

# Genetic analysis of a polymorphism in the human apoA-V gene: effect on plasma lipids

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**Abstract** Recent discovery and characterization of *APOAV* suggests a role in metabolism of triglyceride (TG)-rich lipoproteins. Previously, variation at the *APOAV* locus was shown to modestly influence plasma TGs in normolipidemic samples. The aims of this study were to assess the effects of a polymorphism in *APOAV* (T-1131C) in terms of its frequency among three dyslipidemic populations and a control population, differences of allele frequency across available ethnic groups, and associations with specific lipoprotein TG and cholesterol compartments. We found a striking elevation in the frequency of the rare allele in a Chinese population ( $P = 0.0002$ ) compared with Hispanic and European populations. The rare allele of the polymorphism was associated with elevated plasma TG ( $P = 0.012$ ), VLDL cholesterol ( $P = 0.0007$ ), and VLDL TG ( $P = 0.012$ ), LDL TG ( $P = 0.003$ ), and HDL TG ( $P = 0.016$ ). Linear regression models predict that possession of the rare allele elevates plasma TG by 21 mg/dl ( $P = 0.009$ ) and VLDL cholesterol by 8 mg/dl ( $P = 0.0001$ ), and reduces HDL cholesterol by 2 mg/dl ( $P = 0.017$ ). The association of the polymorphism with altered lipoprotein profiles was observed in combined hyperlipidemia, hypoalphalipoproteinemia, and hyperalphalipoproteinemia, and in controls. These findings indicate that *APOAV* is an important determinant of plasma TG and lipoprotein cholesterol, and is potentially a risk factor for cardiovascular disease.—Aouizerat, B. E., M. Kulkarni, D. Heilbron, D. Drown, S. Raskin, C. R. Pullinger, M. J. Malloy, and J. P. Kane. Genetic analysis of a polymorphism in the human apoA-V gene: effect on plasma lipids. *J. Lipid Res.* 2003. 44: 1167–1173.

**Supplementary key words** lipoprotein • dyslipidemia • ethnicity

Atherosclerosis is the leading cause of morbidity and mortality in both industrialized and developing countries (1). The etiology of coronary artery disease is multifactorial, with both genetic and environmental determinants (2, 3). Abnormalities of lipoprotein metabolism are central to the development of atherosclerosis (4, 5).

Study of premature coronary artery disease has revealed that apolipoproteins are important discriminating factors for distinguishing individuals with coronary artery disease (6–8). The apolipoprotein gene cluster (*APOAI-CIII-AIV*) on human chromosome 11q23 is known to harbor at least three genes that affect the metabolism of plasma lipoproteins (9). The relationship between variations in the gene cluster and plasma lipids has been studied for nearly two decades (9–12). The majority of studies have focused on either apolipoprotein A-I (apoA-I), because of its influence on HDL production, or on apoC-III for its modulation of plasma triglyceride (TG). Recently, study of apoA-IV, an apolipoprotein associated with chylomicron and HDL particles (13), has provided evidence for its role in postprandial lipemia (14) and coronary artery disease (15). While variations in the *APOAI-CIII-AIV* gene cluster have been reported to influence several dyslipidemic states (16–20), the recent characterization of the proximal apoA-V provides evidence for a significant role in the modulation of levels of lipids and lipoproteins (21).

The apoA-V gene (*APOAV*) was recently discovered by comparative sequencing of the *APOAI-CIII-AIV* gene region (21). Pennacchio and colleagues, by construction of both knockout and human transgenic murine models for apoA-V, established its role in modulating plasma TG, a major risk factor for coronary artery disease (21). Employing four single-nucleotide polymorphisms (SNPs) revealed during sequence analysis, significant associations were found between both plasma TG and VLDL mass in two independent human genetic-association studies (21). The minor allele of each of three SNPs, in linkage disequilibrium, was associated with 20–30% higher plasma TG than among individuals homozygous for the major allele. There was no association with a genetic marker in the adjacent apoC-III gene, which is known to also modulate plasma TG (21). Taken together, these data indicate that

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*APOAV* polymorphisms may serve as important prognostic indicators for susceptibility to hypertriglyceridemia.

A recent report of increased plasma TG, associated with an upstream *APOAV* promoter polymorphism in two independent Caucasian populations, suggests that *APOAV* may contribute to certain dyslipidemic states (21). Moreover, variations in *APOAV* may have varying impacts in different ethnic groups. Therefore, we elected to assess the frequency of the same polymorphism in *APOAV* in three dyslipidemic groups and a control population. We also tested for variations in allele frequency in three ethnic groups. The impact of this variation on lipoprotein composition and body mass index (BMI) was examined. A significant difference in minor allele frequency was detected in Chinese in comparison with Hispanic and European populations. Whereas we detected no preferential association with any of the three dyslipidemic phenotypes, the minor *APOAV* allele was associated with elevated plasma TG, VLDL TG, LDL TG, and HDL TG, strikingly elevated VLDL cholesterol, and marginally depressed HDL cholesterol. In addition, linear regression analysis of lipid parameters yielded regression models permitting estimates of adjusted means for plasma TG, VLDL cholesterol, and HDL cholesterol, conditioned on minor allele carrier status.

## METHODS

### The study design

This study was a retrospective analysis of the prevalence of the *APOAV*-T1131C intragenic SNP among three dyslipidemic population samples recruited without bias toward ethnicity. They were selected from the University of California, San Francisco (UCSF) Genomic Resource in Arteriosclerosis (22), a population-based study of atherosclerotic heart disease, using the following criteria: 1) individuals with the combined hyperlipidemia phenotype as having plasma total cholesterol (TC) >200 mg/dl, total plasma TG >200 mg/dl, LDL cholesterol >130 mg/dl, and VLDL cholesterol >30 mg/dl; 2) individuals with hypoalphalipoproteinemia were identified as having HDL cholesterol less than the tenth percentile for their age and gender (23) and TG <150 mg/dl; and 3) those with hyperalphalipoproteinemia had HDL cholesterol greater than 80 mg/dl. The UCSF Committee on Human Research approved the protocols. Informed consent was obtained from all subjects for DNA isolation and plasma collection. Wherever possible, lipid measures were normalized and/or age-gender matched as described previously (22, 23).

Detection of significant linkage disequilibrium between the three intragenic SNPs (rs2266788, rs2072560, and rs662799) (21) led us to adopt a parsimonious genotyping approach using the most informative SNP (rs662799) (24). Patients were selected according to phenotypic profiles consistent with combined hyperlipidemia, hypoalphalipoproteinemia, or hyperalphalipoproteinemia, post hoc, for genotyping at the *APOAV* locus. Unselected controls provided an environmental, age-, and gender-matched baseline population for genotype and genotype-phenotype analyses. Baseline lipoprotein measurements were obtained when patients had received no lipid-lowering medication for at least 1 month.

### The sample

Clinical and demographic data were available on all subjects ( $n = 825$ ). Several exclusion criteria were applied prior to analyses of

lipid values. One hundred and fifty-two subjects were excluded because they had diabetes or hypothyroidism. Among the remaining subjects, 14 individuals were excluded because they were taking medications or had secondary conditions that could affect lipid values at the time of baseline lipid measurement. Finally, 32 subjects who did not pass a consistency check on cholesterol fractions (an absolute difference less than 15 mg/dl for TC vs. the sum of all measured cholesterol fractions: HDL, LDL, and VLDL) were excluded, leaving a total of 627 subjects in the study population.

Regression models for the lipid measures included a data-driven selection among indicators for the *APOAV* polymorphism. The polymorphism was examined in the context of possible effects of the following predictors: age, gender, lipoprotein profiles of clinical populations versus control, angiographic findings, hypertension, smoking, alcohol use, exercise, prior myocardial infarction, premenopausal status (nonmenopausal females aged greater than 12.5 years were also included in this class), and menopause. BMI was calculated as weight (kg)/height (m)<sup>2</sup>. Quantitative self-report of smoking status and frequency was collapsed into a qualitative assignment of smoking status (25). Positive exercise status was qualitatively assigned for individuals expending a minimum of 7.5 kcal/min for 30 min twice a week (25). In addition to myocardial infarction, the presence of cardiovascular disease was qualitatively assigned when an individual had undergone either coronary angioplasty and/or bypass surgery. The presence of hypertension was defined as systolic blood pressure >140 mm Hg and/or diastolic blood pressure >90 mm Hg and/or taking antihypertensive medication.

### Lipoprotein studies

Blood was drawn after a 10 h fast for ultracentrifugal separation of the  $d < 1.006 \text{ g/cm}^3$  and  $d > 1.006 \text{ g/cm}^3$  fractions (26). HDL cholesterol was measured after precipitation of apoB-containing lipoproteins with dextran sulfate and magnesium (27). Cholesterol and TG levels were measured in plasma and in lipoprotein fractions by either automated fluorescence method (28) or automated chemical analysis (29). LDL cholesterol was calculated as the difference of the content of the LDL cholesterol plus HDL cholesterol fraction ( $d > 1.006 \text{ g/cm}^3$ ) and the plasma HDL cholesterol. Standards were provided by the Centers for Disease Control (Atlanta, GA).

### Determination of the apoA-V genotype

Genomic DNA was prepared from whole blood obtained from patients in the Lipid Clinic of UCSF (22). The presence or absence of the polymorphism (rs662799) 1,131 bp upstream of the transcription start site (T-1131C) of the *APOAV* gene was determined as described previously (21), when it was given the arbitrary designation "SNP3." Briefly, site-specific primers were used to amplify a 187 bp region of DNA by PCR encompassing the polymorphism. The penultimate base of the 3' oligonucleotide was changed to incorporate an *MseI* site (TTAA) in the common allele. Next, 10 units of the enzyme *MseI* in 15  $\mu\text{l}$  of buffer was added directly to the PCR products and incubated at 37°C for 3 h. The products were resolved on 5% polyacrylamide gels and visualized using ethidium bromide and UV light.

### Statistical methods

The SAS (2000, 2001) system for statistical analysis was used, principally procedures Regression and general linear model (GLM) for linear regression models, and LOGISTIC for logistic regression models (30, 31). On screened predictors, possible subset regression models were ranked by the Cp criterion (32). Power transformations of response variables were selected using a SAS macro implementing the methods of Box and Cox (33). Transformations of potential predictor variables were examined to

maximize the explanatory power of the overall model (by maximizing the F statistic). One transformation among a small set (square, square root, log, reciprocal, reciprocal squared) was selected that best met these aims, ignoring trivial improvements. Selected interaction effects and covariate-adjusted means of the transformed responses for levels of categorical factors were tested using procedure GLM. Interaction effects with  $P < 0.10$  were retained. Expected means on the untransformed scale were estimated using the "smearing" method (34). Two-group comparisons of means of untransformed variables used the Wilcoxon two-sample test. For multiple comparisons between factor levels, Bonferroni-corrected  $P$  values are reported.

## RESULTS

### Baseline characteristics of the clinical populations

Report of a modest impact by the *APOAV* locus on plasma TG in two normolipidemic samples prompted our study of the effect of this locus in dyslipidemia. Based on the prediction that *APOAV* variation would be associated with dyslipidemia, particularly in the TG and cholesterol compartments of lipoproteins, we screened patients from the Lipid Clinic at UCSF and control subjects (35) for an *APOAV* polymorphism previously associated with elevated TG (21). The clinical characteristics of the four sample populations are described in **Table 1**. In addition to elevated BMI, individuals with combined hyperlipidemia displayed significantly elevated VLDL TG, LDL TG, and HDL TG, and depressed HDL cholesterol. Individuals with hypoalphalipoproteinemia displayed significantly elevated BMI, TC, VLDL cholesterol, VLDL-TG, LDL cholesterol, and LDL-TG, while the HDL-TG compartment was significantly depressed. Individuals with hyperalphalipoproteinemia presented significantly elevated TC, VLDL cholesterol, VLDL-TG, LDL cholesterol, and LDL-TG.

### Frequencies of the SNP

Overall, the genotype frequency for individuals carrying the minor allele (CC homozygotes + CT heterozygotes) at T-1131C was 0.295. Genotype frequencies were

analyzed to assess effects of clinical population, gender, and ethnicity. Chinese ( $n = 85$ ) and Hispanics ( $n = 34$ ) were compared with Europeans ( $n = 443$ ). Because of unacceptably diminished sample size when clinical population, gender, and ethnicity were cross-tabulated, CC+CT was not analyzed with respect to all three factors simultaneously. Instead, separate analyses were carried out with respect to 1) ethnicity and gender and 2) clinical population and gender within the European group.

In the first of these analyses, we found significant differences in minor allele frequencies (CC+CT) among the predominant ethnic groups ( $P < 0.0003$ ) represented in the study sample. No significant gender ( $P = 0.62$ ) or interaction effects ( $P = 0.17$ ) were observed. In multiple comparisons with Europeans, CC+CT was significantly higher for Chinese ( $P = 0.0002$ ). **Table 2** displays CC+CT by ethnic category.

In the analysis of CC+CT versus clinical population and gender within the European group, we found no significant difference in minor allele carrier frequency between the four clinical groups ( $P = 0.85$ ), nor were any significant gender ( $P = 0.93$ ) or interaction ( $P = 0.099$ ) effects evident. Because of the prior hypothesis that genotype frequencies might differ between populations, as well as the nonsignificance of gender effects, CC+CT was reanalyzed with respect to clinical population only ( $P = 0.92$ ). There were no significant multiple comparisons with the control population. **Table 3** displays minor allele carrier frequency versus clinical population within the European group and within the total study population.

Given the weakly significant interaction effect ( $P = 0.099$ ) noted above, the analysis of minor allele carrier frequency among clinical population and gender was examined further for exploratory purposes. Among the three multiple comparisons for specific interactions (each population vs. control by female vs. male), the comparison for individuals with hypoalphalipoproteinemia was significant ( $P = 0.049$ ). Between the two genders, the group with hypoalphalipoproteinemia had the larger minor allele carrier frequency for males, while controls had the larger value for females.

TABLE 1. Clinical characteristics of the study population for lipid analyses

Variable	Control	Combined Hyperlipidemia	Hypoalphalipoproteinemia	Hyperalphalipoproteinemia
Number	191	167	75	194
Female (%)	62	50	44 <sup>a</sup>	48 <sup>a</sup>
Age (years)	51 ± 16	43 ± 18 <sup>a</sup>	44 ± 18	54 ± 15
TC	207 ± 33	<b>317 ± 75</b>	238 ± 72 <sup>a</sup>	285 ± 77 <sup>a</sup>
TG	124 ± 68	<b>339 ± 302</b>	<b>189 ± 118</b>	<b>184 ± 218</b>
VLDL cholesterol	15 ± 15	<b>60 ± 42</b>	31 ± 32 <sup>a</sup>	28 ± 45 <sup>a</sup>
VLDL-TG	66 ± 61	218 ± 104 <sup>a</sup>	130 ± 114 <sup>a</sup>	107 ± 149 <sup>a</sup>
LDL cholesterol	131 ± 29	<b>209 ± 53</b>	166 ± 57 <sup>a</sup>	178 ± 73 <sup>a</sup>
LDL-TG	34 ± 12	82 ± 203 <sup>a</sup>	48 ± 21 <sup>a</sup>	45 ± 26 <sup>a</sup>
HDL cholesterol	60 ± 19	44 ± 13 <sup>a</sup>	<b>36 ± 8</b>	<b>76 ± 20</b>
HDL-TG	18 ± 6	22 ± 9 <sup>a</sup>	15 ± 5 <sup>a</sup>	22 ± 14
BMI	24 ± 4	27 ± 6 <sup>a</sup>	28 ± 6 <sup>a</sup>	25 ± 4

BMI, body mass index; TC, total cholesterol; TG, triglyceride. Values are expressed as mean ± SD, except for Female (%). All lipoprotein parameters are expressed in mg/dl. Boldface values correspond to variables used to define each clinical population.

<sup>a</sup>  $P < 0.05$  (Bonferroni corrected) for comparison of mean or percentage versus normal value.

TABLE 2. Genotype frequencies for carriers of the minor allele within the study population<sup>a</sup>

Ethnicity	CT+CC	N
Chinese	0.471	85
Hispanic	0.235	34
European	0.253	443

<sup>a</sup> Genotype frequency within major ethnic groups.

### Genetic association of lipid parameters with APOAV

To examine the effects of the polymorphism on lipoprotein metabolism, significant changes in mean measures of total TG and cholesterol and total TG and cholesterol within VLDL, LDL, and HDL compartments were evaluated (Table 4). As observed previously (21), total TGs were significantly elevated in carriers of the APOAV minor allele. In addition, evaluation of the TG in the VLDL, LDL, and HDL compartments revealed that presence of the minor allele significantly increased all mean measures.

The T-1131C genotype was found to impact cholesterol levels in a manner that differed from previous report; this observation is likely due to differences in the study populations. Mean cholesterol was significantly elevated in the VLDL compartment ( $P = 0.0007$ ) while apparently not associated with changes in mean LDL in this sample. Carriers of the minor allele displayed a trend toward decreased HDL cholesterol that did not reach significance ( $P = 0.093$ ). The association of the polymorphism with altered lipoprotein profiles was observed in combined hyperlipidemia, hypoalphalipoproteinemia, and hyperalphalipoproteinemia, as well as in controls.

### Linear regression analysis of the effects of APOAV on lipid measures

Regression models for lipid measures included data-driven selection among the polymorphism categories CC, CT, and CT+CC (carriers of the minor allele). The impacts of the potential predictors included age, gender, three categories of dyslipidemia, prior angioplasty, hypertension, BMI, smoking, alcohol use, exercise, prior myocardial infarction, menstruation, and menopause. In the subset comprising the Chinese, Hispanic, and European ethnic groups, ethnicity was also considered; however, age and gender were not considered predictors for the normalized or age-gender-matched lipid measures.

Interaction effects were tested between clinical population and genotype categories, and between clinical popu-

TABLE 3. Genotype frequency within clinical populations, within the European group and overall

Clinical Population	European		Overall	
	CT+CC	n	CT+CC	n
Control	0.240	171	0.330	191
Combined hyperlipidemia	0.274	145	0.323	167
Hypoalphalipoproteinemia	0.259	66	0.240	75
Hyperalphalipoproteinemia	0.242	180	0.258	194

TABLE 4. Plasma concentrations of lipids and BMI in subjects grouped according to APOAV genotype

		TT <sup>b</sup>	CT+CC <sup>b</sup>	P
TG <sup>a</sup>	BMI	25 ± 5 (442)	25 ± 5 (185)	0.35
	Total	198 ± 198 (442)	230 ± 267 (185)	0.012
	VLDL	130 ± 126 (322)	162 ± 135 (127)	0.012
	LDL	48 ± 26 (321)	75 ± 224 (127)	0.003
C <sup>a</sup>	HDL	20 ± 10 (436)	22 ± 12 (181)	0.016
	Total	264 ± 77 (442)	264 ± 84 (185)	0.50
	VLDL	33 ± 36 (322)	47 ± 52 (127)	0.0007
	LDL	175 ± 62 (408)	173 ± 68 (156)	0.43
	HDL	59 ± 24 (436)	55 ± 19 (181)	0.093

<sup>a</sup> Lipid measurements expressed in mg/dl.

<sup>b</sup> Sample size indicated in parentheses.

lation and any indicator of coronary disease (angioplasty, hypertension, or myocardial infarction), the effects of which could be less severe among controls. The models always included clinical population. Models in which a polymorphism effect did not achieve  $P < 0.10$  are not reported.

Among the normalized or age-gender-matched lipid measures, a logarithmic transform was found to be generally appropriate for use in the models; the square-root transform was also examined for normalized LDL cholesterol. No significant models were indicated for TC or LDL cholesterol. In the models that follow, the polymorphism effect CT+CC versus TT was evaluated. For transformed lipid measures, estimated adjusted means on the original scale for CC+CT and TT are presented in Table 5.

For total TG ( $n = 617$ ), the best model selected CC+CT versus TT ( $P = 0.009$ ). Selected effects include clinical population ( $P < 0.0001$ ), hypertension (positive slope,  $P = 0.003$ ), alcohol use (negative slope,  $P = 0.001$ ), exercise (negative slope,  $P = 0.066$ ), and BMI (positive slope,  $P = 0.003$ ). The adjusted  $R^2$  for the model was 0.446.

For VLDL cholesterol ( $n = 448$ ), the best model selected CX versus TT ( $P = 0.0001$ ). Effects include clinical population ( $P < 0.0001$ ), hypertension (positive slope,  $P = 0.096$ ), angioplasty (negative slope,  $P = 0.077$ ), and BMI (positive slope,  $P = 0.001$ ). The adjusted  $R^2$  was 0.477.

For HDL cholesterol ( $n = 607$ ), CC+CT versus TT ( $P = 0.017$ ) was selected in the second best model. Effects include clinical population ( $P < 0.0001$ ), myocardial infarction (negative slope,  $P = 0.016$ ), alcohol use (positive slope,  $P < 0.0001$ ), and BMI (negative slope,  $P < 0.0001$ ).

TABLE 5. Estimated adjusted means for plasma concentrations of normalized/age/gender-matched lipids, grouped according to APOAV genotype

Genotype	TT <sup>a</sup>	CT+CC <sup>a</sup>	P <sup>b</sup>
TG	175	196	0.009
VLDL cholesterol	25	33	0.0001
HDL-CI	43	41	0.017

<sup>a</sup> Lipid measurements expressed in mg/dl.

<sup>b</sup> The  $P$  values are for the corresponding adjusted means on the log scale.

The adjusted  $R^2$  was 0.5597. In the best model for HDL cholesterol, the polymorphism effect selected was CT versus CC and TT combined ( $P = 0.013$ ). Other effects were as above, with slightly smaller  $P$  values. The adjusted  $R^2$  was 0.5601, only slightly higher than for the preceding model.

## DISCUSSION

Previous study of *APOAV* variation revealed a modest influence on TG in normolipidemic individuals, yet the impact in dyslipidemic states was unexplored (21). We hypothesized that a common SNP upstream of *APOAV*, previously associated with elevated plasma TG and VLDL mass in two Caucasian populations (normolipidemic and unselected, respectively), would differ among specific dyslipidemic populations in comparison to an unselected control sample. Whereas an increase –1131C carrier frequency was observed in the combined hyperlipidemia versus control populations, this difference did not reach significance. Of interest is the recent report of increased frequency of the –1131C rare allele in Dutch individuals with familial combined hyperlipidemia (FCHL) (36); however, the low frequency of the minor allele in the Dutch population (0.146) resulted in a more modest enrichment of the minor allele in FCHL individuals (0.165) (36). No evidence of allelic association with either hypoalphalipoproteinemia or hyperalphalipoproteinemia was observed. The somewhat different ethnic compositions of the four clinical populations made it desirable to control for ethnicity in the analysis of minor allele frequencies. As this was impossible due to sample size limitations, that analysis was limited to the European group. In comparison with their countries of origin, populations of US citizens belonging to self-identified ethnic groups will have varying levels of admixture; such samples are ideal for identifying global genetic variations contributing to complex diseases. Because the contribution of *APOAV* to the etiology of these dyslipidemic states may be heterogeneous across populations, the lack of association within European clinical populations is inconclusive and may require investigation in a more homogeneous if not larger population sample (37).

In comparison with the –1131C minor allele frequency established in a Caucasian normolipidemic study population (0.169) (21), we found an elevated –1131C frequency of 0.240. The recruitment of random unselected controls, particularly with respect to lipoprotein profile, would result in the inclusion of dyslipidemic individuals at the expected background population prevalence. Indeed, the control population included four individuals with combined hyperlipidemia, 17 individuals with hypoalphalipoproteinemia, and three individuals with hyperalphalipoproteinemia. Because –1131C contributes to a more atherogenic lipoprotein profile, an unselected control sample population could result in elevated susceptibility allele frequencies compared with a constrained normolipidemic population; however, recalculation of minor allele

frequency in the subgroup of controls without dyslipidemia did not differ substantially (0.238).

A common theme in lipoprotein metabolism and cardiovascular disease risk has been the existence of varying degrees of gender differences in underlying susceptibility alleles of candidate genes (38–40). In the European sample, striking differences in minor allele frequencies were observed between gender and clinical population. Female minor allele frequency was elevated in both combined hyperlipidemia and controls, whereas the opposite was observed in hypoalphalipoproteinemia. Others also observed this gender effect, though the extreme percentile segregation approach utilized makes comparison untenable (21).

In both investigations of the role of *APOAV* in lipoprotein metabolism, the –1131C minor allele was associated with elevated fasting plasma TG. In fact, we found evidence of increased TG content in each lipoprotein compartment measured (Table 4). Of particular interest was the observation of a significant increase in VLDL cholesterol in carriers of the minor allele. This finding in combination with the observation of increased VLDL TG in the same group confirms the report of increased VLDL mass in carriers of the minor allele (21). The observation of depressed HDL cholesterol content in these subjects remains equivocal. Given the elevated plasma TG inherent to combined hyperlipidemia and an unselected control population with respect to primary dyslipidemia, depressed levels of HDL cholesterol could be enriched in carriers of the minor allele secondary to elevated TG (unpublished observations) (41, 42).

Regression models for the normalized age-gender-matched lipid measures among carriers of the minor allele (–1131C) versus homozygous carriers of the major allele (T-1131) were examined in the context of possible effects of ethnicity, the three categories of dyslipidemia, and risk factors for cardiovascular disease. Regression models permitting estimates of adjusted means based on genotype were available for TG, VLDL cholesterol, and HDL cholesterol. Possession of the –1131C minor allele produced, on average, a 10.7% (21 mg/dl) elevation in plasma TG. This is a more modest elevation than the 20–30% previously attributed to possession of the minor allele (21).

Perhaps the most striking effect observed in carriers of the minor allele is 24.2% (8 mg/dl) elevation in VLDL cholesterol. VLDL cholesterol is a component of non-HDL cholesterol, a newly recognized measure of an atherogenic profile with strong predictive promise in a clinical setting (43, 44). The significant elevation of VLDL cholesterol observed due to genetic variation at the *APOAV* locus warrants its investigation as a potential clinical genotype.

The limited number of individuals homozygous for the rare allele made it difficult to distinguish two possible regression models for HDL cholesterol. When all three genotype categories were forced into the model, the same covariates were selected, and the genotype factor had overall  $P = 0.0396$ . For CT versus (CC, TT), the heterozygous state resulted in a 6% (2.5 mg/dl) depression in HDL

cholesterol ( $P = 0.0123$ ), while the model for CT+CC versus TT resulted in a 4.9% (2 mg/dl) decrease ( $P = 0.0167$ ). Though it is not possible to distinguish the “best” model given the data, several examples exist in the literature for which heterozygous states have more detrimental profiles than do their homozygous counterparts (45, 46), not the least of which is a report of a complex haplotype across the *APOAI-CIII-AIV* gene cluster contributing to FCHL (12, 18). To that end, complex haplotype analysis integrating linkage information from all four members of the gene cluster in family-based settings will help to delineate the complex inheritance of this locus to lipoprotein metabolism and cardiovascular risk (47–49).

Preliminary investigations of haplotype structure in the region of *APOAV* and the *APOA-CIII-AIV* gene cluster revealed a complex inheritance of genetic determinants of TG (50, 51). Within *APOAV*, a nonsynonymous change at residue 19 (serine→tryptophan) of apoA-V was independently associated with modestly increased plasma TG in carriers of the minor allele (50). Recent analysis of *APOCIII*, *APOAIV*, and *APOAV* has revealed that T-1131C and S19W have an additive effect on plasma TG (51). Moreover, both polymorphisms are in strong allelic association with another polymorphism in *APOC3* (C-482T) found to independently elevate plasma TG (51). Examination of haplotypes spanning the entire *APOAI-CIII-AIV* gene cluster and *APOAV* within a large study population should help delineate the impact of both individual and compound polymorphisms on TG metabolism.

Exploration of potential ethnic and/or gender differences in the total study sample revealed a striking elevation of minor allele frequency in Chinese individuals. The observation of increased allele frequency among Chinese individuals was observed irrespective of clinical population. This trend was readily observed in Japanese subjects, though small sample size precluded accurate estimates of allele frequency (data not shown). Interestingly, association of T-1131C with elevated plasma TG and lower HDL cholesterol was also reported in a study of Japanese schoolchildren (52). Study of Eastern Asian populations consuming a Western diet revealed an increase of coronary disease and prevalence equivalent to that observed in Caucasian populations (53–57). This suggests that the role of *APOAV* in TG metabolism in these populations has a significant impact on coronary disease and warrants further investigation.

The functional role of the T-1131C polymorphism is not clear. In a recent report, –1131C was found to be in strong linkage disequilibrium with an immediate promoter polymorphism, A-3G (50). While A-3G could be hypothesized to disrupt the Kozak consensus sequence necessary for efficient expression of that allele, formal evaluation of the functional significance of both variations using alternate promoter-driven reporter constructs is required (50).

Compelling evidence from meta-analysis of a number of clinical studies on a large number of patients established an increased level of TG as an independent risk factor for atherosclerotic heart disease (41, 42). The finding of TG-rich

lipoproteins in human atheromata provides substantial pathophysiologic evidence for a direct role in atherogenesis (58). Using the *APOAV* variation(s) for early detection, diagnosis, and treatment of genetically determined cardiovascular disease could have a significant clinical impact. Perhaps pharmacological modulation of the levels of this protein in human patients with high VLDL levels could reduce VLDL and TG levels, potentially elevating HDL cholesterol. Such alterations would ameliorate the atherogenic profile, thus reducing the risk of cardiovascular disease. ■■

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