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

Uğur Hodogluğil, Sinan Tanyolac, David W. Williamson, Yadong Huang, and Robert W. Mahley

Gladstone Institute of Cardiovascular Disease, Cardiovascular Research Institute, Graduate Program in Biological and Medical Informatics, Departments of Pathology and Medicine, University of California, San Francisco, San Francisco, CA; and Faculty of Medicine, Department of Internal Medicine, Division of Endocrinology and Metabolism, Istanbul University, Istanbul, Turkey

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, −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, 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.—Hodogluugil, U., S. Tanyolac, 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 multina-

Copyright © 2006 by the American Society for Biochemistry and Molecular Biology, Inc.

This article is available online at http://www.jlr.org
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 (≤20th and ≥80th).

The expectation-maximization algorithm was used to estimate the maximum-likelihood haplotype frequencies from multilocus genotypic data without known genetic 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 \( D^\prime = D/D_{\text{max}} \) or \( D/D_{\text{min}} \) (50).

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 are provided in Table 1.

| Variable                  | Males (n = 1,661) | Females (n = 1,359) | P      |
|---------------------------|------------------|---------------------|--------|
| Age (years)               | 42 ± 13          | 42 ± 15             | NS     |
| Body mass index (kg/m²)   | 26.1 ± 3.9       | 26.6 ± 5.4          | <0.05  |
| HDL cholesterol (mg/dl)   | 35.8 ± 7.5       | 41.2 ± 9.0          | <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 (%) | 29.9% | 5.5% | <0.001 |
| Cigarette smoking (%)     | 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.

*One or more cigarettes per day.

*One or more drinks per week.

Effects of APOA5 SNPs on triglycerides

145
Table 2. Description and frequency of APOA5 polymorphisms in Turks

| Polymorphic Site  | Nucleotide Change | Location in the Gene | Location on Chromosome 11 | Rare Allele | Number | SNP Identifier | Reference |
|-------------------|-------------------|----------------------|---------------------------|-------------|--------|----------------|-----------|
| -1464T>C         | T/C               | Promoter             | -1,456                    | 29.0%       | 1,574/1,304 | rs10750997     | —         |
| -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 | rs602799       | 8         |
| -1099C>T         | C/T               | Promoter             | -1,091                    | 10.3%       | 1,505/1,181 | rs1294111      | 16        |
| -1021G>A         | G/A               | Promoter             | -1,012                    | 5.8%        | 1,596/1,288 | —             | New       |
| -3A>G            | A/G               | 5’ UTR               | 6                        | 13.9%       | 290/188   | rs651821       | 8         |
| C56G (S19W)      | C/G               | Exon 5               | 178                       | 5.6%        | 1,633/1,334 | rs3135506      | 20        |
| C132A (I44I)     | C/A               | Exon 5               | 254                       | 6.1%        | 130/107   | rs12287066     | 20        |
| IVS3+476G>C      | C/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    |
| G533T (G185C)    | G/T               | Exon 4               | 1,193                     | 0.6%        | 201/288   | rs2072591      | 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         | —             | New       |
| 1495T>C         | T/C               | 3’ UTR               | 2,135                     | 4.8         | 136/111   | —             | New       |

APOA5, apolipoprotein AV gene; SNP, single nucleotide polymorphism; UTR, untranslated region.

*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 H4I; 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 ihtSNPs (-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 markers for their clusters. –1275G>A was exclusively on one haplotype and in LD with –1464T>C. Seven ihtSNPs (-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 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 | 1387-1388delAG | S19W | H4I | IVS3+476G>A | V153M | G185C | 1177C>T | 1259T>C | 1495T>C |
|-----------|----------|----------|-----------|-----------|-----------|------|----------------|------|-----|------------|--------|--------|---------|---------|---------|
| 1         | 1        | 1        | 1         | 1         | 1         | 1    | 1              | 1    | 1   | 1          | 1      | 1      | 1       | 1       | 1       |
| 2         | 2        | 2        | 1         | 2         | 1         | 1    | 1              | 1    | 1   | 2          | 1      | 1      | 1       | 1       | 1       |
| 3         | 1        | 1        | 2         | 1         | 1         | 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       | 1       |
| 5         | 1        | 1        | 1         | 2         | 1         | 1    | 1              | 1    | 1   | 1          | 1      | 1      | 1       | 1       | 1       |
| 6         | 2        | 1        | 1         | 1         | 1         | 1    | 1              | 1    | 1   | 2          | 1      | 1      | 1       | 1       | 1       |
| 7         | 1        | 1        | 1         | 1         | 1         | 1    | 1              | 1    | 1   | 2          | 1      | 1      | 1       | 1       | 1       |
| 8         | 1        | 1        | 1         | 1         | 1         | 1    | 1              | 1    | 1   | 2          | 1      | 1      | 1       | 1       | 1       |
| 9         | 1        | 2        | 1         | 1         | 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 H4I are in strong LD.
c V153M, 1177C>T, and 1495T>C are in strong LD.
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 1295T>C) than in AA subjects (P < 0.005). All three of these SNPs had clear gene-dose effects. Additionally, 19SS 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 1295T>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 ≤20th and >80th percentiles (Table 5). The B allele and B allele carriers were significantly more frequent in the >80th percentile than in the ≤20th percentile groups in both males and females with −1464T>C, −1131T>C, S19W, and 1295T>C (P < 0.05),

### Table 4. APOA5 SNPs and plasma triglyceride levels in a random Turkish population

| SNP       | AA (mg/dl) | AB (mg/dl) | BB (mg/dl) | P (AA vs. AB) | P (AA vs. BB) | Difference (BB – AA)* |
|-----------|------------|------------|------------|---------------|---------------|-----------------------|
| 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  | 132 ± 107 (1,431) | 147 ± 87 (158) | 145 ± 11 (7)   | NS            | NS            |                       |
| S19W      | 148 ± 102 (1,438) | 187 ± 132 (193) | 191 ± 6 (2)    | <0.001        | —             | 39 ± 26               |
| V153M     | 151 ± 105 (1,564) | 156 ± 111 (134) | 104 ± 51 (4)   | NS            | NS            |                       |
| 1295T>C   | 149 ± 104 (1,211) | 166 ± 114 (390) | 195 ± 139 (33) | <0.005        | <0.05         | 46 ± 31               |

| Females   |            |            |            |               |               |                       |
| −1464T>C  | 101 ± 50 (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 ± 60 (1,198) | 127 ± 95 (135) | 96 (1)         | <0.001        | —             | 19 ± 18               |
| V153M     | 107 ± 71 (1,062) | 117 ± 79 (97)  | 82 ± 14 (3)    | NS            | NS            |                       |
| 1295T>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.

*For S19W, the difference is between AB and AA.

### Table 5. Frequencies of rare allele (B) and rare allele carriers (AB + BB) in men and women with APOA5 SNPs who are in the ≤20th and >80th percentiles of triglyceride levels

| SNP       | B (mg/dl) | AB + BB (mg/dl) | P (≤20th vs. >80th) | P (≤20th vs. >80th) |
|-----------|-----------|----------------|---------------------|---------------------|
| Males     |           |                |                     |                     |
| −1464T>C  | 0.290     | 0.252          | 0.344               | <0.001              |
| −1131T>C  | 0.126     | 0.163          | 0.136               | >0.001              |
| −1099C>T  | 0.165     | 0.113          | 0.093               | —                   |
| −1021G>A  | 0.054     | 0.051          | 0.054               | —                   |
| S19W      | 0.060     | 0.048          | 0.090               | <0.005              |
| V153M     | 0.047     | 0.051          | 0.043               | NS                  |
| 1295T>C   | 0.140     | 0.111          | 0.165               | <0.01               |

| Females   |           |                |                     |                     |
| −1464T>C  | 0.291     | 0.255          | 0.326               | <0.001              |
| −1131T>C  | 0.131     | 0.102          | 0.158               | <0.01               |
| −1099C>T  | 0.101     | 0.093          | 0.090               | —                   |
| −1021G>A  | 0.063     | 0.075          | 0.060               | —                   |
| S19W      | 0.051     | 0.032          | 0.060               | <0.03               |
| V153M     | 0.044     | 0.033          | 0.056               | —                   |
| 1295T>C   | 0.153     | 0.110          | 0.202               | <0.001              |

Percentages were analyzed by chi-square (2×2) test. Number of subjects is shown in parentheses.
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 6. Additive effects of APOA5 SNPs on plasma triglyceride levels**

| S19W  | −1131T>C | Males | Increase<sup>a</sup> | Females | Increase<sup>a</sup> |
|-------|----------|-------|----------------------|---------|----------------------|
| 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<sup>b</sup> (158) | 43 | 124 ± 94<sup>c</sup> (113) | 22 |
|      | TC       | 218 ± 112<sup>d</sup> (20) | 80     | 129 ± 106 (15) | 27          |

Values shown are mg/dl, means ± SD. Number of subjects is shown in parentheses.

*Triglyceride increase relative to 19SS/−1131TT.

<sup>a</sup> <sup>b</sup> P < 0.005 versus 19SS/−1131TT.

<sup>c</sup> <sup>d</sup> P < 0.05 versus 19SS/−1131TT.

<sup>e</sup> <sup>f</sup> <sup>g</sup> <sup>h</sup> Triglyceride increase relative to 19SS/1259TT.

<sup>i</sup> P < 0.05 versus 19SS/1259TT.

<sup>j</sup> P < 0.05 versus 19SS/1259TC.

---

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

| Haplotype Frequency | Males<sup>a</sup> | Increase versus Haplotype 1 | Females<sup>a</sup> | Increase versus Haplotype 1 | −1464T>C−1131T>C−1099C>T−1021G>A−V153M 1259T>C |
|---------------------|-------------------|-----------------------------|---------------------|-----------------------------|--------------------------------------------------|
| mg/dl               | 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                   |
| 2                   | 0.101             | 174 ± 124<sup>b</sup> (270) | 31                  | 124 ± 90<sup>c</sup> (243) | 15                  | 1                  | 1                  | 1                  | 1                  | 1                  | 2                  |
| 3                   | 0.101             | 151 ± 106 (290)            | 31                  | 100 ± 57 (218)             | 1                   | 1                  | 2                  | 1                  | 1                  | 1                  | 1                  |
| 4                   | 0.101             | 144 ± 101 (312)            | 31                  | 100 ± 58 (211)             | 1                   | 1                  | 2                  | 1                  | 1                  | 1                  | 1                  |
| 5                   | 0.051             | 145 ± 86 (140)             | 31                  | 101 ± 57 (129)             | 1                   | 1                  | 1                  | 2                  | 1                  | 1                  | 1                  |
| 6                   | 0.050             | 193 ± 131<sup>d</sup> (142) | 50                  | 127 ± 90<sup>e</sup> (111) | 18                  | 2                  | 1                  | 1                  | 2                  | 1                  | 1                  |
| 7                   | 0.057             | 154 ± 114 (112)            | 80                  | 118 ± 78 (79)              | 80                  | 1                  | 1                  | 2                  | 1                  | 1                  | 1                  |
| 8                   | 0.021             | 138 ± 90 (56)              | 80                  | 127 ± 97<sup>f</sup> (51)  | 18                  | 2                  | 1                  | 1                  | 1                  | 1                  | 1                  |
| 9                   | 0.015             | 170 ± 80<sup>g</sup> (49)  | 27                  | 107 ± 88 (25)              | 27                  | 2                  | 1                  | 1                  | 1                  | 1                  | 1                  |
| Sum                 | 0.960             |                             |                     |                             | 1                   | 1                   | 1                  | 1                  | 1                  | 1                  | 1                  |

1, common allele; 2, rare allele. Number of subjects is shown in parentheses.

<sup>a</sup> P < 0.05 versus haplotype 1 (t-test).

<sup>b</sup> P < 0.09 versus haplotype 1 (t-test).

<sup>c</sup> P < 0.05 versus haplotype 3, 4, and 5 (t-test).

<sup>d</sup> Values shown are means ± SD.
V153M only on haplotype 7. These haplotypes were not significantly higher in those with haplotype pair 2-2 (172 vs. 131 mg/dl; n = 6). Other haplotype pairs were too infrequent to analyze. In males, triglyceride levels were significantly higher in the 20th percentile group in both sexes (Table 8). Although haplotype 9 was >2-fold more frequent in the 80th 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 80th percentile than in the 20th percentile (29.0, 12.8, and 6.0%, respectively, in white Americans vs. 19.0, 5.9, and 6.0%, respectively, in Turks). The rare –1464T C SNP was not as strongly linked in Turks (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). 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. However, haplotype analysis in the Turks suggested that the –1131T>C SNP derived from the strong LD between –1464T>C and these other three SNPs (Tables 7, 8; 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.

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. However, haplotype analysis in the Turks suggested that the –1131T>C SNP derived from the strong LD between –1464T>C and these other three SNPs (Tables 7, 8; 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.

DISCUSSION

This study shows that three common APOA5 SNPs (–1131T>C, S19W, and 1259T>C) and the haplotypes formed with seven APOA5 hSNPs 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 (≥80th percentile) than in those with the lowest levels (≤20th percentile). The effects of S19W and of –1131T>C and 1259T>C were indepen-

Table 8. Frequency comparison of common haplotypes of APOA5 between the 20th and 80th percentiles of triglyceride

| Haplotype | All Groups | 20th Percentile | 80th Percentile | P (20th vs. 80th 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      | 0.056 (18)     | 0.079 (57)     | 0.004                      |
| 7         | 0.039      | 0.042 (21)     | 0.038 (28)     | NS                         |
| 8         | 0.090      | 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.
dient 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 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 >80th 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 homoyzgotes 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 (15) 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

### Table 9. Plasma triglyceride levels of SNPs and haplotypes of APOA5 in a white American population

| SNP       | AA       | AB       | BB       | Difference (AB – AA) |
|-----------|----------|----------|----------|---------------------|
| –1464T>C  | 132 ± 77 (160) | 175 ± 115° (65) | 170 ± 139° (7) | 43 |
| –1131T>C  | 139 ± 93 (208) | 162 ± 82° (21) | 205 ± 42 (2) | 23 |
| S19W      | 138 ± 83 (191) | 164 ± 88° (23) | 459 ± 300 (2) | 26 |

| 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° (21) | 132 ± 62° (51) | 1 | 0 | 1 |
| Z         | 0.04      | 168 ± 89° (16) | 132 ± 70° (50) | 1 | 1 | 0 |

A, common allele; B, rare allele. Values shown are mg/dl, means ± SD. Number of subjects is shown in parentheses.

\*P < 0.05 versus AA.

\*P < 0.05 versus haplotype W.
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 apoAV. 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 chromo...

References

1. Murray, C. J. L., and A. D. Lopez. 1997. Mortality by cause for eight regions of the world. Global Burden of Disease Study. Lancet. 349: 1209–1276.
2. Peto, R., A. D. Lopez, J. Boreham, M. Thun, C. Heath, Jr., and R. Doll. 1996. Mortality from smoking worldwide. Br. Med. Bull. 52: 12–21.
3. Brenn, T. 1994. Genetic and environmental effects on coronary heart disease risk factors in Northern Norway. The cardiovascular disease study in Finnmark. Ann. Hum. Genet. 58: 369–379.
4. Heller, D. A., U. de Faire, N. L. Pedersen, G. Dahlén, and G. E. McClean. 1993. Genetic and environmental influences on serum lipid levels in twins. N. Engl. J. Med. 328: 1150–1156.
5. Persusse, L., T. Rice, J. P. Desprès, J. Bergeron, M. A. Province, J. Gagnon, A. S. Leon, D. C. Rao, J. S. Skinner, J. H. Wilmore, et al. 1997. Familial resemblance of plasma lipids, lipoproteins and postheparin lipoprotein and hepatic lipases in the HERITAGE family study. Arterioscler. Thromb. Vasc. Biol. 17: 3263–3269.
6. Wyszynski, D. F., D. M. Waterworth, P. J. Barter, J. Cohen, Y. A. Kesâniemi, R. W. Mahley, R. McPherson, G. Waebaer, T. P. Bersot, S.
in individuals with extreme triglyceride levels. Physiol. Res. 53:
225–228.
42. Talmud, P. J., J. Palmen, W. Putt, L. Lins, and S. E. Humphries.
2005. Determination of the functionality of common APOA5
polymorphisms. J. Biol. Chem. 280: 28215–28220.
43. Groenendijk, M., R. M. Cantor, T. W. A. de Bruin, and G. M.
Dallinga-Thie. 2001. The apoAI-CIII-AIV gene cluster. Atherosclero-
sis. 157: 1–11.
44. Kim, J. Q., J. Song, Y. B. Park, and S. H. Hong. 1998. Molecular
bases of coronary heart disease in Koreans. J. Korean Med. Sci.
13: 1–15.
45. van Dijk, K. W., P. C. N. Rensen, P. J. Voshol, and L. M. Havekes.
2004. The role and mode of action of apolipoproteins CIII and AV:
synergistic actors in triglyceride metabolism? Curr. Opin. Lipidol. 15:
239–246.
46. Baroukh, N., E. Bauge, J. Akiyama, J. Chang, V. Afzal, J. C. Fruchart,
E. M. Rubin, J. Fruchart-Najib, and L. A. Pennacchio. 2004. Analysis
of apolipoprotein A5, C3, and plasma triglyceride concentrations
in genetically engineered mice. Arterioscler. Thromb. Vasc. Biol. 24:
1297–1302.
47. Olivier, M., X. Wang, R. Cole, B. Gau, J. Kim, E. M. Rubin, and
L. A. Pennacchio. 2004. Haplotype analysis of the apolipoprotein
gene cluster on human chromosome 11. Genomics. 33: 912–923.
48. Austin, M. A., J. E. Hokanson, and K. L. Edwards. 1998. Hypertri-
glyceridemia as a cardiovascular risk factor. Am. J. Cardiol. 81 (Suppl.):
7B–12B.