Apolipoprotein Polymorphisms Fail to Define Risk of Coronary Artery Disease

Results of a Prospective, Angiographically Controlled Study

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Background Because genetic factors are believed to contribute to the etiology of coronary artery disease (CAD), it has been suggested that DNA polymorphisms at candidate loci might identify individuals at high risk for developing disease. In this regard, apolipoprotein genes represent extremely promising loci because levels of apolipoproteins and their associated lipoproteins represent a major risk factor for CAD, and rare dysfunctional mutations in these genes result in a significant risk for CAD. To date, although some reports indicate that DNA polymorphisms at these loci are associated with increased risk of CAD, other reports have failed to find such associations.

Methods and Results To resolve the question of whether genetic polymorphisms at apolipoprotein loci can be used to identify individuals at increased risk for CAD, we evaluated the distribution of apolipoprotein genetic polymorphisms in a large series of subjects (n=848) undergoing coronary angiography. Blinded assessment of angiograms was used to discriminate between patients with CAD (≥60% stenosis of any major branch, n=444) and control subjects without disease (≤10% stenosis, n=404). A total of 12 polymorphisms were evaluated at the following loci: apolipoprotein (apo) A-I/C-III/A-IV (five restriction site polymorphisms—Msp I, Pst I, Sst I, Pvul I, Pvu II), apo B (three restriction site polymorphisms—Xba I, EcoRI, Msp I, plus an insertion/deletion polymorphism), apo A-I (Msp I polymorphism), apo C-II (Taq I polymorphism), and apo E (protein isoforms revealed by DNA analysis). All subjects were of Northern European (primarily Anglo-Saxon) descent, and, within each sex, patients and control subjects were of comparable age. All 12 loci were in Hardy-Weinberg equilibrium, with no indication of population heterogeneity. As expected, patients were distinguished from control subjects by their lipid profiles and a higher frequency of known risk factors for CAD. However, analysis by log-linear models indicated that there were no significant associations between apolipoprotein polymorphisms and the risk of CAD (P=0.10 to 0.90). The lack of association was maintained irrespective of whether the analysis was carried out for the entire sample or the contrast was made more stringent by comparing patients most likely to have a genetic component to their disease (ie, young patients with early-onset CAD) with the control subjects least likely to have genetic susceptibility (ie, older control subjects who had ample time to develop CAD).

Conclusions Despite the fundamental role of apolipoprotein genes in lipid metabolism, we find no evidence that common genetic polymorphisms of the major apolipoprotein loci have a significant influence on the risk of developing angiographically defined CAD in this representative population. Therefore, at this time we find no support for the hypothesis that mass screening for genetic polymorphisms at candidate loci can reduce the burden of CAD by identifying a substantial proportion of high-risk individuals. Instead, it appears more appropriate to direct attention toward modifying high-risk behaviors to alleviate the consequences of traditional environmental risk factors. (Circulation. 1994;89:567-577.)

Key Words • apolipoproteins • genetics • coronary artery disease • atherosclerosis

There is ample evidence that genetic factors influence the risk for coronary artery disease (CAD), the leading cause of death for both men and women in the United States. In part, this conclusion stems from the fact that a positive family history represents an important risk factor for CAD and genetic factors are known to influence the distribution of lipids and lipoprotein levels, themselves known to be major risk factors for CAD. Although many genes are likely to influence the development of atherosclerosis, because lipid profiles play such a major role in defining CAD risk, it has been suggested that the genes involved in lipid metabolism are an especially important set of “candidate genes” for atherosclerosis. Accordingly, it has been proposed that if polymorphic alleles predisposing to disease can be identified, screening for the presence of the alleles may identify a substantial proportion of high-risk individuals. The appropriate monitoring of these individuals, in conjunction with targeted intervention, could then delay, or avert, the onset of CAD. If this supposition were correct, it would argue in support of adopting the “high-risk” strategy to alleviate the burden of CAD rather than the “population-based” strategy frequently advocated by epidemiologists.

Although many lipid genes have been identified, mapped, and, in many cases, cloned, the genes encoding for the major apolipoproteins represent key candidates for CAD. By maintaining the structural integrity of lipoproteins, acting as cofactors for lipid-processing
enzymes, and serving as ligands for lipoprotein receptors, apolipoproteins play a central and pervasive role in lipid metabolism. Equally important, the two lipoprotein functions that are most predictive of CAD risk—low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C)—are each associated with a major apolipoprotein species: apolipoprotein (apo) A-I with HDL-C and apo B with LDL-C. Thus, the significant elevation of LDL-C levels in patients with CAD compared with control subjects is reflected by an equally significant elevation of apo B levels, whereas the risk of CAD associated with decreased levels of HDL-C is reflected by decreased levels of apo A-I.5,11

Although a number of dysfunctional mutations, resulting in extreme lipoprotein phenotypes and substantially increased risk of CAD, have been identified in various candidate genes, they tend to be rare and account for only a small proportion of individuals with CAD. Of these, even the most common and best understood—mutations at the LDL receptor (LDLR) locus—account for no more than 5% of CAD patients. Because dysfunctional mutations of the apolipoprotein genes tend to be equally rare, less than 10% of CAD patients are likely to have their disease caused by a specific dysfunctional mutation with major effect. However, more subtle defects in candidate genes, resulting in an increased risk for CAD, could occur more frequently in the population and thus account for an appreciable proportion of CAD patients.

A number of initial studies have attempted to address the possibility that polymorphisms at various candidate loci might be associated with increased risk of CAD with variable results. Some studies concluded there was a significant association between hyperlipidemia or clinical CAD and a DNA polymorphism, whereas others found no association. As early as 1983, DNA polymorphisms were described for the apo A-I region,12 laying the foundation for the observation that 32% of 88 patients with premature CAD possessed a Pst I restriction site compared with only 4% of 123 random Massachusetts control subjects (P<.001).7 Accordingly, it was suggested that polymorphisms in the apo A-I/C-III/A-IV genomic region might be useful markers for detecting individuals at high risk for premature CAD and familial hypercholesterolemia.7 Other studies also reported associations between risk of CAD and DNA polymorphisms in this genomic region.13,14 However, a follow-up study of the original patient population failed to find an association between eight restriction fragment length polymorphisms (RFLPs) in the A-I/C-III/A-IV region and CAD in 347 subjects. This follow-up study also reviewed other reported data and, based on the lack of consistent associations, concluded that disease associations with RFLPs in this region were unlikely.15

The apo B locus has been the focus of similar studies after an initial finding from Boston of a high frequency of an allele of the Xba I polymorphism in postinfarction patients compared with control subjects.16 Subsequent studies in Austria and London also found a modest association between disease risk and apo B polymorphisms.17,18 However, although a recent study in Boston found small differences in the allele frequency of two apo B RFLPs in 233 patients and control subjects,19 they concluded that these single-site polymorphisms would impart little useful information about disease risk. Those investigators also concluded that the published data tended to be inconsistent, although the EcoRI polymorphism gave positive results in four of five studies.

Besides these two apolipoprotein genomic regions, RFLPs of the apo E/C-1/C-2 have also been examined for disease association. Although most studies were negative, one study reported a marked association between an Hpa I polymorphism and type III hyperlipidemia.20 While this finding could be attributed to linkage disequilibrium between the Hpa I polymorphism and the apo E-2 isoform, this association and its clinical significance remain to be confirmed.

Thus, although an attractive hypothesis, the inconsistent results from different studies indicate that the association of specific apolipoprotein genetic polymorphisms with CAD remains to be established. Because the inconsistent results observed among previous studies may be the result of small sample size, differences in ethnic background, differences in disease definition (clinical versus angiographic), and chance associations among multiple comparisons,21 an adequately powered, prospective, controlled trial is required. This investigation was designed as a prospective, case-control study in a large, homogeneous sample of patients undergoing coronary angiography, with the objective of having sufficient statistical power to resolve the question of whether there is an association between common apolipoprotein genetic polymorphisms and angiographically documented CAD.

Methods

Subject Selection
A total of 909 subjects were recruited from individuals undergoing selective coronary arteriograms at LDS Hospital (Salt Lake City, Utah). To satisfy our definition of early-onset disease, men had to be between 30 and 64 years old, and women had to be between 35 and 69 years old. The study was restricted to Caucasians residing in Utah, southeastern Idaho, and southwestern Wyoming, a region where 90% of the population are Caucasians of Northern European descent and are genetically representative of the general US Caucasian population.22 To obtain valid lipid profiles, we excluded subjects with unstable angina along with subjects who had experienced a myocardial infarction within the past 6 weeks. The first 791 subjects enrolled in the study were consecutive subjects presenting for angiogram who met the entry criteria. However, because this ascertainment strategy resulted in insufficient control subjects, we retrospectively contacted an additional 118 subjects reported to have had a normal coronary angiogram within the past 2 years and who otherwise met the study criteria. After consenting to participate in the study and following an overnight fast, subjects visited the clinic where they were examined and had blood drawn for lipid and genetic analysis. Data collected included height and weight (used to calculate the body mass index [BMI]—weight in kilograms divided by the square of height in meters), systolic and diastolic blood pressures, and a complete history obtained by a standardized questionnaire. History taking included questions about known CAD risk factors (family history of CAD, smoking habits, alcohol consumption, exercise habits), medical history (hypertension, diabetes, cardiac history), and current medication use.

Coronary Angiography
Coronary angiography was conducted at LDS Hospital using Siemens single-plane radiographic equipment. All angiograms
were evaluated by one of three cardiologists (J.L.A., L.A.K., H.W.M.) using the same format and reporting forms as those described in the CASS study. The angiographic evaluation was blinded so the cardiologist had no knowledge of the clinical history or clinically reported findings in subjects. Coronary stenosis was defined as the linear percentage of smooth walled narrowing of the coronary artery compared with the diameter of the nearest normal segment. Patients were classified into the following three categories: (1) >60% stenosis of at least one major vessel, advanced CAD (patients); (2) <10% stenosis—no disease (control subjects); and (3) between 10% and 60% stenosis—minor CAD (indeterminate patients who were excluded from further consideration). The results of the CASS form were then compared with the patient’s disease status. If discrepancies were noted, a second cardiologist reviewed the films in blinded fashion, and the discrepancy was resolved before classifying a patient’s disease status.

**Lipid Analysis**

Plasma was separated from the fasting blood samples according to LRC guidelines, and lipid profiles were determined in the Cardiovascular Genetics Lipid Research Laboratory. This laboratory participates in the cholesterold standardization program of the Centers for Disease Control and Prevention and also shares samples and standards with several other laboratories, including the Northwest Lipid Research Clinic in Seattle, Wash. All laboratory personnel were blinded as to the patient’s status (patient versus control subject). Cholesterol and triglyceride levels in total plasma and subfractions were measured with a Baker Encore II automated analyzer. Lipoprotein fractions were measured by a microscale procedure. HDL being measured in the supernatant after precipitation of apo B-containing lipoproteins with dextran sulfate/MgCl2, and lipoprotein subclassification in a microfuge, whereas a Beckman TL 100 ultracentrifuge was used to separate very low-density lipoprotein (VLDL) from LDL and HDL. Apo A-I and apo B were measured by radioimmunoassay using Ventrex kits (Ventrex Inc, Portland, Me). Lipoprotein (a) [Lp(a)] levels were measured using a commercial enzyme-linked immunoadsorbent assay method.

**Analysis of DNA Polymorphisms**

DNA was extracted from peripheral lymphocytes by standard procedures, and DNA polymorphisms were determined by either Southern hybridization (seven loci) or polymerase chain reaction (PCR) amplification (five loci). For Southern analysis, to avoid spurious results due to incomplete digestion, 3 to 5 µg of DNA was digested overnight with a 5- to 10-fold excess of the appropriate restriction enzyme. The resulting products were separated by electrophoresis through 0.8% or 1.0% agarose gels and then transferred to nylon filters. The filters were then hybridized overnight with the appropriate 32P dCTP-labeled probe at 65°C in 5× SSC, 1× Denhardt’s, 30 mmol/L tris HCl (pH 8.0) 0.25% SDS, 10 mmol/L EDTA, and 100 mg/mL denatured salmon sperm DNA. Hybridization filters were washed three times for 5 minutes in 0.25× SSC, 0.2% SDS, 10 minutes in 0.25× SSC, 0.2% SDS at room temperature, followed by two 30-minute washes in 0.25× SSC, 0.2% SDS at 65°C. The probe for the Msp I polymorphism, Pst I, and Sac I polymorphisms of the apo A-I gene was the 2.2-kb Pst I fragment contained in the pSVA21 plasmid, whereas the two Pvu II polymorphisms in the 5′ flanking region of the apo A-IV gene were detected by the 2-kb BamHI-EcoRI fragment contained in pCTII-9. The 0.43-kb cDNA fragment in pAIIIE9 was used to detect the Msp I polymorphism in the apo A-II gene, and the 0.5-kb Pst I fragment in pCTII-711 was used to detect the Tag I polymorphism in the apo C-II gene. PCR amplification was used to detect the four polymorphisms at the apo B locus (Xba I, EcoRI, Msp I, and the 9-bp insertion/deletion), using 1 µmol/L of each primer and 7 ng/µL of genomic DNA, followed by restriction digestion. For the Xba I polymorphism, we used the primer pair 5′- CTGCAAGCTTAAGAGACAC-3′ and 5′-CGGCCAC- TGCAGCTACTGT-3′ and 10% DMSO and 30 amplification cycles of 1-minute denaturation at 95°C, 1-minute annealing at 60°C, and 1-minute extension at 70°C. For the EcoRI polymorphism, the primer pair was 5′- CTGAAAGTGCTTCTGAAG-3′ and 5′-CTCGAAAG- GAAGTGTAATCAC-3′ with 30 cycles of 1-minute denaturation at 94°C, 90-second annealing at 58°C, and 2-minute extension at 60°C, finishing with 10 minutes at 70°C. The primers and conditions for detecting the Msp I polymorphism and 9-bp insertion/deletion were as described elsewhere. PCR amplification, followed by Hha I digestion, was also used to determine apo E isotypes.

**Statistical Analysis**

Patients and control subjects were compared with regard to their medical history (as defined by chart review and response to the questionnaire), risk factors for CAD (as defined by response to the questionnaire), lipid profiles, and the frequency of alleles at the 12 apolipoprotein polymorphisms. For categorical variables, patients and control subjects were cross-classified and compared by log-linear analysis using the GLIM software package, with significant differences defined by the likelihood ratio test. Analyses of medical history variables were adjusted for sex, and for all other analyses men and women were analyzed separately. After data were excluded for subjects on lipid-lowering medications, lipid fractions were treated as quantitative variables. Normality and equal variance assumptions were checked for each sex by using q-normal plots and F tests, respectively. Using the S-PLUS statistical package, we applied unpaired t tests to untransformed data in both sexes for cholesterol, LDL, and apo A-I, and for apo B data in women, to log-transformed triglyceride data and to square-root transformed HDL data in both sexes and VLDL data in men. The Aspin-Welch unequal variance t test was used for apo B data in men and VLDL data in women. Lp(a) levels between patients and control subjects were compared using the Wilcoxon rank-sum test and quartile test because even when pooled transformed, the distribution of this variable departed substantially from normality.

Allele and genotypic frequencies for each of the 12 loci were determined from the observed genotypic counts, and χ2 analysis was used to check for departures from Hardy-Weinberg expectations. Allele frequency differences between patients and control subjects were analyzed first separately in men and women and then in both sexes combined, after confirming equality between the sexes. The likelihood ratio test statistic was used to evaluate the difference between patients and control subjects,73 using the log-linear procedures of GLIM. To reduce the experiment-wise error rate that would otherwise accrue by carrying out independent tests on each of 12 polymorphisms (α=1−[1−0.05]12) 46, we compared the resulting statistics with the Bonferroni α statistic by dividing the individual significance levels by 12, using an α level of 10% to be excessively conservative.

**Results**

**Angiographic Classification of Patients**

The angiographic review of the 791 consecutive subjects referred for selective angiography identified 444 individuals with advanced CAD (>60% stenosis in at least one major vessel), 307 disease-free individuals (<10% stenosis), and 40 individuals with indeterminate disease. Evaluation of the 118 retrospectively studied subjects identified an additional 97 disease-free individuals, plus 21 individuals with indeterminate disease. The 61 individuals with indeterminate levels of disease...
were excluded from further consideration, leaving a total of 848 unequivocally characterized subjects: 444 patients (325 men and 119 women) and 404 disease-free control subjects (168 men and 236 women).

### Referral Pattern for Coronary Angiography

In the majority of patients (83.6% of men and 77.3% of women), the primary reason for referral to angiography was chest pain (Table 1), with pain typical of angina pectoris being noted in a higher proportion of male patients than in female patients (74.2% versus 61.3%, P<.01). Conversely, atypical chest pain was a more frequent reason for referral in female patients (16.0%) than in male patients (9.5%). Occurrence of a previous myocardial infarct, the next most common reason for referral of CAD cases, was equally frequent for men (13.5%) and women (14.3%). Ten patients had other signs suggestive of possible CAD, whereas an additional nine patients were initially referred for evaluation of valvular heart disease.

In the control group, chest pain was also the primary reason for referral for angiography (Table 1), although less frequently than among patients. In control subjects, chest pain was recorded in roughly equal proportions for both sexes irrespective of whether the chest pain was typical of angina (33.3% of men and 28.0% of women) or was atypical (16.7% of men and 18.2% of women). However, referral for angiography because of valvular heart disease was more frequent in female control subjects than in male control subjects (24.6% versus 14.9%, P<.05), accounting for roughly one fifth (20.5%) of all referrals in the control group. The remaining 32.8% of the disease-free subjects were referred for a variety of reasons, including unexplained heart failure, atrial fibrillation or atrial or ventricular ectopy, syncope, and myocardial infarction. In addition, there were four control subjects referred because of a myocardial infarct confirmed by ECG and enzyme changes. Because their coronary arteries were completely normal, the infarct was presumed to be due to coronary artery spasm or to a thrombus or thromboembolus.

### Medical History

The distribution of pertinent cardiological conditions at the time of angiography was compared between patients and control subjects using sex-adjusted log-linear analysis (Table 2). Compared with subjects without disease, patients with CAD were significantly more likely to have experienced angina (71% versus 30%, P<.001), to have had a myocardial infarct (44% versus 1%, P<.001), or to have had previous bypass surgery (25% versus 0%, P<.001). However, atypical chest pain was experienced by similar proportions of patients with CAD and disease-free subjects. The distribution of demographic parameters and cardiovascular risk factors is given in Table 3. Among men, although neither mean weight nor mean BMI (kg/m²) differed between the groups, control subjects were slightly taller than male patients (P<.05). Also, although the age difference between patients and control subjects was within our target of no more than 5 years, the mean difference of 3.2 years was statistically significant. Female patients and control subjects were similar with respect to all demographic variables. However, women made up a greater proportion of the control group (58%) than the patient group (27%), as expected from the known sex-related difference in disease prevalence. Because of this imbalance, the genetic analysis was carried out by sex separately as well as overall.

### Table 1. Pattern of Referral for Coronary Artery Angiography in 444 Patients With Coronary Artery Disease and 404 Control Subjects

<table>
<thead>
<tr>
<th>Primary Referral Category</th>
<th>Patients</th>
<th>Control Subjects</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
<td>Men</td>
</tr>
<tr>
<td>Chest pain</td>
<td>272</td>
<td>84</td>
<td>356</td>
</tr>
<tr>
<td>Typical</td>
<td>241</td>
<td>56</td>
<td>297</td>
</tr>
<tr>
<td>Atypical</td>
<td>31</td>
<td>28</td>
<td>59</td>
</tr>
<tr>
<td>Valvular disease</td>
<td>5</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>Previous myocardial infarction</td>
<td>44</td>
<td>3</td>
<td>47</td>
</tr>
<tr>
<td>Arrhythmia/syncope</td>
<td>2</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td>Heart failure/cardiomyopathy</td>
<td>1</td>
<td>12</td>
<td>13</td>
</tr>
<tr>
<td>Positive stress test</td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Miscellaneous*</td>
<td>0</td>
<td>24</td>
<td>24</td>
</tr>
</tbody>
</table>

*Miscellaneous reasons included unexplained ECG changes, pulmonary hypertension, chronic obstructive pulmonary disease, peripheral artery disease, and evaluation of morbid obesity.

### Table 2. Cardiological Conditions of 444 Patients With Coronary Artery Disease and 404 Disease-Free Control Subjects Compared Using Sex-Adjusted Log-Linear Analysis

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Control Subjects</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Angina</td>
<td>316</td>
<td>71</td>
<td>122</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>194</td>
<td>44</td>
<td>4</td>
</tr>
<tr>
<td>Atypical angina</td>
<td>50</td>
<td>11</td>
<td>71</td>
</tr>
<tr>
<td>Bypass surgery</td>
<td>110</td>
<td>25</td>
<td>0</td>
</tr>
</tbody>
</table>
TABLE 3. Demographic Attributes and Risk Factors for Coronary Artery Disease in 444 Patients With Disease and 404 Disease-Free Control Subjects*

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Control Subjects</th>
<th>P</th>
<th>Patients</th>
<th>Control Subjects</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample size</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>56.0±0.40</td>
<td>52.8±0.73</td>
<td>&lt;.001</td>
<td>59.0±0.98</td>
<td>57.3±0.69</td>
<td>NS</td>
</tr>
<tr>
<td>Height, cm</td>
<td>177.4±0.35</td>
<td>178.8±0.52</td>
<td>&lt;.05</td>
<td>163.6±0.61</td>
<td>163.2±0.41</td>
<td>NS</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>86.3±0.78</td>
<td>87.8±1.2</td>
<td>NS</td>
<td>71.4±1.4</td>
<td>71.4±1.1</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>27.4±0.22</td>
<td>27.4±0.34</td>
<td>NS</td>
<td>27.0±0.50</td>
<td>26.7±0.39</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Non smokers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td>42%</td>
<td>60%</td>
<td>&lt;.001</td>
<td>69%</td>
<td>76%</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Hypertensive</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td>30%</td>
<td>5%</td>
<td>&lt;.001</td>
<td>25%</td>
<td>8%</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><strong>Hypertensive and medicated†</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td>43%</td>
<td>30%</td>
<td>&lt;.01</td>
<td>55%</td>
<td>41%</td>
<td>NS</td>
</tr>
<tr>
<td>Family history</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Father</td>
<td>41%</td>
<td>33%</td>
<td>NS</td>
<td>44%</td>
<td>39%</td>
<td>NS</td>
</tr>
<tr>
<td>Brother</td>
<td>26%</td>
<td>14%</td>
<td>&lt;.01</td>
<td>33%</td>
<td>22%</td>
<td>NS</td>
</tr>
</tbody>
</table>

BMI indicates body mass index.

*Sex-specific mean±SEM values compared by t test, and coronary artery disease risk factors compared by log-linear analysis.
†Refers to antihypertensive drugs including β-blockers and diuretics.

More male control subjects were nonsmokers (never smoked) than were male patients (60% versus 42%, P<.001), although among women, no difference was detected between diseased and control subjects. For both sexes, significantly more patients than control subjects were afflicted with diabetes or hypertension (P<.001 and P<.01, respectively). Among hypertensive subjects, male patients were more likely to use antihypertensive drugs than control subjects (P<.01), but in females the increase in medication among patients was not significant.

Patients with CAD were significantly more likely than control subjects to have a brother with CAD (Table 3; P<.01 for men and P>.05 for women). However, although more patients than control subjects reported fathers with CAD, this difference was not significant, nor was it for the presence of CAD in any other relative.

Serum Lipid Levels

Sixteen individuals (10 patients and 6 control subjects) had incomplete lipid profiles and were excluded from analysis. In addition, data were excluded from subjects who were on lipid-lowering medication: 33 male patients, 17 female patients, 5 male control subjects, and 19 female control subjects. Lipid profiles were then compared among the remaining 384 patients and 374 control subjects grouped by sex (Table 4). Compared with control subjects, patients of each sex had significantly higher mean concentrations of serum cholesterol, LDL, apo B, triglyceride, and VLDL but significantly lower mean HDL levels. However, although apo A-I concentrations were lower among patients, this difference was not significant, regardless of the test used (t test and Wilcoxon rank-sum test). Median Lp(a) levels were significantly higher among

TABLE 4. Lipid and Lipoprotein Levels in 384 Patients With Coronary Artery Disease and 374 Control Subjects*

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Control Subjects</th>
<th>P</th>
<th>Patients</th>
<th>Control Subjects</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Triglycerides</strong></td>
<td>164.8±5.6</td>
<td>146.7±8.8</td>
<td>&lt;.01</td>
<td>200.3±12.0</td>
<td>139.5±7.0</td>
<td>&lt;.001</td>
</tr>
<tr>
<td><strong>Total cholesterol</strong></td>
<td>205.9±2.3</td>
<td>186.7±2.7</td>
<td>&lt;.001</td>
<td>219.5±4.6</td>
<td>190.2±3.1</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>HDL</td>
<td>32.5±0.5</td>
<td>35.5±0.8</td>
<td>&lt;.01</td>
<td>37.9±1.3</td>
<td>43.5±0.9</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>LDL</td>
<td>138.2±2.0</td>
<td>122.2±2.6</td>
<td>&lt;.001</td>
<td>137.4±4.1</td>
<td>118.1±2.7</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>VLDL</td>
<td>34.4±1.3</td>
<td>28.5±1.6</td>
<td>&lt;.01</td>
<td>43.4±3.0</td>
<td>27.8±1.5</td>
<td>&lt;.001†</td>
</tr>
<tr>
<td>Apo A-I</td>
<td>96.0±1.1</td>
<td>99.9±1.8</td>
<td>NS</td>
<td>109.9±2.3</td>
<td>113.6±1.8</td>
<td>NS</td>
</tr>
<tr>
<td>Apo B</td>
<td>88.8±1.2</td>
<td>77.6±1.5</td>
<td>&lt;.001†</td>
<td>90.3±2.5</td>
<td>75.4±1.7</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>10.8</td>
<td>5.4</td>
<td>&lt;.001‡</td>
<td>13.8</td>
<td>10.3</td>
<td>NS‡</td>
</tr>
</tbody>
</table>

HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein; Apo, apolipoprotein; and Lp(a), lipoprotein (a).

*Values given are sex-specific mean±SEM values with exclusion of values for 16 individuals with incomplete lipid data and 74 individuals on lipid-lowering medication. Significance was evaluated using unpaired t tests except as noted.
†Aspin-Welch unequal-variances t test.
‡Median values and Wilcoxon rank-sum test.
male patients than control subjects (10.8 versus 5.4 mg/dL, \( P < .001 \)). Also, more male patients had Lp(a) concentrations in the upper quartile than did male control subjects (30% versus 16%, \( P < .025 \)). Among women, although the median Lp(a) level was higher for patients than for control subjects (13.8 versus 10.3 mg/dL) and more patients than control subjects were in the upper quartile of Lp(a) concentrations (32% versus 22%) these differences were not significant (\( P > .10 \) for both tests).

**Allele and Genotype Frequencies in Patients and Control Subjects**

Before testing whether apolipoprotein polymorphisms are associated with increased risk of CAD, we evaluated whether genotypic frequencies were in Hardy-Weinberg proportions. For each of the 12 loci, the \( \chi^2 \) evaluation was carried out separately by sex and by disease status. Within patients and control subjects, we tested for equality of allele frequencies between the sexes and, finding no sex-specific differences, tested for Hardy-Weinberg for both sexes combined. In the 48 sex-specific evaluations, there were no trends in the deviations from Hardy-Weinberg expectation and only two instances of nominally significant deviations—the apo A-II Msp I site in female control subjects (\( P < .01 \)) and the apo B EcoRII site in female patients (\( P < .005 \)). However, after applying the Bonferroni correction for multiple tests, neither of these results approached statistical significance. When the data were combined by sex, all 24 comparisons had nominal \( P \) values of >.10. Furthermore, there was no indication of a consistent heterozygote excess, or heterozygote deficiency, irrespective of whether the comparison was made across polymorphisms, or across sex-disease specific groups. Therefore, despite the low power of the \( \chi^2 \) test to detect deviations from Hardy-Weinberg expectations,\(^{40} \) we can conclude there is no evidence of genetic heterogeneity within our sample. Similarly, there is no evidence of locus-specific perturbations that might indicate inaccurate genotypic assignment.

Given the sex imbalance in patients and control subjects, we tested the hypothesis of disease association by comparing allele frequencies between patients and control subjects separately for each sex and then with sexes combined (Table 5). Of the 36 comparisons, only 3 approached a nominal significance level of 5%: apo A-I/Pvu IIa in men (\( P = .05 \)), apo B/Msp I in women (\( P = .04 \)), and apo B/Msp I in men and women combined (\( P = .02 \)). However, after correction for multiple tests, with the Bonferroni tables,\(^{36} \) none of these allele frequency differences between patients and control subjects was statistically significant (for an overall \( \alpha = .10 \), each individual \( \alpha \) should be .003). In fact, inspection of Table 5 reveals a remarkable conformity of allele frequencies between patients and control subjects, whether considered separately by sex or when sexes are combined. The distribution of confidence intervals around the odds ratio further reinforces the conclusion that at these loci there is no genetic difference between patients and control subjects (Figure). Note that because the confidence intervals around the 11 odds ratios do not reflect the Bonferroni correction, the probability that the true odds ratios simultaneously are caused by the values depicted in the Figure is only approximately 45% (1-.11[\( \alpha = .05 \)] = .45\(^9 \)).

Because genotypes were in Hardy-Weinberg proportions and there were no sex-specific differences in allele
frequencies, the comparison of genotypic frequencies between patients and control subjects was carried out for both sexes combined (Table 6). Of the 12 genotypic comparisons, only the apo B/Msp I data were suggestive of a possible association between the “−” allele and disease \( P < .08 \). However, this difference lacked even nominal statistical significance. Overall, the distribution of genotypes is remarkably similar for both patients and control subjects (Table 6).

Because it could be argued that analysis of the full data set suffers from possible misclassification (some of the younger control subjects might possess genetic susceptibility without having developed overt disease, whereas older patients lacking genetic susceptibility might have developed disease due to behavioral factors), we carried out a more stringent analysis by only comparing younger patients (112 men <55 years old and 95 women <60 years old) with older control subjects (71 men ≥55 years old and 47 women ≥60 years old). This more stringent comparison failed to find any differences between patients and control subjects for any of the 12 apolipoprotein polymorphisms. In particular, the nominal statistical significance of the apo B/Msp I and apo B/EcoRI polymorphisms in women disappeared when the analysis was restricted to cases with early-onset CAD compared with older control subjects.

**Discussion**

**Summary of Study Results**

Our study was designed to evaluate the feasibility of reducing the burden of CAD in the general population by using mass screening of common DNA polymorphisms to identify high-risk individuals before the clinical onset of disease. These individuals could then be targeted for intervention strategies to modify their disease risk rather than attempting to modify the risk profile of the entire population—the “high-risk approach” versus the “population approach.” However, the critical assumption underlying this proposition is that DNA polymorphisms of candidate loci can be used to detect individuals with a high risk of developing CAD. Because polymorphisms of the apolipoprotein loci have been identified as being potentially useful in this context, our study focused on common DNA polymorphisms of the eight major apolipoprotein loci. All 12 polymorphisms tested at these sites were in Hardy-Weinberg equilibrium with no indication of population heterogeneity. Despite the fundamental role played by these genes in lipid metabolism, our study found no association between any of these polymorphisms and the risk of developing angiographic CAD. This lack of association held for both allele frequencies and genotypic frequencies, whether sexes were analyzed separately or together. Although the subset analyses had less statistical power than the full analysis, the overall conclusion that there was no biologically meaningful association between these polymorphisms and risk of CAD was strengthened by our failure to detect any association in the analysis involving younger patients and older control subjects. In all comparisons, patients and control subjects exhibited a remarkable similarity in allele frequencies and in genotypic frequencies with no trends being detectable. We conclude that the few minor departures from uniformity between patients and control subjects are due to chance occurrences; the differences were not consistently observed in subgroups and in no case were they statistically significant after correcting for multiple comparisons.

Because the genetic composition of the Utah population reflects the gene pool of North American Caucasians and because the relative absence of environmental risk factors in Utah will tend to enhance the contribution of genetic risk factors, we presume this lack of association between risk of CAD and the common DNA polymorphisms of the major apolipoprotein loci will be generally true for North American Caucasians. Therefore, the development of a “high-risk” approach based on using genetic strategies to reduce the risk of CAD in the general population seems infeasible. Instead, the population approach that aims to reduce the burden of CAD by focusing on behaviors that influence the prevalence of common risk factors seems to be a better strategy.
### Attributes of This Study

A feature of this study is that despite analyzing a relatively large number of candidate gene polymorphisms, we consistently failed to find any significant association with disease. This contrasts with the inconsistencies scattered through the accumulated literature.\(^{15,21}\) We surmise that the consistency of this study is largely due to our ability to reduce, or eliminate, factors that could lead to a biased comparison between patients and control subjects and therefore give rise to a spurious result. An important attribute was the fact that when forming the samples for patients and control subjects, we were able to avoid subtle biases in ethnic ancestry or genetic composition. This is largely because the Utah Caucasian population is not only representative of the US Caucasian gene pool\(^ {22}\) but also tends to be genetically homogenous with respect to geographic region and other sociodemographic factors that might otherwise differentiate patient populations. The consistent fit of genotypic arrays to Hardy-Weinberg expectations confirms the genetic homogeneity of the Utah Caucasian population. Therefore, it is unlikely that our case-control comparison was contaminated by subtle differences in ethnic ancestry. By contrast, in older, more established populations where geographic neighborhoods are frequently correlated with genetic microdifferentiation (eg, eastern US seaboard and Europe), such differences are hard to eliminate (eg, the initial association between CAD and the apo A-I/Pst I polymorphism).\(^ {22}\)

This study was also strengthened by use of the same clinical criteria—angiographic evaluation—to discriminate between patients and control subjects. Use of stringent criteria to classify patients (>60% stenosis) and control subjects (<10% stenosis) minimized contamination between groups. However, because considerations of cost and safety prohibit angiographic assessment of asymptomatic subjects from the general population, our control sample was necessarily drawn from subjects referred for angiography rather than being a randomly selected population sample. Subjects referred for angiography and subsequently found to have normal coronary arteries may not be representative of “normal,” disease-free individuals in the population. As noted in Table 1, however, control subjects were characterized by a diverse group of diagnoses (ie, valvular heart disease, cardiomyopathies, and chest pain syndromes), whereas patients were predominantly referred because of signs indicative of CAD. Despite these differences in referral patterns, the two samples

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**Table 6. Genotype Frequencies for 12 Apolipoprotein Loci in 444 Patients With Coronary Artery Disease and 404 Disease-Free Control Subjects**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Site</th>
<th>Patient</th>
<th>Control subject</th>
<th>Patient</th>
<th>Control subject</th>
<th>Patient</th>
<th>Control subject</th>
<th>Patient</th>
<th>Control subject</th>
<th>Patient</th>
<th>Control subject</th>
<th>Patient</th>
<th>Control subject</th>
<th>Patient</th>
<th>Control subject</th>
<th>Patient</th>
<th>Control subject</th>
<th>Patient</th>
<th>Control subject</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo A-I</td>
<td>Msp I</td>
<td>0.00</td>
<td>0.00</td>
<td>0.18</td>
<td>0.14</td>
<td>0.82</td>
<td>0.85</td>
<td>0.51</td>
<td>0.54</td>
<td>0.48</td>
<td>0.91</td>
<td>0.09</td>
<td>0.00</td>
<td>0.48</td>
<td>0.61</td>
<td>0.32</td>
<td>0.06</td>
<td>0.84</td>
<td>0.03</td>
</tr>
<tr>
<td>Apo A-II</td>
<td>Msp I</td>
<td>0.04</td>
<td>0.27</td>
<td>0.28</td>
<td>0.45</td>
<td>0.68</td>
<td>0.69</td>
<td>0.14</td>
<td>0.98</td>
<td>0.25</td>
<td>0.52</td>
<td>0.23</td>
<td>0.98</td>
<td>0.23</td>
<td>0.51</td>
<td>0.28</td>
<td>0.52</td>
<td>0.45</td>
<td></td>
</tr>
<tr>
<td>C-II</td>
<td>Taq I</td>
<td>0.35</td>
<td>0.45</td>
<td>0.28</td>
<td>0.45</td>
<td>0.21</td>
<td>0.20</td>
<td>0.14</td>
<td>0.28</td>
<td>0.05</td>
<td>0.30</td>
<td>0.65</td>
<td>0.31</td>
<td>0.03</td>
<td>0.30</td>
<td>0.67</td>
<td>0.01</td>
<td>0.21</td>
<td></td>
</tr>
<tr>
<td>Apo B</td>
<td>I/D</td>
<td>0.67</td>
<td>0.31</td>
<td>0.65</td>
<td>0.10</td>
<td>0.31</td>
<td>0.52</td>
<td>0.98</td>
<td>0.44</td>
<td>0.45</td>
<td>0.11</td>
<td>0.52</td>
<td>0.45</td>
<td>0.45</td>
<td>0.10</td>
<td>0.05</td>
<td>0.30</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>Apo E</td>
<td>Msp I</td>
<td>0.34</td>
<td>0.33</td>
<td>0.34</td>
<td>0.34</td>
<td>0.34</td>
<td>0.34</td>
<td>0.70</td>
<td>0.01</td>
<td>0.21</td>
<td>0.78</td>
<td>0.07</td>
<td>0.01</td>
<td>0.21</td>
<td>0.78</td>
<td>0.01</td>
<td>0.14</td>
<td>0.85</td>
<td></td>
</tr>
<tr>
<td>Apo E</td>
<td>Msp I</td>
<td>0.11</td>
<td>0.59</td>
<td>0.23</td>
<td>0.23</td>
<td>0.80</td>
<td>0.23</td>
<td>0.80</td>
<td>0.08</td>
<td>0.62</td>
<td>0.23</td>
<td>0.80</td>
<td>0.08</td>
<td>0.62</td>
<td>0.23</td>
<td>0.80</td>
<td>0.08</td>
<td>0.62</td>
<td></td>
</tr>
</tbody>
</table>

*Genotype frequencies presented for both sexes combined. Genotype frequencies of patients and control subjects compared by log-linear models.*
were comparable in their distribution of age, height, weight, and BMI. Therefore, it is unlikely that this method of selecting control subjects introduced bias into the study. Furthermore, the validity of using these control subjects as surrogates of "normal" individuals in the general population is strengthened by the fact that control subjects referred because of signs suggestive of CAD had the same genotypic frequencies as control subjects referred for other reasons, such as valvular heart disease (P > .05, all comparisons). Similarly, there were no differences in genotypic frequencies between control subjects with angina (typical or atypical) and those without. In addition, a small sample of 75 randomly drawn asymptomatic community control subjects also had the same distribution of allele frequencies as the angiographic control subjects (unpublished observations). Therefore, lacking indication of genetic differences between the diagnostic subgroups of the control subjects or with the random community sample, there is no evidence that the use of angiographically defined control subjects has resulted in either bias or contamination with "early CAD" individuals.

In this connection, we note that although 30% of control subjects presented with a complaint of chest pain, believed to represent angina, angiography revealed that these complaints were not due to "occult" CAD. This suggests that chest pain diagnosed as "angina" is an inadequate surrogate for coronary atherosclerosis. In general, angiographic disease substantially precedes clinical disease, generally requiring ≥70% diameter stenosis to cause classic angina. Therefore, we view the strict angiographic definitions of disease and normal groups as a strength of this study compared with studies that rely on softer clinical findings to define disease and thereby tend to obscure the difference between patients and control subjects. A similar criticism can be leveled at studies that include subjects with intermediate angiographic degrees of disease (stenoses of 20% to 50%).

Finally, although levels of lipids (total cholesterol, LDL cholesterol, and so on) were generally lower in our study subjects than in some US populations, the differences between patients and control subjects were consistent with other reports.41-46 With the exception of apo A-I, all established lipid risk factors were significantly different in patients and control subjects for both men and women. Because other studies have also found that apo A-I is more weakly associated with risk of coronary disease,11 this result does not suggest that our samples were somehow unrepresentative. Also, although the small effect of hospitalization on lipid levels47 may have contributed to the generally low lipid profiles we observed, the fact that we obtained similar data from the small community sample (unpublished results) suggests that the observed lipid values are more likely to reflect the lower rates of coronary disease rates in Utah compared with the rest of the United States.48 Although not the primary focus of our study, we recognize that the magnitude of the difference in lipid profiles between patients and control subjects may have been partly influenced by differences in demographic and clinical factors not controlled for explicitly. These include age (in men), prevalence of smoking, alcohol use, and diabetes and probably diet as well. However, there is no evidence that these factors interact with the distribution of apolipoprotein polymorphisms because subgroup analyses (eg, between smokers and non-smokers and so on) failed to reveal any discernible differences in genotypic frequencies.

Conclusions and Implications

Despite the fundamental role played by apolipoproteins in lipid metabolism, common genetic polymorphisms at the major DNA loci were not found to influence the risk of developing angiographic CAD. These conclusions are unlikely a result of a significant β-error, given the relatively large sample size compared with previous reports. In addition, the stringent definitions for diseased and control subjects plus the simultaneous assessment of several polymorphisms at all of the known apolipoprotein gene loci lend strength to this conclusion.

The study tested for several but not every polymorphism in each apolipoprotein region. For example, 5 polymorphisms were evaluated in the A-I/C-III/A-IV region, whereas at least 11 are known. However, because of linkage disequilibrium,30,51 these other polymorphisms are not independent, with the consequence that the 5 polymorphisms in this study fail to detect only haplotypes that are individually rare and that collectively account for only 20% of the population distribution described by Benlian et al.51 Furthermore, addition of any single polymorphism would uniquely identify only a small segment of the population (1% to 4%). Thus, although we cannot exclude the possibility that an undetermined polymorphism in the A-I/C-III/A-IV region might be associated with disease, this seems an unlikely eventuality. The present study has taken the most comprehensive look at variation in DNA loci in coronary disease by simultaneously assessing multiple polymorphisms in all 5 apolipoprotein regions, with the result that a substantial portion of the commonly existing genetic variability in these loci has been examined.

The study had sufficient power to detect substantial, clinically significant differences in risk by polymorphism. Despite the multiple comparisons, we found no overall associations between these apolipoprotein polymorphisms and disease and no suggestive trends in the data. We would argue that to be useful as a population screening tool, a DNA polymorphism needs to identify 10% to 15% of high-risk individuals with reasonable specificity. Given the range of allele frequencies at these apolipoprotein loci, this would require a minimum risk ratio of 1.6 to 3.0. In terms of the combination of allele frequency and risk ratio required to identify 10% of individuals at high risk for CAD, this study had sufficient power to identify such associations if they existed. Although we cannot exclude the possibility of relative risk in the range of 1.2 to 1.5 for alleles with a frequency of 10% or less, such associations would account for less than 5% of high-risk individuals and would require sample sizes of upward of 2500 clinically defined patients and control subjects to be detected. Therefore, our results suggest it would be counterproductive to develop disease prevention strategies based on these apolipoprotein DNA polymorphisms. On a priori grounds, this is not completely unexpected because any single polymorphism is unlikely to be associated with more than a fraction of the heritable variability in lipid profiles, and lipid profiles themselves
determine only a portion of CAD risk. A similar comment applies to the usefulness of mass screening for the rare genetic lesions that lead to CAD (eg, LDL receptor defects and similar mutations\(^b\)). It should be stressed that these conclusions do not gainsay the clinical importance of screening relatives of individuals known to possess such a mutation or of screening individuals whose lipid profile gives them an indisputably high probability of carrying the mutation. Nevertheless, although a useful adjunct for evaluating members of high-risk families, the impact of these mutations on the prevalence of CAD in the general population (<5% overall\(^c,d\)) is far too small to justify screening for their presence in a public health setting.

Overall, the results of this study support the view of Rose.\(^2\) Coronary heart disease, like hypertension, is a “mass” disease to which nearly all individuals are susceptible in varying degrees. Although different levels of its precursors may be genetically determined to a variable degree, incidence rates, population differences, and time trends appear to be largely determined by environment and lifestyle. Genetic and environmental approaches to research will continue to be interdependent, but the contribution of environmental control measures has been and will continue to be substantially greater than genetic control measures, which are confined to less common conditions or to individuals at exceptionally high risk. Our findings suggest that at this time and given our current level of understanding of DNA polymorphisms, a broad-based approach to risk reduction is likely to be the most effective strategy on a population scale.

Acknowledgments

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Apolipoprotein polymorphisms fail to define risk of coronary artery disease. Results of a prospective, angiographically controlled study.


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