Associations of Allelic Differences at the A-I/C-III/A-IV Gene Cluster With Carotid Artery Intima-Media Thickness and Plasma Lipid Transport in Hypercholesterolemic-Hypertriglyceridemic Humans

Wolfgang Patsch, A. Richey Sharrett, Iou Y. Chen, Yen-Chiu Lin-Lee, Spencer A. Brown, Antonio M. Gotto, Jr, Eric Boerwinkle

Abstract Individuals with elevated levels of plasma cholesterol and triglyceride may be at higher risk for coronary artery disease than those with isolated elevations of either cholesterol or triglyceride. Sequence variation in the A-I/C-III/A-IV gene cluster has been implicated in the etiology of some disorders associated with premature atherosclerosis and/or hypertriglyceridemias with or without elevations of cholesterol. This led to the hypothesis that allelic variation at this gene locus alters plasma lipid transport and affects susceptibility for atherosclerosis. The study population, from the Atherosclerosis Risk in Communities (ARIC) Study, consisted of 50 normolipidemic individuals, 48 subjects with elevated plasma cholesterol, 47 subjects with elevated plasma triglyceride, and 123 subjects with both elevated plasma cholesterol and triglyceride who were used to evaluate associations between an Xmn I polymorphic site 2.5 kilobase pairs (kbp) upstream of the structural gene for apolipoprotein (apo) A-I, intimal-medial thickening of the extracranial carotid arteries, and several plasma lipid factors. The relative allele frequencies of the 8.3-kbp allele and the 6.6-kbp allele were .86 and .14, respectively, in the entire study population and did not differ among the lipid phenotypes. In the group with elevated plasma cholesterol and triglyceride, subjects possessing the 6.6-kbp allele exhibited a greater carotid artery intimal-medial thickness (P<.034) and higher plasma levels of apoA-I, high-density lipoprotein (HDL) cholesterol, and HDL, cholesterol (P<.02) than subjects homozygous for the 8.3-kbp allele. In contrast, subjects with the 6.6-kbp allele displayed lower mean ratios of apolipoproteins C-II to C-III, C-II to A-IV, and E to A-IV in plasma (P<.05) and a lower mean ratio of apolipoprotein C-II to C-III in the triglyceride-rich lipoproteins (P=.026). Sequence variation in or near the genes encoding apolipoproteins A-I, C-III, and A-IV may therefore identify a group of hypercholesterolemic-hypertriglyceridemic persons who are at higher risk for atherosclerosis than others with the same lipoprotein phenotype.

Key Words • carotid atherosclerosis • hyperlipidemia • apolipoproteins • gene expression

Interest in disorders of plasma lipid transport derives mainly from their association with atherosclerosis and its clinical sequelae. Based on the lipoprotein class(es) elevated in plasma, disorders of lipid transport have been classified into six phenotypes, but each of these phenotypes may result from a number of genetic diseases and environmental factors affecting lipoprotein metabolism. Distinct genetic defects of phenotypically similar diseases can be approached or identified by screening unrelated individuals for common sequence variations in or near candidate genes. Genes that are likely to affect lipoprotein phenotypes include the genes coding for apolipoproteins, lipoprotein receptors, and key enzymes of plasma lipoprotein metabolism.

Received December 3, 1993; revision accepted March 7, 1994.
From the Department of Medicine (W.P., I.Y.C., Y.-C.L.-L., S.A.B., A.M.G.), Baylor College of Medicine, Houston, Tex; the Epidemiology and Biometry Program (A.R.S.), National Heart, Lung, and Blood Institute, Bethesda, Md; and the Genetics Center (E.B.), The University of Texas Health Science Center, Houston.
Correspondence to Wolfgang Patsch, MD, Department of Laboratory Medicine, Landeskrankenanstalten Salzburg, A-6020 Salzburg, Austria.

Several polymorphic sites within a 31.4-kilobase pair (kbp) DNA segment of chromosome 11 that contains the genes encoding apolipoproteins A-I, C-III, and A-IV are associated with hypertriglyceridemia, hypopalphalipoproteinemia, and premature coronary artery disease. A variant Xmn I site located 2.5 kbp upstream of the apolipoprotein (apo) A-I gene can be detected by apoA-I cDNA probes that reveal 8.3- and 6.6-kbp fragments for the major and minor alleles, respectively. In a study of hyperlipidemic patients, the frequency of the minor allele was increased in phenotypes IIb, III, and V. Furthermore, an association of this restriction fragment length polymorphism (RFLP) with familial combined hyperlipidemia (FCH) has been described and was substantiated in family studies. Among the lipid disorders, elevation of both low-density lipoprotein (LDL) cholesterol and plasma triglyceride is a frequent lipid abnormality that greatly enhances the risk for coronary artery disease (CAD). In the Helsinki Heart Study, patients with such a lipoprotein abnormality exhibited a higher incidence of coronary heart disease than patients with isolated elevations of LDL cholesterol or plasma triglyceride.

To ascertain whether sequence variation in or near the A-I/C-III/A-IV gene cluster influences plasma lipid
transport and susceptibility for atherosclerotic disease, we determined genotypes with respect to the variant Xmn I site in selected participants of the Atherosclerosis Risk in Communities (ARIC) Study who were normolipidemic or exhibited elevations of plasma cholesterol, plasma triglyceride, or both. While the frequency of this RFLP was similar among the lipoprotein phenotype types studied, the RFLP showed an association with carotid intima-media thickness in subjects with elevations of plasma cholesterol and triglyceride. Among hypercholesterolemic-hypertriglyceridemic subjects, subtle differences existed in the plasma expression levels of the apoA-I, apoC-III, and apoA-IV genes between subjects homozygous for the major allele and those possessing one or two minor alleles. Thus, structural variation in the A-I/C-III/A-IV gene cluster may modify lipoprotein metabolism, thereby enhancing the risk of CAD in subjects with hypercholesterolemia and concomitant hypertriglyceridemia.

Methods

Study Population

Subjects for this cross-sectional study were selected from the ARIC Study, which combines cardiovascular surveillance of adults aged 35 through 74 years in four US communities with a prospective study of examined cohorts aged 45 through 64 years to represent these communities. The total cohort comprises 15,800 adults, approximately one quarter in each of the four communities: Forsyth County, NC; the city of Jackson, Miss; the northwestern suburbs of Minneapolis, Minn; and Washington County, Md. Each sample except for that of Jackson, which included only black residents, was designed as a probability sample of residents of the entire community. The participation rate (i.e., the proportion of enumerated age eligibles who completed the baseline examination) was 46% in Jackson and approximately 66% in the other three communities. Baseline examinations were conducted from November 1986 through February 1990. Each ARIC participant underwent a baseline examination that included sitting blood pressure, anthropometry, B-mode ultrasound examination of the carotid arteries, fasting blood sampling, and a set of interviews (medical history, medication use, smoking history, and others).

Subjects of the present study were ARIC participants whose lipid values placed them in one of four categories: (1) normolipidemic (LDL cholesterol 160 mg/dL and triglyceride <250 mg/dL); (2) hypercholesterolemic (lipoprotein phenotype IIa; LDL cholesterol 160 mg/dL and triglyceride <250 mg/dL); (3) hypertriglyceridemic (phenotype IV; triglyceride 250 mg/dL but not 600 mg/dL and either LDL cholesterol 160 mg/dL or total cholesterol 250 mg/dL with triglyceride >400 mg/dL); and (4) hypercholesterolemic-hypertriglyceridemic (phenotype IIB and possibly phenotype III; triglyceride 250 mg/dL but not >600 mg/dL and LDL cholesterol 160 mg/dL or total cholesterol 250 mg/dL with triglyceride >400 mg/dL). Exclusion criteria included race other than black or white; data missing on carotid arteries, lipid values placed them in one of four categories: (1) normo-

Clinical and Laboratory Studies

Carotid wall thickness was measured by B-mode ultrasound images as described.20 Measurements were made at three sites prone to atherosclerosis: the distal 10 mm of the common carotid, the bifurcation, and the proximal 10 mm of the internal carotid on both sides of the neck. The current analysis used the mean of far-wall measurements (deep to the skin) over all six sites of the combined thickness of the intima and media. When inadequate imaging prevented measurements at any of the six sites, the mean of the six sites was derived after imputing values from measured to unmeasured sites by multiple regression analysis using observed relations among thicknesses at various sites.21

Sitting blood pressure was measured after a 5-minute rest, three times, using a random-zero sphygmomanometer. The systolic blood pressure value used was the average of the second and third readings. Hypertension was defined by a systolic or diastolic blood pressure >160 mm Hg or >95 mm Hg, respectively, or by current use of antihypertensive medication. Body mass index (BMI; kilograms per meters squared) was calculated from measurements of weight (to the nearest pound) and height (to the nearest centimeter). Smoking status was assessed by interview. Presence of cardiovascular disease was defined as either a history of angiography or intermittent claudication by Rose questionnaire; self-reported physician-diagnosed history of myocardial infarction or stroke or a prevalent Q wave on electrocardiogram; or self-reported history of cardiovascular surgery, angioplasty, or carotid endarterectomy. Subjects were considered diabetic if they identified themselves as such, were using hypoglycemic medications, or had elevated fasting serum glucose (≥140 mg/dL [7.8 mmol/L]). Fasting serum glucose was measured by a hexokinase/glucose-6-phosphate dehydrogenase method.

Venous blood was collected after a 12-hour fast into tubes containing EDTA. Plasma was separated by centrifugation at 4°C. Aliquots of plasma and buffy coats were stored at each field center at -70°C and shipped on dry ice at weekly intervals to the ARIC Central Lipid Laboratory in Houston, where they were stored at -70°C until analysis, usually within 6 weeks.

Plasma cholesterol and triglyceride were measured by enzymatic procedures24,25 using a Cobas-Bio or Cobas-Fara centrifugal analyzer (Roche Diagnostics) and the respective enzymatic kits (catalogue Nos. 236691 and 701912, Boehringer Mannheim Diagnostics). Cholesterol was measured in total HDL, and the HLDL fraction was separated by the polyanion precipitation method as described.26 LDL cholesterol values were calculated according to Friedewald et al.26 ApoA-I and apoB levels in plasma were determined by radioimmunoassays. ApoA-II, apoC-II, apoC-III, and apoE levels in plasma were measured by immunodiffusion using antisemir-containing plates and standards obtained from Dainabot Pure Chemicals Ltd. ApoC-II and apoC-III concentrations were also determined in the HDL-containing supernatant obtained by polyanion precipitation, which allowed calculation of the apo-C-II and apo-C-III associated with very-low-density lipoprotein (VLDL). Apo-A-I was measured by rocket immunoelectroforesis. Apo-A-I antisera were kindly provided by Dr K. Weisgraber, Gladstone Foundation, San Francisco, Calif, and the apo-A-I standard was a gift of Dr A. Weinberg, Bowman Gray School of Medicine, Winston-Salem, NC.
Plasma levels of lipoprotein[a] (Lp[a]) were determined by an enzyme-linked immunoassay and represented the total protein moiety of Lp[a], including apo[a] and apoB, in milligrams per deciliter.

The coefficients of laboratory variation measured using aliquots of internal quality-control pools were 2.5% for cholesterol, 2.7% for triglyceride, 5.2% for LDL cholesterol, 3.7% for HDL cholesterol, 8.2% for HDL cholesterol, and 10.5% for HDL cholesterol and ranged from 8% to 11% for apo-lipoproteins A-I, A-II, A-IV, B, C-III, C-IV, E, and Lp[a].

Genomic DNA was extracted from leukocytes obtained from 10 mL venous blood. DNA (10 µg) was digested with 40 U Asp 700 (Xmn I) (Boehringer Mannheim Co) using the buffer provided by the manufacturer. Southern blotting was performed by standard procedures, and membranes were hybridized with full-length 32P-labeled apoA-I cDNA that was provided by Dr. L. Chan, Baylor College of Medicine, Houston. The apoE genotype was determined by restriction isotyping using the primers described by Emi et al. and Hha I (GIBCO BRL) restriction was determined as suggested by Hixson and Vernier.

Statistical Analyses

Frequencies of the Xmn I alleles were estimated by gene counting. Agreement of Xmn I genotype frequencies with Hardy-Weinberg equilibrium expectations was tested using a x² goodness-of-fit test. Differences in allele frequencies among lipoprotein-phenotype groups were tested by using a contingency x² statistic.

Fasting lipid, lipoprotein, and apolipoprotein levels were adjusted before the analysis for the concomitant effects of sex, age, ethanol consumption, cigarette smoking, and prevalence of diabetes mellitus and hypertension. Adjustments were also made for BMI in view of the mounting evidence suggesting a major gene for BMI. By eliminating possible effects of the putative BMI gene on lipid phenotypes, such an adjustment would amplify the effects of primary lipid genes. Both ANOVA and a more robust nonparametric test, the Kruskal-Wallis test, were used to test the equality of adjusted lipid, lipoprotein, and apolipoprotein levels among different strata. The strata of primary interest were Xmn I genotypes and lipoprotein phenotypes. The relations between plasma lipid, lipoprotein, and apolipoprotein levels and carotid artery wall thickness were summarized using Spearman’s rank correlation. As suggested by Lehmann and Sokal and Rohlf, Fisher’s z transformation was used to test hypotheses concerning these correlations.

Results

No statistically significant associations were found between the lipoprotein phenotype and sex, age, ethanol consumption, and prevalence of hypertension or CAD, though CAD prevalence was somewhat greater in the groups with elevated cholesterol. However, BMI, smoking history, and prevalent diabetes differed among the diagnostic groups (Table 1), with the hypertriglyceridemic groups showing significantly greater mean BMI and prevalence of diabetes. Hypertension prevalence was insignificantly elevated in the hypertriglyceridemic groups.

The lipid and apolipoprotein profiles of the four phenotypes were consistent with the selection criteria (Table 2). Genotype frequencies (Table 3) did not differ among the four diagnostic groups (x² = 3.61, P > .73). Allele frequencies obtained by gene counting were also similar among the four groups (Table 3). The frequency of the common X1 allele (8.3-kbp allele) ranged from .82 in the normolipidemic group to .89 in the hypercholesterolemic group. The genotype frequencies within each diagnostic group were in Hardy-Weinberg equilibrium.

Although the average wall thickness in normolipidemic subjects was lower than in the other groups, differences in carotid far-wall intimal-medial thickness among the four lipid phenotypes were not statistically significant irrespective of whether measurements were adjusted for age, sex, BMI, ethanol consumption, smoking, or prevalence of diabetes or hypertension (unadjusted data, P = .18 by ANOVA and Kruskal-Wallis test; adjusted data, P = .38 by ANOVA and P = .21 by Kruskal-Wallis test). However, the average carotid intimal-medial thickness was significantly greater in the hypercholesterolemic-hypertriglyceridemic group than in normolipidemic subjects in the total ARIC Caucasian cohort (mean [SD] = 0.745 [0.192] mm, n = 9354, P = .004). With or without adjustment for age, sex, BMI, ethanol intake, smoking, or prevalence of diabetes or hypertension, wall thickness differed by genotype in the mixed-hyperlipidemia group but in none of the other lipid groups. Consistent with a dominant effect, mean and median wall thickness was greater in subjects possessing the 6.6-kbp allele compared with subjects homozygous for the 8.3-kbp allele (Table 4). The low frequency of the X2X2 genotype did not permit testing for gene-dosage effects. A marginally significant genotype effect was also found in the entire study group (Table 4). However, after excluding the mixed-hyper-
In the entire sample, no associations were found between \( Xmn \) I genotypes and the factors listed in Tables 1 and 2 (data not shown). In the hypercholesterolemic-hypertriglyceridemic group, a borderline significant association was found between apoE genotype and intimamedia thickness whether stratified by \( Xmn \) I genotype class \((P<.01)\). In the hypercholesterolemic-hypertriglyceridemic group, no significant difference in BMI was observed, but no association between genotype and age, sex, ethanol consumption, smoking, or prevalence of hypertension, diabetes, or CAD were detected \((P=1.958\) by \( \text{ANOVA} \) and \( P=.2141 \) by \( \text{Kruskal-Wallis} \) test). Thus, the borderline significant associations of genotypes with wall thickness in the entire sample reflected the genotype contribution of the mixed-hyperlipidemic group.

The frequencies of the \( e2/e2, e2/e3, e3/e3, e3/e4, e4/e4 \), and \( e2/e4 \) genotypes were \( .01, .20, .52, .15, .04, \) and \( .08 \), respectively, in subjects homozgyous for the common \( Xmn \) I genotype and \( Xmn \) I genotype and apoE genotype with respect to carotid intimamedia thickness was noted. Only plasma concentrations of apoE and apoC-III appeared to be affected by the \( Xmn \) I allele. These frequencies were \( .15, 0, .52, .33, 0, \) and \( 0 \), respectively, in subjects homozygous for the common \( Xmn \) I allele and those heterozygous for the \( 8.3 \)-kb allele \((P<.01)\). Among the apolipoproteins encoded by genes on chromosome 11, the ratios did not differ between subjects homozgyous for the \( 8.3 \)-kb allele \((P<.001)\). To directly compare the expression levels of the other apolipoproteins encoded by the genes located on chromosome 11 near the \( Xmn \) I site, plasma apoC-III and apoA-IV levels were determined by immunoassay. These were significantly elevated in subjects homozygous or heterozygous for the \( 6.6 \)-kb allele. In addition, levels of other apolipoproteins that are encoded by genes located on different chromosomes but that may have complementary or opposing functions to apolipoproteins A-I, C-III, and A-IV were measured. Plasma levels of apoC-II, apoE, and apoA-II did not differ significantly between the two genotype groups \((P<.001)\).

Since plasma levels of these apolipoproteins not only depend on their rate of synthesis and secretion but also are regulated by postsecretory processing and clearance of lipoproteins from the circulation, plasma ratios of apolipoproteins were calculated. A distinct pattern of apolipoprotein ratios emerged from these calculations. Among the apolipoproteins encoded by genes on chromosome 11, the ratios did not differ between subjects homozgyous for the \( 8.3 \)-kb allele and those heterozygous or homozgyous for the uncommon allele at the \( Xmn \) I restriction site. Likewise, ratios of apolipoprotein levels in the mixed-hyperlipidemic group revealed significantly higher plasma concentrations of HDL cholesterol, HDL\(_3\) cholesterol, and apoA-I in subjects possessing the \( 6.6 \)-kb allele than in those homozgyous for the \( 8.3 \)-kb allele \((P<.001)\). To directly compare the expression levels of the other apolipoproteins encoded by the genes located on chromosome 11 near the \( Xmn \) I site, plasma apoC-III and apoA-IV levels were determined by immunoassay. These were significantly elevated in subjects homozygous or heterozygous for the \( 6.6 \)-kb allele. In addition, levels of other apolipoproteins that are encoded by genes located on different chromosomes but that may have complementary or opposing functions to apolipoproteins A-I, C-III, and A-IV were measured. Plasma levels of apoC-II, apoE, and apoA-II did not differ significantly between the two genotype groups \((P<.001)\).

### Table 2. Plasma Lipid, Lipoprotein, and Apolipoprotein Levels by Lipoprotein Phenotype

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normolipidemic</th>
<th>Hypercholesterolemic</th>
<th>Hypercholesterolemic-hypertriglyceridemic</th>
<th>HTG</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC</td>
<td>196±27</td>
<td>262±23</td>
<td>290±32</td>
<td>222+26</td>
</tr>
<tr>
<td>TG</td>
<td>105±48</td>
<td>132±47</td>
<td>326±74</td>
<td>327±67</td>
</tr>
<tr>
<td>LDL-C</td>
<td>121±23</td>
<td>186±21</td>
<td>191±28</td>
<td>123±22</td>
</tr>
<tr>
<td>HDL-C</td>
<td>53.9±17.8</td>
<td>49.2±13.9</td>
<td>38.4±8.4</td>
<td>36.4±10.5</td>
</tr>
<tr>
<td>HDL(_2)-C</td>
<td>14.6±9.1</td>
<td>11.7±7.5</td>
<td>8.8±5.5</td>
<td>9.6±4.8</td>
</tr>
<tr>
<td>HDL(_3)-C</td>
<td>39.3±10.8</td>
<td>37.5±9.5</td>
<td>29.6±8.2</td>
<td>26.8±10.1</td>
</tr>
<tr>
<td>ApoA-I</td>
<td>133±34</td>
<td>128±26</td>
<td>118±24</td>
<td>111±28</td>
</tr>
<tr>
<td>ApoB</td>
<td>87±23</td>
<td>117±28</td>
<td>138±34</td>
<td>104±24</td>
</tr>
<tr>
<td>Lipoprotein[a]</td>
<td>7.5±7.3</td>
<td>15.5±13.6</td>
<td>6.8±7.4</td>
<td>5.5±7.0</td>
</tr>
</tbody>
</table>

Hypercholesterolemic; HTG, hypertriglyceridemic; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; and Apo, apolipoprotein.

Values are mean±SD and are expressed in milligrams per deciliter.

*Probability that mean values are the same in the four lipid groups.

### Table 3. Genotype Distribution and Relative Allele Frequencies of the RFLP Detected With Enzyme \( Xmn \) I

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Genotype Distribution, n (Frequency)</th>
<th>Relative Allele Frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( X1X1 )</td>
<td>( X1X2 )</td>
</tr>
<tr>
<td>Normolipidemic</td>
<td>34 (.68)</td>
<td>14 (.28)</td>
</tr>
<tr>
<td>Hypercholesterolemic</td>
<td>38 (.79)</td>
<td>9 (.19)</td>
</tr>
<tr>
<td>Hypercholesterolemic-hypertriglyceridemic</td>
<td>93 (.76)</td>
<td>27 (.22)</td>
</tr>
<tr>
<td>HTG</td>
<td>34 (.72)</td>
<td>13 (.28)</td>
</tr>
</tbody>
</table>

RFLP indicates restriction fragment length polymorphism; \( X1 \), 8.3-kilobase pair (kbp) allele; \( X2 \), 6.6-kilobase allele; hypercholesterolomic; and HTG, hypertriglyceridemic.
Our selection criteria for the hypercholesterolemic-hypertriglyceridemic group included lipoprotein phenotype IIb and possibly III. To determine a possible contribution of type III hyperlipoproteinemia to the Xmn I polymorphism associations observed in the group with mixed hyperlipidemia, we used the apoE genotype to identify subjects likely to have type III rather than type IIb. The techniques of ultracentrifugation and/or lipoprotein electrophoresis commonly used for diagnosis of type III hyperlipoproteinemia were not feasible because only frozen plasma specimens were available. Four subjects homozygous for the e2 allele were identified among the subjects with genotype X1X2 or X2X2, whereas only one subject was homozygous for the e2 allele among the subjects with genotype X1X1. Notwithstanding this difference in the constituency of the A-I/C-III/A-IV genotype classes by subjects likely to have type III hyperlipoproteinemia, omission of the five e2/2 cases from the analyses did not substantially change the earlier observations. Mean carotid intimal-medial thickness remained significantly greater in subjects with genotype X1X2 or X2X2 than in those with genotype X1X1 (P = .010), and the mean ratio of apoC-II to apoC-III in VLDL remained higher in the X1X1 group than in the X1X2 plus X2X2 group (P = .029).

### Table 4. Carotid Far-Wall Intimal-Medial Thickness by Lipoprotein Phenotype and Xmn I Genotype

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n Normolipidemic</th>
<th>n Hypercho</th>
<th>n Hypercho/HTG</th>
<th>n HTG</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>X1X1</td>
<td>34 0.736±0.153</td>
<td>38 0.779±0.153</td>
<td>93 0.771±0.178</td>
<td>34 0.747±0.134</td>
<td>199 0.762±0.162</td>
</tr>
<tr>
<td>(0.733±0.142)</td>
<td>(0.785±0.136)</td>
<td>(0.764±0.172)</td>
<td>(0.759±0.114)</td>
<td>(0.761±0.151)</td>
<td></td>
</tr>
<tr>
<td>X1X1/X2X2</td>
<td>16 0.730±0.177</td>
<td>10 0.790±0.137</td>
<td>30 0.872±0.238</td>
<td>13 0.776±0.229</td>
<td>69 0.810±0.214</td>
</tr>
<tr>
<td>(0.762±0.127)</td>
<td>(0.819±0.148)</td>
<td>(0.847±0.216)</td>
<td>(0.792±0.202)</td>
<td>(0.813±0.186)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>50 0.735±0.163</td>
<td>48 0.781±0.148</td>
<td>123 0.796±0.198</td>
<td>47 0.755±0.163</td>
<td>266 0.775±0.178</td>
</tr>
<tr>
<td>(0.742±0.137)</td>
<td>(0.792±0.136)</td>
<td>(0.784±0.186)</td>
<td>(0.768±0.142)</td>
<td>(0.775±0.162)</td>
<td></td>
</tr>
</tbody>
</table>

Hypercholesteremic (Hypercho) and hypertension. Values are mean±SD and are expressed in millimeters.

Probabilities that mean or median carotid wall thickness is the same among genotype groups: *P = .015 (ANOVA) and P = .037 (Kruskal-Wallis test); †P = .034 (ANOVA) and P = .086 (Kruskal-Wallis test); ‡P = .100 (t test) and P = .138 (Kruskal-Wallis test); §P = .041 (t test) and P = .089 (Kruskal-Wallis test).

### Table 5. Characteristics of Hypercholesterolemic-Hypertriglyceridemic Subjects by Genotype

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>X1X1</th>
<th>X1X2/X2X2</th>
<th>Total</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>59</td>
<td>30</td>
<td>123</td>
<td>NS</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>55/38</td>
<td>18/12</td>
<td>73/50</td>
<td>NS</td>
</tr>
<tr>
<td>Age, y</td>
<td>54.1±5.5</td>
<td>55.3±4.5</td>
<td>54.4±5.3</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>27.9±4.1</td>
<td>29.7±4.5</td>
<td>28.4±4.2</td>
<td>.045</td>
</tr>
<tr>
<td>Ethanol Intake, g/wk</td>
<td>42.4±67.0</td>
<td>30.6±72.0</td>
<td>39.5±68.1</td>
<td>NS</td>
</tr>
<tr>
<td>Cigarette use, cigarette-y</td>
<td>432±446</td>
<td>486±446</td>
<td>445±445</td>
<td>NS</td>
</tr>
<tr>
<td>Hypertension†</td>
<td>0.120</td>
<td>0.267</td>
<td>0.156</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes†</td>
<td>0.151</td>
<td>0.233</td>
<td>0.171</td>
<td>NS</td>
</tr>
<tr>
<td>CAD†</td>
<td>0.159</td>
<td>0.100</td>
<td>0.144</td>
<td>NS</td>
</tr>
</tbody>
</table>

X1 indicates 8.3-kilobase pair (kbp) allele; X2, 6.6-kbp allele; BMI, body mass index; and CAD, coronary artery disease. Values are mean±SD where appropriate.

*Probability that mean values are the same in the two genotype groups; P > .05, not significant (NS). †Prevalent disease expressed as a proportion.

Proteins encoded by genes located on chromosomes 1 (apoA-II), 2 (apoB), and 19 (apoC-II and apoE) were similar between the two genotype groups studied (data not shown). However, significant differences existed in plasma ratios of apolipoproteins encoded by genes on chromosome 19 to apolipoproteins encoded by genes on chromosome 11. The mean ratios of apoA-II to apoC-III, apoC-II to apoA-IV, and apoE—with or without adjustments for the known effects of the apoE genotype on apoE plasma levels—to apoA-IV were significantly lower in subjects with one or two uncommon alleles (Table 7).

Since apolipoproteins C-II and C-III regulate the catabolism of triglyceride-rich lipoproteins, we determined the ratio of apoA-II to apoC-III in VLDL. As in plasma, this ratio was substantially lower in subjects possessing the 6.6-kbp allele irrespective of whether adjustment was made for confounding variables (Table 8). The ratio of apoC-III to apoE in VLDL, thought to be of importance for the removal of remnants of triglyceride-rich lipoproteins from the circulation, could not be measured in triglyceride-rich lipoproteins because HDL particles that contain apoE are coprecipitated with VLDL by the reagents used for separation of HDL and apoB-containing lipoproteins.46
TABLE 6. Adjusted Plasma Lipid, Lipoprotein, and Apolipoprotein Levels by Genotype

<table>
<thead>
<tr>
<th>Variable</th>
<th>n</th>
<th>TC</th>
<th>TG</th>
<th>LDL-C</th>
<th>HDL-C</th>
<th>HDLj-C</th>
<th>HDL3-C</th>
<th>ApoA-I</th>
<th>ApoA-II</th>
<th>ApoA-IV</th>
<th>ApoB</th>
<th>ApoC-II</th>
<th>ApoC-III†</th>
<th>ApoC-III‡</th>
<th>ApoE</th>
<th>Lipoprotein[a]</th>
</tr>
</thead>
<tbody>
<tr>
<td>X1X1</td>
<td>93</td>
<td>287±28</td>
<td>322±66</td>
<td>189±27</td>
<td>38±9</td>
<td>9±5</td>
<td>29±9</td>
<td>116±23</td>
<td>29.4±9</td>
<td>13.9±4.3</td>
<td>136±32</td>
<td>4.34±1.46</td>
<td>17.3±5.1</td>
<td>5.87±2.10</td>
<td>7.15±7.90</td>
<td></td>
</tr>
<tr>
<td>X1X2/X2X2</td>
<td>30</td>
<td>296±36</td>
<td>328±66</td>
<td>192±28</td>
<td>43±8</td>
<td>10±8</td>
<td>33±5</td>
<td>129±24</td>
<td>31.8±6.4</td>
<td>15±1.3</td>
<td>136±38</td>
<td>4.25±1.35</td>
<td>18.9±4.9</td>
<td>5.67±1.53</td>
<td>6.23±6.14</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>123</td>
<td>289±31</td>
<td>324±79</td>
<td>190±27</td>
<td>39±9</td>
<td>9±6</td>
<td>30±8</td>
<td>119±24</td>
<td>30.1±8.9</td>
<td>14.2±4.2</td>
<td>136±38</td>
<td>4.25±1.35</td>
<td>18.9±4.9</td>
<td>5.67±1.53</td>
<td>6.91±7.48</td>
<td></td>
</tr>
</tbody>
</table>


Pt | .181 | .277 | .407 | .008 | .477 | .016 | .005 | .005 | .005 | .005 | .145 | .145 | .741 | .153 | .648 | .750 |

X1 indicates 8.3-kilobase pair (kbp) allele; X2, 6.6-kbp allele; TC, total cholesterol; TG, triglyceride; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; and Apo, apolipoprotein. Values are adjusted for age, sex, body mass index, ethanol consumption, smoking history, and prevalence of hypertension and diabetes. Values are mean±SD and are expressed as milligram per deciliter where appropriate.

**Discussion**

This study suggested that sequence variations in the A-I/C-III/A-IV gene cluster on chromosome 11 influence atherogenesis in subjects with type IIb hyperlipoproteinemia. This conclusion is based on an association of the minor allele of the Xmn I polymorphism 2.5 kbp upstream of the structural gene for apoA-I with carotid intimal-medial thickness. It is supported by an association of the minor allele with distinct changes in lipid transport, ie, differences in the plasma ratios of apoC-II to apoC-III, apoC-II to apoA-IV, and apoE to apoA-IV as well as in the ratio of apoC-II to apoC-III in VLDL. This relation of allelic variation at the A-I/C-III/A-IV gene cluster with differences in expression levels of the apolipoproteins encoded by genes at this locus relative to levels of apolipoproteins encoded by genes located on chromosome 19 is plausible from both biochemical and pathophysiological viewpoints. That plasma levels of apoA-I were higher in subjects with the minor allele provides some direct evidence that altered expression of the apolipoprotein genes clustered on chromosome 11 is associated with the allelic variation identified by the variant Xmn I site 5’ to this locus.

Associations of genetic markers with disease susceptibility warrant cautious interpretation, in particular because DNA markers are usually not identical with the DNA sequences that affect expression or function of a protein but depend on the phenomenon of linkage disequilibrium. Since linkage disequilibria may vary among races and environmental and other genetic factors may affect the expression of a disorder (in this case,

TABLE 7. Adjusted Plasma Apolipoprotein Ratios in Hypercholesterolemic-Hypertriglyceridemic Subjects by Genotype

<table>
<thead>
<tr>
<th>Apolipoproteins</th>
<th>X1X1</th>
<th>X1X2/X2X2</th>
<th>Total</th>
<th>P*</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoC-II/ apoC-III</td>
<td>0.263±0.095</td>
<td>0.230±0.059</td>
<td>0.255±0.088</td>
<td>.027</td>
<td>.141</td>
</tr>
<tr>
<td>ApoC-II/ apoA-IV</td>
<td>0.344±0.166</td>
<td>0.290±0.091</td>
<td>0.330±0.152</td>
<td>.026</td>
<td>.104</td>
</tr>
<tr>
<td>ApoE/ apoC-III‡</td>
<td>0.358±0.134</td>
<td>0.315±0.109</td>
<td>0.348±0.129</td>
<td>.132</td>
<td>.063</td>
</tr>
<tr>
<td>ApoE/ apoA-IV</td>
<td>0.481±0.265</td>
<td>0.372±0.151</td>
<td>0.454±0.246</td>
<td>.009</td>
<td>.048</td>
</tr>
</tbody>
</table>

X1 indicates 8.3-kilobase pair (kbp) allele; X2, 6.6-kbp allele; and Apo, apolipoprotein. Apolipoprotein levels were corrected for age, sex, body mass index, ethanol consumption, cigarette smoking, and disease status. Values are mean±SD.

*Probability that mean values are the same in the two genotype groups.
†Probability that median values are the same in the two genotype groups.
‡ApoE and apoC-III plasma levels were corrected for apoC genotype.
lipoprotein phenotype and/or carotid artery wall thickness), stringent criteria for selecting study subjects and rigorous statistical tests to control for confounding variables must be applied. Because of the higher frequencies of the minor allele of the Xmn I RFLP in black subjects was quite similar to that in white control populations in Boston, London, Austria, France, and the Mediterranean. Furthermore, our design excluded use of drugs or hormones that could affect lipoprotein levels and carotid intima-media thickness. Other factors (e.g., age, sex, BMI, smoking history, hypertension, or diabetes) that might affect lipoprotein phenotype or arterial wall thickness showed only limited associations with carotid wall thickness, lipid transport was observed in this study. If the association between sequence variation at the A-I/C-III/A-IV gene cluster and atherosclerosis reflects causality, the increased intimal-medial thickness of carotid arteries would be expected to result from anomalies in lipid transport that are caused by functional defects or changes in the expression of apolipoproteins encoded by the genes on chromosome 11. ApoA-I, the major structural protein of HDL, activates lecithin:cholesterol acyltransferase and may mediate the uptake of HDL by hepatocytes. ApoC-III inhibits the activity of lipoprotein lipase and decreases apoE-mediated remnant removal and may thus reduce the catabolism of triglyceride-rich lipoproteins. Indeed, the catabolism of triglyceride-rich lipoproteins is accelerated in patients lacking apoC-III due to an inversion of the apoA-I and apoC-III genes, and overexpression of apoC-III in transgenic mice is associated with hypertriglyceridemia. While apoA-IV, like apoA-I, may activate lecithin:cholesterol acyltransferase and mediate uptake of HDL, its principal function is not known. A role of apoA-IV in the metabolism of triglyceride-rich lipoproteins is supported by animal studies showing a correlation between apoA-IV expression and triglyceride synthesis. Even though the function of apoA-IV in triglyceride metabolism is not fully understood, apoA-IV may, in contrast to apoC-III, enhance the catabolism of triglyceride-rich lipoproteins by facilitating the transfer of apoC-II to triglyceride-rich lipoproteins.

A number of mechanisms that would be consistent with the known or suspected functions of apoC-III and apoA-IV could underlie the associations found in the present study between sequence variations at the DNA level and altered apolipoprotein ratios and increased carotid wall thickness. A striking finding in subjects with the minor allele was their markedly reduced apoC-II to apoC-III ratio in VLDL, a characteristic that would diminish hydrolysis of their triglycerides by lipoprotein lipase. Overexpression of apoC-III or a structural defect
in this protein that would make it less amenable for transfer to HDL may cause an alteration in the ratio of apoC-II to apoC-III in VLDL. However, the ratio of plasma apoC-II to apoA-IV was also reduced as was the ratio of apoC-II in VLDL to apoA-IV in plasma (not shown; P = 0.06). Since apoA-IV facilitates acquisition of apoC-II by triglyceride-rich lipoproteins, the altered apoC-II to apoC-III ratio may only reflect a defect in apoA-IV function. Since the apoE/apoC-III ratio, thought to be critical for receptor-mediated removal of triglyceride-rich lipoproteins, tended to be lower in subjects with the minor allele, a larger fraction of VLDL may have been converted into LDL, and this could account for the increased plasma concentrations of both VLDL and LDL.

A higher mean apoA-I level among subjects with the minor allele was associated with higher levels of HDL cholesterol, particularly HDL₃ cholesterol. Since increases in HDL₃ cholesterol are likely the result of increased apolipoprotein synthesis, whereas increased HDL₂ levels are more likely the result of reduced apolipoprotein catabolism, this HDL subclass distribution is consistent with enhanced expression of apoA-I. Given the similarity between subjects with or without the minor allele in ratios for apolipoproteins encoded by genes on chromosome 11, it is possible that the apoA-I, apoC-III, and apoA-IV genes are all expressed at higher levels in subjects with the minor allele. In animal models, the expression of the apoA-I, apoC-III, and apoA-IV genes is usually not coordinated in response to hormonal and metabolic stimuli, but it is possible that transcription of the entire gene cluster is stimulated by a mutation in a cis-regulatory element.

That apoA-I and HDL cholesterol levels and carotid intimal-medial thickness were increased in subjects possessing the minor allele was an unexpected finding. However, in FCH subjects the minor Xmn I allele is also associated with higher plasma apoA-I levels. It therefore seems possible that elevations of apoA-I and/or HDL cholesterol per se are not always protective. Indeed, some hypotheses propose a primary role of triglyceride-rich lipoproteins or their remnants in atherogenesis. In subjects with the minor Xmn I allele, the altered apolipoprotein composition of triglyceride-rich lipoproteins could have increased their atherogenic potential, thereby abrogating the beneficial effect of higher HDL levels.

Even though plausible from a mechanistic view, the apolipoprotein changes associated with the RFLP in the hypercholesterolemic-hypertriglyceridemic group may not be involved in the elevation of plasma cholesterol and triglyceride. Rather, changes in function and/or expression of apoA-I, apoC-III, or apoA-IV may affect a metabolism of triglyceride-rich lipoproteins that is ineffective due to other causes and may thereby increase their atherogenicity. Our studies do not permit resolution of this question, but they may provide a framework for clinical and biochemical studies to understand the molecular mechanisms underlying the associations reported.

Acknowledgments

This work was supported under contracts (N01-HC55015, N01-HC55016, N01-HC55018, N01-HC55019, N01-HC55020, N01-HC55021, and N01-HC55022) with the National Heart, Lung, and Blood Institute and by grant HL-27341 from the National Institutes of Health. Dr Boerwinkle is an Established Investigator of the American Heart Association. We thank Suzanne Simpson for the expert editorial assistance and Kim Lawson for assistance in statistical analyses. We are indebted to Jeannette Bensen, Catherine Paton, Amy Haire, and Delilah Posey (Forsyth County Field Center); to Bobbie J. Alliston, Faye A. Blackburn, Catherine W. Britt, and Barbara L. Davis (Jackson Field Center); to John O’Brien, Linda Goldman, Barbara Kuehl, and Anne Murrill (Minneapolis Field Center); to Carol Christman, Sonny Harrel, Lowell Hill, and Joan Nelling (Washington County Field Center); to Valerie Stinson, Pam Pfle, Hoang Pham, and Teri Trevino (Central Hemostasis Laboratory); and to Charles E. Rhodes, Doris Epps, Selma Sosy, and Maria L. Messi (Central Lipid Laboratory).

References


and presence in intron-7 of a 40-million-year-old Alu sequence. 


Associations of allelic differences at the A-I/C-III/A-IV gene cluster with carotid artery intima-media thickness and plasma lipid transport in hypercholesterolemic-hypertriglyceridemic humans.


doi: 10.1161/01.ATV.14.6.874

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/14/6/874