Association Between the Coronary Artery Disease Risk Locus on Chromosome 9p21.3 and Abdominal Aortic Aneurysm

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Background—Recent genome-wide studies have shown a significant association of a locus on chromosome 9p21.3 and coronary artery disease. We performed a case-control study to investigate the association between this locus and abdominal aortic aneurysm (AAA).

Methods and Results—A total of 1714 patients (899 patients with AAA and 815 controls) were genotyped for the lead single-nucleotide polymorphism, rs1333049, on chromosome 9p21. The frequency of the C (risk) allele of rs1333049 in the control group was 0.471. There was a significant association between the C allele and AAA (odds ratio, 1.22; 95% confidence interval, 1.06 to 1.39; P=0.004). The genotypic-specific odds ratios (compared with the GG genotype) were 1.17 (95% confidence interval, 0.93 to 1.47; P=0.191) for the GC genotype and 1.50 (95% confidence interval, 1.14 to 1.97; P=0.004) for the CC genotype. In logistic regression modeling, the association of the CC genotype with AAA was independent of the presence of clinical coronary artery disease (odds ratio, 1.46; 95% confidence interval, 1.11 to 1.94; P=0.008).

Conclusions—Our study shows that the recently identified chromosome 9 variant that increases risk of coronary artery disease is also associated with the presence of AAA. The findings suggest that the effect of this locus on risk of cardiovascular disease extends beyond the coronary circulation.

Key Words: aneurysm ■ aorta ■ cardiovascular diseases ■ genetics

Abdominal aortic aneurysm (AAA) is a major cause of death in the Western world and accounts for ≈10,000 deaths per annum in England and Wales, mostly from rupture. The underlying final pathological cause is proteolytic destruction of the aortic wall, but the factors that initiate this proteolytic process are unknown. There is a positive association of AAA with some atherosclerotic risk factors such as smoking and hypertension but a negative association with diabetes mellitus. In addition, there is a strong genetic component to AAA, with first-degree relatives having a 10-fold increased risk of the disease. The mode of inheritance of AAA is unknown, although pedigree studies have suggested that it is probably polygenic. To date, most studies of candidate genes have proven to have disappointing results.

Clinical Perspective see p 42

Recent studies using high-density genotyping have reported an association between coronary artery disease (CAD) and a locus on chromosome 9p21.7–9 That has also been shown to be associated with AAA. We investigated the association between this locus and the presence of an AAA to determine whether or not this was a genuine link.
with asymptomatic but angiographically demonstrated coronary artery stenoses of ≥50% were included in the CAD group.

Ethical approval for the study was obtained from the Leicestershire Research Ethics Committee, and each participant consented to inclusion in the study.

Genotyping
DNA was extracted from whole blood with the use of a commercially available kit (Puregene, Gentra). We typed rs1333049, the lead single-nucleotide polymorphism associated with CAD on chromosome 9p21 in our previous genome-wide association study, using a custom TaqMan single-nucleotide polymorphism allelic discrimination genotyping assay. Each assay used 15 ng of DNA, 36 mmol/L of each primer pair, 8 mmol/L of both allele-specific fluorescent probes, and TaqMan genotyping master mix, containing AmpliTaq Gold DNA Polymerase, dNTPs, and ROX passive reference (Applied Biosystems [ABI], Foster City, Calif). Polymerase chain reaction was performed on a GeneAmp polymerase chain reaction system 9700 (ABI) with 384 well plates, with a cycling protocol of 95°C for 10 minutes followed by 45 cycles of 92°C for 15 seconds and 60°C for 1 minute. Fluorescence was detected after polymerase chain reaction with the use of the ABI Prism 7900HT Sequence Detector System, and genotypes were called with the use of ABI Prism SDS software version 2.1 (ABI).

Statistical Analysis
Tests for deviance from Hardy-Weinberg equilibrium and comparisons between genotype frequency in the case and control groups were determined with the use of binary logistic regression. Binary logistic regression (backward stepwise) was also used to construct separate models for genotype as a risk for AAA adjusted for age and other recorded patient demographics. Covariates were included in the model if they had a statistically significant effect (P < 0.05).

Because the majority of phenotypic data recorded was binary (e.g., smoking status, presence of diabetes, hypertension), only those participants with complete data sets for all of the factors entered into the model could be analyzed in this manner, and the total number of participants included in this analysis was 921 (471 controls and 450 AAA) (Table 4). Genotype frequencies remained in Hardy-Weinberg equilibrium in these groups (AAA, P = 0.354; control, P = 0.345; Fisher exact test), and in the total analysis (Table 1) and rs1333049 genotype were entered into a logistic regression model to determine which of these were independently associated with AAA (Table 3). Only those participants with complete data sets for all of the factors entered into the model could be analyzed in this manner, and the total number of participants included in this analysis was 921 (471 controls and 450 AAA) (Table 4). Genotype frequencies remained in Hardy-Weinberg equilibrium in these groups (AAA, P = 0.354; control, P = 0.345, Fisher exact test), and in the

Results
In total, 1714 participants were included in the study, 899 with AAA and 815 controls (Table 1). Median age in the control group was 66 years (range, 51 to 98 years) and in the AAA group was 73 years (range, 48 to 96 years). More than 90% of subjects were men in both groups. Median aneurysm size (maximum diameter) in the AAA group was 5.2 cm (range, 3 to 12 cm).

Genotype frequencies for rs1333049 are shown in Table 2. There was no evidence of deviation from Hardy-Weinberg equilibrium in either the AAA or control group (AAA, P = 0.350; control, P = 0.122, Fisher exact test). The frequency of the C allele in the control group was 0.471. The overall distributions of the genotypes were significantly different between cases and controls (P = 0.015). There was a significant association between the C allele and AAA (odds ratio, 1.22; 95% confidence interval [CI], 1.06 to 1.39; P = 0.004, Cochran-Armitage trend test). The genotypic-specific odds ratios (compared with the GG genotype) were 1.17 (95% CI, 0.93 to 1.47; P = 0.191) for the GC genotype and 1.50 (95% CI, 1.14 to 1.97; P = 0.004) for the CC genotype, which represent dominant and recessive genetic models, respectively. Under a codominant genetic model, the odds ratio (for C allele positivity) was 1.26 (95% CI, 1.01 to 1.57; P = 0.040).

Those factors found to be associated with AAA on univariate analysis (Table 1) and rs1333049 genotype were entered into a logistic regression model to determine which of these were independently associated with AAA (Table 3). Only those participants with complete data sets for all of the factors entered into the model could be analyzed in this manner, and the total number of participants included in this analysis was 921 (471 controls and 450 AAA) (Table 4). Genotype frequencies remained in Hardy-Weinberg equilibrium in these groups (AAA, P = 0.354; control, P = 0.345, Fisher exact test), and in the
control group rs1333049 C allele frequency was 0.454. Factors that were independently associated with AAA and retained in the final model were age, reported family history of AAA, history of tobacco use (prior or current), hyperlipidemia, CAD, and the rs1333049 CC genotype (Table 3).

To further explore whether the association of the locus with AAA was independent of the presence of clinical CAD, logistic regression modeling was performed in the whole data set with these 2 variables alone entered into the regression model. The odds ratio for the GC genotype in this analysis was 1.27 (95% CI, 1.00 to 1.62; P=0.008) and for the CC genotype was 1.46 (95% CI, 1.11 to 1.94; P=0.008). In this model, the odds ratio for CAD was 3.16 (95% CI, 2.44 to 4.10; P<0.001).

To examine any relationship between genotype and size, the AAA group was divided into quartiles (3 to 4 cm, 4 to 5.4 cm, 5.4 to 6.5, and >6.5 cm), and genotype frequencies were compared between the 4 groups. No significant association (P=0.723, 6 df, \( \chi^2 \)) was identified, and furthermore, when median aortic size was compared between the 3 genotypes, again no association was seen (P=0.861, Kruskal-Wallis test).

Discussion

This study demonstrates a significant association between a locus on chromosome 9p and presence of AAA. This locus has recently been identified through genome-wide association and follow-up studies as a robust risk locus for CAD.7–11 The finding suggests that the effect of this locus on risk of cardiovascular disease extends beyond the coronary circulation into other vascular beds.

Given the strong relationship between CAD and AAA, we tried to assess whether the association of the locus with AAA was independent of its association with CAD. Adjusting for the presence of clinically apparent CAD by logistic regression did not attenuate the association of the locus with AAA. However, it is impossible to exclude the possibility that the association marks the presence of occult CAD in the patients. Nonetheless, our data are consistent with a recent large study in multiple cohorts, which also found that the association of the chromosome 9 locus with AAA was independent of clinical CAD and also reported an association with intracranial aneurysms.12

There are both similarities and differences in the pathogenesis of CAD and AAA. Both involve inflammation and increased smooth muscle turnover. CAD is a disease largely of the intima and media of coronary vessels, with lipid deposition and plaque formation as key features. AAA is a disease of the media and adventitia of aorta associated with proteolytic degradation of elastin together with increased collagen turnover, an inflammatory infiltrate, and smooth muscle cell apoptosis. The fact that the same allele (C) of the rs1333049 is associated with increased risk in both conditions suggests that the mechanism by which the locus affects the risk of the 2 conditions is similar. The association with intracranial aneurysms12 perhaps points to a mechanism involving vascular remodeling that is common to all 3 conditions.

The chromosome 9p region, marked by rs1333049 and associated with AAA and CAD, spans \( \approx \)50 to 60 kb and has no known protein coding genes within it. However, recent studies have shown that the locus colocalizes with a large noncoding RNA, ANRIL, which is expressed in atherosclerotic tissue as well as walls of AAAs.11 Furthermore, expression of ANRIL is coordinated with that of p14/ARF and possibly also the cyclin-dependent kinases p16/CDKN2A and

Table 3. Results of Binary Logistic Regression Modeling

<table>
<thead>
<tr>
<th></th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior/current tobacco use</td>
<td>4.43</td>
<td>2.79 to 7.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Family history of AAA</td>
<td>2.53</td>
<td>1.41 to 4.54</td>
<td>0.002</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>1.90</td>
<td>1.35 to 2.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CAD</td>
<td>3.54</td>
<td>2.36 to 5.30</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age</td>
<td>1.15</td>
<td>1.12 to 1.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>rs1333049 GC genotype (vs GG)</td>
<td>1.33</td>
<td>0.89 to 1.99</td>
<td>0.161</td>
</tr>
<tr>
<td>rs1333049 CC genotype (vs GG)</td>
<td>2.09</td>
<td>1.31 to 3.31</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Table 4. Distribution of Demographic and Other Phenotypes in Those AAA Cases and Controls Used for Regression Modeling

<table>
<thead>
<tr>
<th></th>
<th>Control (n=471)</th>
<th>AAA (n=450)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>462 (98)</td>
<td>416 (92)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tobacco use (prior or current)</td>
<td>326 (69)</td>
<td>412 (92)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Family history of AAA</td>
<td>24 (5)</td>
<td>49 (11)</td>
<td>0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>179 (38)</td>
<td>237 (53)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>128 (27)</td>
<td>214 (48)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CAD</td>
<td>50 (11)</td>
<td>179 (40)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>56 (12)</td>
<td>56 (12)</td>
<td>0.718</td>
</tr>
</tbody>
</table>

Numbers and percentages (in parentheses) of cases and controls with associated previously diagnosed pathology are shown. The P values are from univariate comparisons of cases and controls (all \( \chi^2 \) test, 1 df).
p15/CDKN2B in both physiological and pathological conditions.\textsuperscript{13} p16/CDKN2A and p15/CDKN2B lie in an adjacent segment of chromosome 9 to rs1333049 and ANRIL, and p14/ARF is encoded by an alternative exon 1 and by exons 2 and 3 of p16/CDKN2A. This suggests that the coordinated expression of ANRIL with these genes may reflect regulation of these genes by ANRIL through a mechanism such as RNA interference. Importantly, in the context of the present finding, the cyclin-dependent kinases as well as p14/ARF are known to play a central role in the regulation of the cell cycle and may be implicated in the pathogenesis of atherosclerosis through their role in transforming growth factor-\(\beta\)-induced growth inhibition.\textsuperscript{14,15} Cell growth and inhibition and apoptosis, especially of smooth muscle cells, are also clearly of relevance to the pathogenesis of AAA.\textsuperscript{16} Further work is required to establish whether ANRIL-mediated cyclin-kinase–dependent effects on cell growth are the mechanism by which the chromosome 9p locus affects risk of AAA. If this is the case, it could provide a novel therapeutic target to prevent their development or progression in those at high risk. In summary, we report strong evidence from a case-control study with ultrasound-assessed controls that a locus on chromosome 9p is associated with AAA.

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Disclosures

None.

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