Association Between Apolipoprotein E Alleles and Calcific Valvular Heart Disease

Gian M. Novaro, MD; Ravish Sachar, MD; Gregory L. Pearce, MS; Dennis L. Sprecher, MD; Brian P. Griffin, MD

Background—Studies on apolipoprotein E (apoE) alleles have reported an increased risk of coronary heart disease in patients with the apoE4 allele. Given the risk factor and histological similarities between coronary and calcific valvular heart disease (aortic stenosis [AS] and mitral annular calcification [MAC]), we postulated that apoE alleles might be associated with the development of these valvular lesions.

Methods and Results—We evaluated the association between apoE alleles and calcific valvular lesions in 802 patients undergoing transthoracic echocardiography using logistic regression analyses. No difference was noted in genotype distribution (P = 0.59) or prevalence of apoE4 between those with or without MAC (30% versus 27%, respectively; P = 0.57). Compared with patients without AS, the genotype distribution of patients with AS differed significantly (P = 0.03), with increasing prevalences of the apoE 4 allele (27% in those without versus 40% in those with AS; P = 0.01). In multivariate analyses adjusting for age, gender, low-density lipoprotein cholesterol levels, and coronary artery disease, increasing age and the apoE4 allele were significant independent predictors of AS (odds ratio, 1.94; 95% confidence interval, 1.01 to 3.71; P = 0.046), whereas the apoE4 allele was not predictive of MAC.

Conclusions—These findings support extension of the importance of the apoE4 allele beyond atherosclerosis and Alzheimer’s disease to calcific AS. (Circulation. 2003;108:1804-1808.)

Key Words: stenosis ■ apolipoproteins ■ calcification ■ genomics

Calcific aortic stenosis (AS) and mitral annular calcification (MAC) are common valvular conditions in the elderly population, both of which are associated with significant cardiovascular morbidity and mortality.1–4 The development of AS has been associated with atherosclerotic risk factors, including elevated total cholesterol5,6 and low-density lipoprotein cholesterol (LDL-c) levels.2 Microscopically, a chronic inflammatory process involving lipids, lipoproteins, and macrophages characterizes its appearance.7 Similarly, MAC has been associated with atherosclerotic risk factors and, histologically, demonstrates the presence of lipids and inflammatory cells.8 Although clinical risk factors have been identified in association with calcific valvular disease, no specific genetic markers exist that serve to identify patients at risk for their development.

Recently, in a case-control study, Ortlepp and colleagues9 analyzed the distribution of a vitamin D receptor polymorphism in patients with and without AS and demonstrated a higher prevalence of the B allele in those with stenotic aortic valves. Because the presence of this polymorphism coupled with clinical risk factors would fail to entirely explain an individual’s propensity to develop the disease, other yet-unidentified markers of susceptibility potentially exist. The gene for apolipoprotein E (apoE) contains 3 alleles that encode for 6 different protein genotypes (apoE 2/2, 2/3, 2/4, 3/3, 3/4, 4/4), of which apoE 3/3 accounts for ≈60% of the population’s genotype in the United States.10 In comparison to the apoE3 allele, apoE4 is associated with higher total and LDL-c levels and lower high-density lipoprotein (HDL-c) levels; opposite effects are seen with the apoE2 allele.11,12 Studies on apoE polymorphisms have suggested a higher prevalence of coronary heart disease in patients with the apoE4 allele, present in ≈20% of the population, implicating allele-specific properties in the development of atherosclerosis, possibly beyond their effects on circulating lipid levels.13 As a result of their role in the transport of lipoproteins14 and differing oxidative properties,15 certain apoE genotypes and alleles could favor the development of calcific AS and MAC, with a risk attributable to factors beyond that imposed by their associated serum lipoprotein levels. We postulated that specific apoE alleles, like in coronary disease, are associated with calcific valvular disease. To investigate this hypothesis, we analyzed the distribution and prevalence of apoE alleles in an echocardiographic cohort of patients with and without AS and MAC.
Demographic and clinical data were obtained by database review or from the patients’ medical records. Clinical data recorded included the following: prior evidence of coronary artery disease (history of myocardial infarction, angioplasty, coronary artery bypass grafting, or coronary artery disease by angiography [epicardial coronary stenosis >50%]); history of hypertension; current smoking; and history of diabetes mellitus. Complete lipid panels were collected in the fasting state and included total cholesterol, LDL-c, HDL-c, and triglyceride levels. Study approval was obtained from our institutional review board and ethics committee.

### Statistical Methods

Comparisons between groups for apoE distributions were made with χ2 tests. Comparisons of presentation characteristics between patients with and without AS and MAC were made with either unpaired t tests (for continuous measures) or χ2 tests (categorical measures). Multivariate logistic regression was used to estimate the adjusted relative risk of having the apoE4 allele. P values of ≤0.05 were considered statistically significant.

### Results

#### Patient Characteristics

Of the total 802 patients, 43 (5%) had AS and the remaining 759 (95%) did not. With regard to MAC, 165 patients (21%) of the total cohort had MAC; the remaining 637 (79%) did not. The prevalence of MAC, as expected, was greater in patients with AS than without (54% versus 19%, respectively; P<0.001) (Figure 1). Baseline characteristics based on valvular disease are shown in Table 1. On average, patients with AS were significantly older and had a higher prevalence of coronary artery disease than those without AS. Other traditional cardiac risk factors, including LDL-c levels, were similar between the groups. Mean aortic valve area for the AS cohort was 1.3±0.3 cm², with an average peak and mean gradient of 26±12 mm Hg and 15±6 mm Hg, respectively. Two patients in the AS group had a suspected congenital bicuspid aortic valve. Characteristics based on status of the mitral annulus demonstrated that patients with MAC were significantly older and had a higher prevalence of hypertension, diabetes mellitus, and coronary artery disease. Again, lipid levels were similar between the groups based on the status of MAC.

### Methods

#### Study Patients

From the Preventive Cardiology database of apoE genotypes obtained at our institution from November 1995 to December 2001 (n=2957), a total of 802 patients with available transthoracic echocardiographic studies were identified. Patients underwent echocardiographic studies for the following indications: coronary disease without infarction (18%), myocardial infarction (15%), valvular heart disease (12%), cardiomegaly (9%), cardiomyopathy (8%), acute coronary insufficiency (8%), undiagnosed heart murmur (7%), congestive heart failure (6%), atrial fibrillation (5%), postcardiac surgery (4%), vascular disease (3%), hypertensive heart disease (1%), syncope (1%), and miscellaneous (3%). Patients were categorized on the basis of the presence or absence of echocardiographically determined AS and/or MAC. AS was defined as thickened leaflets with restricted systolic motion, a pressure gradient of ≥26 mm Hg, and/or calcified aortic leaflets. Calculation of aortic valve area was performed with the use of a Hhal digest, as described elsewhere.17 During the study period, apoE analysis was performed as a risk parameter as part of the general assessment of cardiovascular risk. Although patients were aware of the analysis and were provided the results and appropriate counseling, specific informed consent for genetic testing was not routinely obtained.

#### Clinical and Laboratory Data Including ApoE

Analysis of apoE genotypes was performed with the use of a polymerase chain reaction amplification of DNA followed by Hhal digestion, as described elsewhere.17 During the study period, apoE analysis was performed as a risk parameter as part of the general assessment of cardiovascular risk. Although patients were aware of the analysis and were provided the results and appropriate counseling, specific informed consent for genetic testing was not routinely obtained.

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**Figure 1.** Diagram of cohort distribution on the basis of valve disease status.

**Table 1.** Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>AS Absent (n=759)</th>
<th>AS Present (n=43)</th>
<th>P</th>
<th>MAC Absent (n=637)</th>
<th>MAC Present (n=165)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>60±12</td>
<td>68±10</td>
<td>&lt;0.001</td>
<td>59±11</td>
<td>67±10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>36</td>
<td>33</td>
<td>0.63</td>
<td>35</td>
<td>41</td>
<td>0.12</td>
</tr>
<tr>
<td>Hypertension</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>62</td>
<td>70</td>
<td>0.31</td>
<td>60</td>
<td>73</td>
<td>0.002</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>21</td>
<td>0.46</td>
<td>24</td>
<td>33</td>
<td>0.01</td>
</tr>
<tr>
<td>Tobacco use</td>
<td>10</td>
<td>7</td>
<td>0.79</td>
<td>10</td>
<td>6</td>
<td>0.09</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>74</td>
<td>86</td>
<td>0.08</td>
<td>73</td>
<td>82</td>
<td>0.02</td>
</tr>
<tr>
<td>LDL-c, mg/dL</td>
<td>124±50</td>
<td>129±43</td>
<td>0.59</td>
<td>124±46</td>
<td>127±60</td>
<td>0.55</td>
</tr>
<tr>
<td>HDL-c, mg/dL</td>
<td>44±15</td>
<td>42±11</td>
<td>0.24</td>
<td>45±14</td>
<td>44±15</td>
<td>0.21</td>
</tr>
<tr>
<td>White race</td>
<td>85</td>
<td>88</td>
<td>0.91</td>
<td>86</td>
<td>85</td>
<td>0.54</td>
</tr>
<tr>
<td>Asian race</td>
<td>&lt;1</td>
<td>0</td>
<td>…</td>
<td>&lt;1</td>
<td>0</td>
<td>…</td>
</tr>
</tbody>
</table>

Values are shown as mean±SD when continuous or percentages when categorical.
TABLE 2. Lipid Parameters by ApoE Allele

<table>
<thead>
<tr>
<th></th>
<th>ApoE2</th>
<th>ApoE3</th>
<th>ApoE4</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-c, mg/dL</td>
<td>109 (76–150)</td>
<td>122 (91–156)</td>
<td>119 (90–151)</td>
<td>0.18</td>
</tr>
<tr>
<td>HDL-c, mg/dL</td>
<td>41 (33–50)</td>
<td>42 (34–52)</td>
<td>42 (33–53)</td>
<td>0.79</td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>213 (146–416)</td>
<td>161 (118–247)</td>
<td>167 (110–274)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Values are shown as median values (interquartile range).

The total cohort apoE genotype distribution was similar to that found in the general US population, and conformed with Hardy-Weinberg equilibrium: apoE 2/2 (1%), 2/3 (9%), 2/4 (2%), 3/3 (62%), 3/4 (23%), and 4/4 (2%). The genotype distribution of the Preventive Cardiology database patients not meeting criteria for the study (n=2155) was compared with our study cohort’s distribution and found not to be significantly different (P=0.22). In keeping with previous reports, lipid levels differed across apoE alleles (Table 2).

Distribution of ApoE Genotypes

ApoE genotype distributions by status of AS and MAC are shown in Table 3. The genotype distributions among patients with and without MAC were not significantly different (P=0.59). In contrast, genotype distributions for those with and without AS differed significantly (P=0.03), with a greater prevalence of the apoE 2/4 (2% versus 9%) and 3/4 (23% versus 28%) genotypes in those with AS. With regard to the apoE4 allele (Figure 2), as compared with those without any valvular lesion or with those without AS, the prevalence of apoE4 was increased in AS patients regardless of the status of MAC. The prevalence of apoE4 in those with and without MAC, however, was similar (P=0.57).

Multivariate Predictors of AS and MAC

In multivariate analysis, adjusting for age, gender, coronary artery disease, and LDL-c, increasing age and the apoE4 allele significantly and independently predicted the presence of AS (Table 4). Independent predictors of MAC included only increasing age and a trend toward diabetes mellitus, whereas the apoE4 allele failed to predict the presence of MAC.

Discussion

In this investigation of apoE alleles and calcific valvular disease, we found that the apoE 2/4 and 3/4 genotypes were more prevalent in those with calcific AS. In multivariate analysis, the apoE4 allele was a significant and independent predictor of AS. These findings remained significant despite adjusting for age, gender, coronary artery disease, and LDL-c. ApoE alleles, however, were not independently predictive of MAC without AS.

To date, specific genetic markers for the development of calcific valvular disease are not known. The degenerative valvular process appears to be active and involves a chronic inflammatory infiltrate with lipid and lipoprotein deposition, including apoE, and dystrophic calcification. Multiple clinical atherosclerotic risk factors such as hypercholesterolemia and hypertension have been linked to the development of calcific valvular disease. Whether the genesis of AS and MAC has a genetic contribution in addition to clinical risks is not known. Ortlepp et al recently put forth the initial report suggesting a potential genetic marker of AS. The B allele of the vitamin D receptor, a polymorphism associated with rapid bone loss and lower bone mineral density, had a significantly greater prevalence in patients with AS versus control subjects. This observation provides further insight into the association between osteoporosis and AS, and possibly the observed heterotopic ossification noted on diseased aortic leaflets. Recent investigations have shown an osteoblast-like phenotype on calcific, nonrheumatic aortic valves, with evidence of mature lamellar bone, active bone remodeling, and bone matrix proteins. Furthermore, in experimental animal models, aortic valve stenosis seemed to develop in hypercholesterolemic rabbits only if supplemented with vitamin D(2). Hence, although increasing age and atherosclerotic factors are important acknowledged risks, it seems that additional factors such as calcium handling and genetic predisposition could be needed to develop calcific AS. To that effect, despite our study’s high prevalence of traditional cardiac risk factors and coronary artery disease, the apoE4
The apoE4 allele was still found to be a significant independent predictor of AS.

Reports of the various apoE genotypes and their relationship to the various atherosclerotic diseases have had inconsistent results. On the basis of a meta-analysis by Wilson et al, the apoE4 allele appears to be associated with a higher prevalence of coronary heart disease. However, its association with atherosclerotic disease in other arterial distributions such as the carotid arteries has been less consistent. Specifically, the apoE 3/4 genotype was shown to have no association with carotid intima–media thickness and plaque size, whereas apoE 2/3 had a protective effect. Other studies have shown contrasting results. To our knowledge, the only prior study of apoE alleles and calcific AS reported negative results with regard to the apoE4 allele. This evaluation, however, was small in sample size and included only non-diabetic, severe AS patients.

Establishing a mechanistic relationship between specific alleles and an increased likelihood of disease development remains challenging. The development of AS is associated with an inflammatory infiltrate and increased oxidative stress, because oxidatively modified LDL-c, in colocalization with dystrophic calcification, has been demonstrated on diseased aortic leaflets. The various apoE genotypes are associated with divergent effects on plasma lipids. Additionally, they exert differing antioxidant effects, with apoE4 possessing the least antioxidant properties. At the molecular level, apoE has ancillary properties, including regulating migration of lymphocytes, an important cellular process in diseased aortic leaflets. Mechanistically, these alternative properties, which differ at the cellular level and across the apoE alleles, could selectivity predispose to increased lipid deposition and inflammation on susceptible endothelial targets. Importantly, the effect of apoE4 on the risk of AS in our study was present despite adjusting for levels of LDL-c.

**Limitations**

Our study’s principal limitation is the relatively small sample of AS subjects, specifically in a genetic association study, which can potentially increase the risk of a false-positive association. Thus, further larger studies are required to validate our findings. Additional factors such as renal disease were not captured and, thus, it was not possible to adjust for all potential confounders. The high prevalence of coronary disease and cardiac risk factors in our entire cohort, as a result of selection bias inherent to a preventive cardiology population, limit our ability to apply these findings to the general population. The high prevalence is evident by the lack of association between diabetes, tobacco use, and hypertension with AS, factors previously linked to the development of AS.

**Conclusions**

In summary, the present study provides evidence to suggest a genetic marker associated with calcific valvular heart disease, identifying the apoE4 allele as a significant independent predictor of AS. These findings support extension of the importance of the apoE4 allele beyond atherosclerosis and Alzheimer’s disease to calcific AS. As has been hypothesized for the development of coronary artery disease, calcific AS could also represent a polygenic disorder modulated by a host of nongenic metabolic and environmental factors. Understanding the potential genetic underpinning of common disorders such as AS could be important in developing new strategies to alter the natural history of disease and allow aggressive risk factor modification in those most at risk.

**References**

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Circulation. 2003;108:1804-1808; originally published online October 6, 2003;
doi: 10.1161/01.CIR.0000097560.96431.3E
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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World Wide Web at:
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