were novel (1387–1388delAG and 1495T>C) and two were published previously (1177C>T and 1259T>C). The IVS3+476G>A intronic SNP was also identified previously.

LD for *APOA5*

The LD between polymorphic sites was calculated using unphased genotypes from 14 SNPs from 215 randomly chosen unrelated Turkish subjects (**Table 3**; see supplementary Table II). Three clusters of *APOA5* polymorphic sites were in strong LD: –3A>G, IVS3+476G>A, and 1259T>C; S19W and I44I; and V153M, 1177C>T, and 1495T>C. 1259T>C, S19W, and V153M were chosen as markers for their clusters. –1275G>A was exclusively on one haplotype and in LD with –1464T>C. Seven htSNPs (–1464T>C, –1131T>C, –1099C>T, –1021G>A, S19W, V153M, and 1259T>C) were selected to assess the association between *APOA5* polymorphisms and plasma triglyceride levels in ~3,000 Turkish subjects.

The initial sequencing results suggested that the 1387–1388delAG variant was completely linked to the V153M, 1177C>T, and 1495T>C variants. This linkage was further supported by sequencing three additional 153MM subjects. Because it was in LD with and a marker for V153M, the 1387–1388delAG variant was not analyzed further.

The frequency of the rare G185C allele, which is significantly associated with high triglyceride levels in the Chinese population (15), was 0.6% (n = 487) in the Turkish population. Only five Turkish males and one female

TABLE 3. Common *APOA5* haplotypes in a random Turkish population

Haplotype	–1464T>C	–1275G>A	–1131T>C	–1099C>T	–1021G>A	–3A>G[a]	S19W[b]	I44I[b]	IVS3+476G>A[a]	V153M[c]	G185C	1177C>T[c]	1259T>C[a]	1495T>C[c]
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	2	1	2	1	1	2	1	1	2	1	1	1	2	1
3	1	1	1	2	1	1	1	1	1	1	1	1	1	1
4	2	2	1	1	1	1	1	1	1	1	1	1	1	1
5	1	1	1	1	2	1	1	1	1	1	1	1	1	1
6	2	1	1	1	1	1	2	2	1	1	1	1	1	1
7	1	1	1	1	1	1	1	1	1	2	1	2	1	2
8	2	1	1	1	1	2	1	1	2	1	1	1	2	1
9	1	1	2	1	1	1	1	1	1	1	1	1	1	1

1, common allele; 2, rare allele.
[a] −3A>G, IVS3+476G>A, and 1259T>C are in strong linkage disequilibrium (LD).
[b] S19W and I44I are in strong LD.
[c] V153M, 1177C>T, and 1495T>C are in strong LD.

TABLE 4. *APOA5* SNPs and plasma triglyceride levels in a random Turkish population

SNP	AA	AB	BB	*P* (AA vs. AB)	*P* (AA vs. BB)	Difference (BB – AA)[a]	
	mg/dl	*mg/dl*	*mg/dl*			*mg/dl*	*%*
Males							
–1464T>C	139 ± 94 (774)	159 ± 108 (688)	187 ± 144 (112)	<0.001	<0.001	48	35
–1131T>C	144 ± 99 (1,220)	170 ± 117 (357)	230 ± 148 (24)	<0.001	<0.001	86	60
–1099C>T	151 ± 104 (1,198)	148 ± 104 (298)	161 ± 97 (9)	NS	NS		
–1021G>A	152 ± 107 (1,431)	147 ± 87 (158)	145 ± 111 (7)	NS	NS		
S19W	148 ± 102 (1,438)	187 ± 132 (193)	191 ± 6 (2)	<0.001	—	39	26[a]
V153M	151 ± 105 (1,364)	156 ± 111 (134)	104 ± 51 (4)	NS	NS		
1259T>C	149 ± 104 (1,211)	166 ± 114 (390)	195 ± 139 (33)	<0.005	<0.05	46	31
Females							
–1464T>C	101 ± 59 (649)	115 ± 78 (551)	122 ± 98 (104)	<0.001	<0.03	21	21
–1131T>C	104 ± 64 (983)	121 ± 87 (298)	135 ± 107 (21)	<0.002	<0.05	31	30
–1099C>T	109 ± 73 (947)	102 ± 70 (229)	120 ± 61 (5)	NS	NS		
–1021G>A	110 ± 73 (1,137)	103 ± 56 (141)	98 ± 49 (10)	NS	NS		
S19W	108 ± 69 (1,198)	127 ± 95 (135)	96 (1)	<0.001	—	19	18[a]
V153M	107 ± 71 (1,062)	117 ± 79 (97)	82 ± 14 (3)	NS	NS		
1259T>C	104 ± 64 (961)	124 ± 86 (326)	150 ± 118 (40)	<0.001	<0.002	46	44

A, common allele; B, rare allele. Values shown are means ± SD. Number of subjects is shown in parentheses.
[a]For S19W, the difference is between AB and AA.

with 185GC heterozygosity were identified, and all had very high plasma triglyceride levels (372 ± 250 mg/dl for males, 499 mg/dl for the female). However, because of its low frequency, this SNP is unlikely to have a significant impact in the Turkish population.

APOA5 htSNPs and plasma triglyceride levels

The seven htSNPs and their associations with plasma triglyceride levels are presented in **Table 4**, where A denotes common alleles and B denotes rare alleles. In both males and females, triglyceride levels were significantly higher in AB and BB subjects (–1464T>C, –1131T>C, and 1259T>C) than in AA subjects ($P < 0.005$). All three of these SNPs had clear gene-dose effects. Additionally, 19SW subjects had significantly higher triglyceride levels than those with 19SS ($P < 0.001$), and this effect was more prominent in males. There were too few 19WW subjects for statistical analysis. In males, the –1131T>C SNP had the greatest effect on plasma triglycerides; the difference between the BB and AA genotypes was 86 mg/dl (60%). Interestingly, the rare –1131T>C allele had a much greater effect in males than in females (60% vs. 30% increase). The 1259T>C polymorphism had the largest impact in females: the triglyceride level was 46 mg/dl (44%) greater in the BB group than in the AA group.

Because clear gene-dose effects were observed (Table 4), both AB and BB individuals were combined into a group of B allele carriers (AB + BB), and both allele and genotype frequency distributions were determined for subjects with triglyceride levels in the $\leq 20^{th}$ and $\geq 80^{th}$ percentiles (**Table 5**). The B allele and B allele carriers were significantly more frequent in the $\geq 80^{th}$ percentile than in the $\leq 20^{th}$ percentile groups in both males and females with –1464T>C, –1131T>C, S19W, and 1259T>C ($P < 0.05$),

TABLE 5. Frequencies of rare allele (B) and rare allele carriers (AB + BB) in men and women with *APOA5* SNPs who are in the $\leq 20^{th}$ and $\geq 80^{th}$ percentiles of triglyceride levels

	B				AB + BB			
SNP	All	$\leq 20^{th}$ Percentile	$\geq 80^{th}$ Percentile	*P* ($\leq 20^{th}$ vs. $\geq 80^{th}$ Percentile)	All	$\leq 20^{th}$ Percentile	$\geq 80^{th}$ Percentile	*P* ($\leq 20^{th}$ vs. $\geq 80^{th}$ Percentile)
Males								
−1464T>C	0.290	0.252	0.344	<0.001	0.508 (800)	0.434 (118)	0.590 (233)	<0.001
−1131T>C	0.126	0.090	0.163	<0.001	0.238 (381)	0.176 (48)	0.304 (121)	<0.001
−1099C>T	0.105	0.113	0.093	—	0.204 (307)	0.218 (57)	0.179 (68)	—
−1021G>A	0.054	0.051	0.054	—	0.103 (165)	0.096 (26)	0.103 (41)	—
S19W	0.060	0.048	0.090	<0.005	0.119 (195)	0.096 (27)	0.177 (75)	<0.01
V153M	0.047	0.051	0.043	—	0.092 (138)	0.094 (24)	0.087 (33)	—
1259T>C	0.140	0.111	0.165	<0.01	0.259 (423)	0.208 (57)	0.304 (130)	<0.01
Females								
−1464T>C	0.291	0.235	0.326	<0.001	0.502 (655)	0.418 (104)	0.559 (170)	<0.001
−1131T>C	0.131	0.102	0.158	<0.01	0.245 (319)	0.201 (50)	0.292 (88)	<0.02
−1099C>T	0.101	0.093	0.090	—	0.198 (234)	0.186 (44)	0.172 (47)	—
−1021G>A	0.063	0.075	0.060	—	0.117 (151)	0.137 (34)	0.110 (33)	—
S19W	0.051	0.032	0.060	<0.03	0.102 (136)	0.063 (16)	0.120 (38)	<0.05
V153M	0.044	0.033	0.056	—	0.086 (100)	0.066 (16)	0.112 (30)	—
1259T>C	0.153	0.110	0.202	<0.001	0.276 (366)	0.212 (53)	0.351 (112)	<0.001

Percentages were analyzed by chi-square (2×2) test. Number of subjects is shown in parentheses.

TABLE 6. Additive effects of *APOA5* SNPs on plasma triglyceride levels

S19W	–1131T>C	Males	Increase[a]	Females	Increase[a]
SS	TT	138 ± 93 (1,041)	—	102 ± 58 (852)	—
	TC	165 ± 111 (332)	27	119 ± 86 (279)	17
	CC	228 ± 151 (23)	90	138 ± 110 (20)	36
SW	TT	181 ± 125[b] (158)	43	124 ± 94[b] (113)	22
	TC	218 ± 112[c] (20)	80	129 ± 106 (15)	27
S19W	1259T>C	Males	Increase[d]	Females	Increase[d]
SS	TT	142 ± 96 (1,028)	—	102 ± 58 (826)	—
	TC	162 ± 112 (364)	20	122 ± 84 (310)	20
	CC	195 ± 139 (33)	53	150 ± 118 (40)	48
SW	TT	187 ± 133[e] (164)	45	125 ± 93[e] (121)	23
	TC	221 ± 109[f] (22)	79	146 ± 119 (13)	44

Values shown are mg/dl, means ± SD. Number of subjects is shown in parentheses.
[a] Triglyceride increase relative to 19SS/–1131TT.
[b] $P < 0.005$ versus 19SS/–1131TT.
[c] $P < 0.005$ versus 19SS/–1131TC.
[d] Triglyceride increase relative to 19SS/1259TT.
[e] $P < 0.05$ versus 19SS/1259TT.
[f] $P < 0.05$ versus 19SS/1259TC.

further supporting the association of these SNPs with increased triglycerides.

Two SNP pairs, S19W/–1131T>C and S19W/1259T>C, had significant additive and independent effects on triglyceride levels (**Table 6**). –1131T>C and 1259T>C were each associated significantly with increased triglyceride levels in 19SS homozygous males and females. The largest effects were a 90 mg/dl difference between the 19SS/–1131CC and 19SS/–1131TT genotypes in males and a 48 mg/dl difference between the 19SS/1259CC and 19SS/1259TT genotypes in females. There were too few 19SW/–1131CC and 19SW/1259CC subjects for statistical analysis (data not shown). Notably, for both SNP pairs examined, double heterozygotes always had higher triglyceride levels than single heterozygotes. The –1099C>T, –1021G>A, and V153M SNPs were not associated with plasma triglyceride levels.

APOA5 haplotypes and plasma triglyceride levels

The nine most common *APOA5* haplotypes (frequency > 1.0%) accounted for 96.0% of all 36 predicted haplotypes (**Table 7**). Plasma triglyceride levels associated with haplotype 1, the most frequent haplotype possessing the common alleles for all seven htSNPs, were compared with the mean triglyceride levels for the other haplotypes (Table 7). Haplotype 2, characterized by the rare alleles for –1464T>C, –1131T>C, and 1259T>C, was associated with significantly higher triglyceride levels in both males and females than haplotype 1. Notably, the rare –1464T>C SNP occurred in isolation on haplotype 4, and its triglyceride level was not different from that associated with haplotype 1, suggesting that –1464T>C by itself had no effect. However, haplotype 6, which possessed the rare alleles of –1464T>C and S19W, was associated with higher triglyceride levels than haplotype 1 in both males and females. The triglyceride levels associated with haplotypes 2 and 6 were significantly higher in males than in females (haplotype 2, 22% vs. 14%; haplotype 6, 35% vs. 17%). Additionally, haplotype 9, with the –1131T>C rare allele in isolation, was associated with higher triglyceride levels than haplotype 1 in males only [27 mg/dl

TABLE 7. Plasma triglyceride levels of common haplotypes of *APOA5* and their frequencies in a random Turkish population

Haplotype	Frequency	Males[d]	Increase versus Haplotype 1		Females[d]	Increase versus Haplotype 1		–1464T>C	–1131T>C	–1099C>T	–1021G>A	S19W	V153M	1259T>C
		mg/dl	*mg/dl*	*%*	*mg/dl*	*mg/dl*	*%*							
1	0.481	143 ± 98 (1,341)	—	—	109 ± 75 (1,104)	—	—	1	1	1	1	1	1	1
2	0.101	174 ± 124[a] (270)	31	22	124 ± 90[a] (243)	15	14	2	2	1	1	1	1	2
3	0.101	151 ± 106 (290)			100 ± 57 (218)			1	1	2	1	1	1	1
4	0.104	144 ± 101 (312)			100 ± 58 (211)			2	1	1	1	1	1	1
5	0.051	145 ± 86 (140)			101 ± 57 (120)			1	1	1	2	1	1	1
6	0.050	193 ± 131[a] (142)	50	35	127 ± 90[a] (111)	18	17	2	1	1	1	2	1	1
7	0.037	154 ± 114 (112)			118 ± 78 (79)			1	1	1	1	1	2	1
8	0.021	138 ± 90 (56)			127 ± 97[b,c] (51)	18	17	2	1	1	1	1	1	2
9	0.015	170 ± 80[a] (49)	27	19	107 ± 88 (25)			1	2	1	1	1	1	1
Sum	0.960													

1, common allele; 2, rare allele. Number of subjects is shown in parentheses.
[a] $P < 0.05$ versus haplotype 1 (*t*-test).
[b] $P < 0.09$ versus haplotype 1 (*t*-test).
[c] $P < 0.05$ versus haplotypes 3, 4, and 5 (*t*-test).
[d] Values shown are means ± SD.

(19%); $P < 0.05$]. However, haplotype 8, containing the –1464T>C and 1259T>C rare alleles, was associated with higher triglyceride levels than haplotype 1 in females [18 mg/dl (17%); $P = 0.09$]. The –1099C>T SNP was found only on haplotype 3, –1021G>A only on haplotype 5, and V153M only on haplotype 7. These haplotypes were not associated with differences in plasma triglyceride levels.

Haplotypes 2 and 6 were 1.6- to 2.1-fold more frequent in the $\geq 80^{th}$ than in the $\leq 20^{th}$ percentile group in both sexes (**Table 8**). Although haplotype 9 was >2-fold more frequent in the $\geq 80^{th}$ percentile group in males, the difference was not statistically significant, possibly because of the low number of subjects tested. However, when triglyceride tertiles were used, haplotype 9 was significantly more frequent in the $\geq 67^{th}$ percentile than in the $\leq 33^{rd}$ percentile [2.8% (n = 29) vs. 0.9% (n = 7); $P < 0.01$]. In females, haplotype 8 was more common in the $\geq 80^{th}$ than in the $\leq 20^{th}$ percentile (Table 8). These findings further substantiate the association between the –1131T>C, S19W, and 1259T>C SNPs and increased plasma triglyceride levels in Turks.

To assess the additive effects of haplotypes, we examined the mean triglyceride values of subjects with haplotype pairs 1-1, 1-2, 1-6, 2-2, and 2-6 (other haplotype pairs were too infrequent to analyze). In males, triglyceride levels were higher in those with haplotype pairs 2-2 (252 ± 190 mg/dl; n = 8) and 2-6 (261 ± 138 mg/dl; n = 8) than in those with haplotype pair 1-1 (133 ± 89 mg/dl; n = 288), 1-2 (150 ± 114 mg/dl; n = 155), or 1-6 (185 ± 131 mg/dl; n = 90). In females, triglyceride levels were significantly higher in those with haplotype pair 2-2 (172 ± 140 mg/dl; n = 10) than in those with haplotype pair 1-1 (108 ± 74 mg/dl; n = 262) or 1-2 (128 ± 87 mg/dl; n = 127). Also in females, haplotype pair 2-6 (128 ± 101 mg/dl; n = 9) was associated with higher triglyceride levels than haplotype pair 1-1. These findings suggest that *APOA5* haplotypes 2 and 6 had additive effects, particularly in males.

In addition to *t*-test comparisons, analysis of covariance (covariates were HDL-C, age, BMI, smoking, and alcohol consumption) confirmed the significance of the SNP and haplotype effects on triglyceride levels (see supplementary Table III). Bonferroni post hoc analysis showed that this significance originated principally from haplotypes 2, 6, and 9 in males and from haplotypes 2, 6, and 8 in females.

TABLE 8. Frequency comparison of common haplotypes of *APOA5* between the $\leq 20^{th}$ and $\geq 80^{th}$ percentiles of triglyceride

Haplotype	All Groups	Triglyceride Subgroups: $\leq 20^{th}$ Percentile	Triglyceride Subgroups: $\geq 80^{th}$ Percentile	P ($\leq 20^{th}$ vs. $\geq 80^{th}$ Percentile)
Males				
1	0.474	0.516 (254)	0.439 (316)	0.01
2	0.096	0.069 (34)	0.125 (90)	0.02
3	0.103	0.108 (53)	0.093 (67)	NS
4	0.110	0.118 (58)	0.103 (74)	NS
5	0.050	0.045 (22)	0.050 (36)	NS
6	0.050	0.036 (18)	0.079 (57)	0.004
7	0.039	0.042 (21)	0.038 (28)	NS
8	0.020	0.016 (8)	0.015 (11)	NS
9	0.017	0.008 (4)	0.018 (13)	NS
Females				
1	0.491	0.543 (246)	0.491 (260)	NS
2	0.108	0.084 (38)	0.132 (70)	0.02
3	0.097	0.090 (41)	0.076 (40)	NS
4	0.094	0.102 (46)	0.068 (36)	NS
5	0.054	0.066 (30)	0.047 (25)	NS
6	0.049	0.029 (13)	0.059 (31)	0.035
7	0.036	0.024 (11)	0.049 (26)	NS
8	0.023	0.009 (4)	0.032 (17)	0.021
9	0.012	0.013 (6)	0.008 (4)	NS

Values shown are frequencies. Number of subjects is shown in parentheses. Percentages were analyzed by chi-square test.

White American study population and the –1464T>C SNP

Haplotype analysis in the Turks suggested that the –1464T>C SNP was a marker for the –1131T>C, S19W, and 1259T>C SNPs and that the associated phenotype seen with the –1464T>C SNP derived from the strong LD between –1464T>C and these other three SNPs (Tables 7, 8; see supplementary Table II). To confirm this phenomenon in another population, we analyzed the distribution of the –1464T>C, –1131T>C, S19W, and 1259T>C SNPs in 802 self-reported white non-Hispanic Americans. Initial analysis showed that the –1131T>C and 1259T>C SNPs were almost in complete LD in white Americans (only 3 of 228 paired genotypes were different; $D' = 0.935$), as in other European populations; therefore, 1259T>C was not genotyped further. In contrast, the –1131T>C and 1259T>C SNPs were not as strongly linked in Turks ($D' = 0.698$; see supplementary Table II). The rare allele frequencies for the –1464T>C, –1131T>C, and S19W SNPs were 19.0, 5.9, and 6.0%, respectively, in white Americans and 29.0, 12.8, and 5.6%, respectively, in Turks (Table 2). Triglyceride levels were significantly higher in AB and BB subjects with –1464T>C and in AB subjects with both –1131T>C and S19W than in AA subjects ($P < 0.05$) (**Table 9**). Haplotype analysis suggested LD between –1464T>C and the –1131T>C and S19W SNPs, and that –1464T>C might be a marker for these other SNPs in white Americans as in Turks. The rare –1464T>C SNP occurred in isolation on haplotype X, and the triglyceride level associated with this haplotype was not different from that associated with haplotype W (Table 9), suggesting that –1464T>C by itself had no effect.

DISCUSSION

This study shows that three common *APOA5* SNPs (–1131T>C, S19W, and 1259T>C) and the haplotypes formed with seven *APOA5* htSNPs were significantly associated with increased plasma triglyceride levels in Turks, regardless of sex. No other associations with lipid parameters (HDL-C, LDL, total cholesterol, or total cholesterol/HDL-C ratio) were found. The rare SNP alleles were significantly more frequent in subjects with the highest plasma triglyceride levels ($\geq 80^{th}$ percentile) than in those with the lowest levels ($\leq 20^{th}$ percentile). The effects of S19W and of –1131T>C and 1259T>C were indepen-

TABLE 9. Plasma triglyceride levels of SNPs and haplotypes of *APOA5* in a white American population

SNP	AA	AB	BB	Difference (AB – AA)		
Males						
–1464T>C	132 ± 77 (160)	175 ± 115[a] (65)	170 ± 139[a] (7)	43		
–1131T>C	139 ± 93 (208)	162 ± 82[a] (21)	205 ± 42 (2)	23		
S19W	138 ± 83 (191)	164 ± 88[a] (23)	459 ± 300 (2)	26		
Females						
–1464T>C	116 ± 67 (364)	131 ± 87[a] (176)	137 ± 107[a] (23)	15		
–1131T>C	120 ± 76 (505)	139 ± 78[a] (63)	211 ± 78 (3)	19		
S19W	121 ± 77 (500)	138 ± 75[a] (58)	150 ± 105 (4)	17		
Haplotype	Frequency	Males	Females	−1464T>C	−1131T>C	S19W
W	0.76	132 ± 73 (335)	117 ± 69 (844)	0	0	0
X	0.12	135 ± 75 (30)	122 ± 98 (107)	1	0	0
Y	0.05	174 ± 96[b] (21)	132 ± 62[b] (51)	1	0	1
Z	0.04	168 ± 89[b] (16)	132 ± 70[b] (50)	1	1	0

A, common allele; B, rare allele. Values shown are mg/dl, means ± SD. Number of subjects is shown in parentheses.
[a] $P < 0.05$ versus AA.
[b] $P < 0.05$ versus haplotype W.

dent of each other and additive, each showing a dose-dependent association with phenotype. Turks have low HDL-C levels (26, 31–34), and the inverse relationship between plasma HDL-C and triglyceride levels is well established (35, 36). When plasma triglyceride levels were adjusted for covariates (HDL-C, age, BMI, smoking, and alcohol consumption), the –1131T>C, S19W, and 1259T>C SNPs and haplotypes were significantly associated with increased plasma triglyceride levels, suggesting that the primary associations were between these polymorphisms and triglyceride levels.

The rare allele of –1464T>C was associated with increased plasma triglyceride levels in Turks. However, the mean triglyceride level for the haplotype with the rare –1464T>C allele in isolation (haplotype 4; Table 7) was not significantly different from that for the most frequent haplotype (haplotype 1; Table 7), suggesting that the association between –1464T>C and triglyceride level was primarily attributable to –1131T>C, 1259T>C, and S19W. Additionally, the frequency of haplotype 4 was not higher in the $\geqslant 80^{th}$ than in the $\leqslant 20^{th}$ percentile group. These results suggest that –1464T>C might only be a marker for those three SNPs and not a direct modulator of triglyceride levels. The same conclusion was reached in analyzing the effect of –1464T>C in white Americans (Table 9).

Two *APOA5* SNPs, –1131T>C and S19W, have been studied extensively, and their rare allele frequencies vary greatly among populations. The frequency of the –1131T>C rare C allele was 27–37% in East Asians (12–14, 16, 19), 13–16% in Hispanics (12, 20), 6–9% in African-Americans and western Europeans (or their descendants) (8, 17, 20, 21), and 12.8% in Turks. The allele frequency of the S19W SNP was very rare (<0.1%) in Chinese (16) and Japanese (13), 4–8% in African-Americans and western Europeans (17, 18, 20, 21), 15% in Hispanics (20), and 5.6% in Turks. More importantly, the high plasma triglyceride levels associated with these rare alleles also vary among populations. Japanese (19) and Malay (16) homozygotes for the –1131T>C rare allele had 10% and 69% higher triglyceride levels, respectively, than homozygotes for the common allele. Intermediate increases in triglycerides have been associated with this polymorphism in other populations (12, 16, 17, 20). The impact of the –1131T>C rare allele on plasma triglyceride levels was comparatively high in Turks. The –1131T>C subjects had a 60% (86 mg/dl) increase in triglyceride levels in Turkish males and a 30% (31 mg/dl) increase in Turkish females. Compared with 19SS, 19SW was associated with 8–16% higher plasma triglyceride levels among Caucasians (21) and 20–30% higher levels in African-Americans (20) and with 26% (39 mg/dl) higher levels in Turkish males and 18% (19 mg/dl) higher levels in Turkish females. Interestingly, associations were not found for –1131T>C in African-American males or females (20, 22) or for S19W in the LOCAT study (24) or in African-American males or white females from the CARDIA study (22) or in CAD patients from Vancouver, Canada (23). However, the associations of –1131T>C and S19W with triglyceride levels in Turks are significant and some of the highest reported for the *APOA5* locus. In our analysis of non-Hispanic white Americans, the alleles had effects similar to those reported for Caucasian and European populations.

The 1259T>C SNP has also been investigated for its association with triglyceride levels. It was associated with 37% higher triglyceride levels in a Japanese-American population (13) and with 33–53% higher levels in three Singaporean populations (16). We found similar increases of 31% (46 mg/dl) in Turkish males and 44% (46 mg/dl) in Turkish females.

The –1131T>C, S19W, and 1259T>C polymorphisms explained 18.6, 10.7, and 8.6% of the variance in triglyceride levels, respectively, in Turkish males and 9.3, 3.8, and 12.5%, respectively, in Turkish females. The magnitudes of these variances are consistent with the higher percentage increase in triglyceride levels associated with both –1131T>C and S19W and with the lower percentage increase associated with 1259T>C in Turkish males (Table 4). Previously, we showed that gender has a much greater effect on HDL-C levels in Turks, especially males, than in other populations (32). Similarly, the combined

effect of the nine common *APOA5* haplotypes explained 16.2% of the variance in triglyceride levels in Turkish males and 12.8% in females, and the percentage increases associated with haplotypes 2 and 6 were higher in males (Table 7). These results suggest that gender-specific influences may interact with these polymorphisms to modulate triglyceride levels in Turks.

In European populations, four SNPs (–1131T>C, –3A>G, IVS3+476G>A, and 1259T>C) constituted a single haplotype (17, 20). However, in Turks, three Singaporean populations, and African-Americans, the *APOA5* haplotype structure was more complex (16, 22). –1131T>C was in strong, but not complete, LD with the three other SNPs in Turks and Singaporeans (16), and 1259T>C was very rare in African-Americans (<0.001%) (22). Haplotypes containing both the –1131T>C and 1259T>C rare alleles modulated triglyceride levels in Turks, and the effect of these SNPs may be independent of each other and gender-specific, because triglyceride increase was associated with haplotype 9 (–1131T>C in isolation) only in males and with haplotype 8 (1259T>C in isolation) only in females. On the other hand, –1131T>C was not associated with triglyceride levels in African-Americans, in whom 1259T>C is extremely rare (22). Association studies, including studies of *APOA5*, have shown gender differences in lipid metabolism (37–40), but the mechanism is not fully understood. Functional studies should be conducted to determine how the –1131T>C and 1259T>C SNPs modulate triglycerides.

The G185C SNP, with an allelic frequency of 4.2%, was significantly associated with increased triglyceride levels in a Chinese population (15) but was extremely rare or absent in Caucasians (20, 41). Although G185C was very rare in the Turkish population (0.6% allelic frequency), all six GC heterozygotes had very high plasma triglyceride levels.

Plasma triglyceride levels were decreased significantly by overexpression of *APOA5* (8, 9) and increased significantly in *Apoa5* knockout mice (8). Because *APOA5* polymorphisms have been associated with high plasma triglyceride levels, SNP-associated increases may reflect the impaired function of apoA-V. In HepG2 cells, the W19-encoded signal peptide was secreted into the medium at significantly lower levels than the S19-encoded signal peptide (42). Potentially, the –1131T>C, –3A>G, and 1259T>C SNPs may also affect the function of *APOA5*. –1131T>C is located in the promoter region and may alter *APOA5* expression, and 1259T>C, located in the 3′ UTR, might affect the stability of *APOA5* mRNA. Alternatively, 1259T>C, which is in complete LD in Turks, may be a marker for –3A>G; however, expression assays did not support a biological function for –3A>G (42). Although in vitro studies did not show individual effects of these three SNPs, cooperative effects cannot be excluded. Except for the two studies of African-American males and females in whom the 1259T>C SNP was very rare (20, 22), the –1131T>C SNP was shown to be associated with increased plasma triglyceride levels in several studies (8, 12–14,16–22) and supports the idea of cooperation between *APOA5* SNPs.

APOA5 is located downstream of the *APOA1/C3/A4* gene cluster in a small 60 kb region on human chromosome 11. *APOA1* variants are primarily associated with altered HDL-C levels (43, 44) and *APOC3* variants with altered triglyceride levels (43–45). Transgenic and knockout studies suggest that *APOA5* and *APOC3* independently influence plasma triglyceride levels in an opposite manner (46). A recent study in Caucasians suggested a high degree of LD across the entire gene cluster; nevertheless, *APOA5* was separated from the other apolipoprotein genes by a region of low LD (47). Additionally, some individual *APOA5* SNPs (haplotype *APOA5**2) were in strong LD with *APOC3* SNPs, whereas S19W exerted its effect on triglyceride levels independently of *APOC3* SNPs (47). The structure of the *APOA1/C3/A4/A5* cluster and its association with triglyceride levels should be examined in other populations.

Hypertriglyceridemia is an independent risk factor for CAD (1, 2). For every 1 mmol/l (~88.5 mg/dl) increase in plasma triglycerides, the risk of CAD was increased significantly by 14% in males and 37% in females after adjustment for HDL-C and other factors (48). In Turks, the *APOA5* SNP-associated triglyceride increase was 19–86 mg/dl, depending on sex and the polymorphism, in a population in which ~40% carry at least one rare allele of –1131T>C, S19W, or 1259T>C. The magnitude of the change in triglyceride levels and the relatively high frequencies of these rare *APOA5* alleles are important considerations in assessing the risk of CAD in Turks, particularly those with low plasma HDL-C levels.

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