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. (Circ Cardiovasc Genet. 2008;1:39-42.)

Key Words: aneurysm ■ aorta ■ cardiovascular diseases ■ genetics

A bdominal aortic aneurysm (AAA) is a major cause of death in the Western world and accounts for \( \approx 10,000 \) deaths per annum in England and Wales, mostly from rupture.1 The underlying final pathological cause is proteolytic destruction of the aortic wall, but the factors that initiate this proteolytic process are unknown.2 There is a positive association of AAA with some atherosclerotic risk factors such as a smoking and hypertension but a negative association with diabetes mellitus.3 In addition, there is a strong genetic component to AAA, with first-degree relatives having a 10-fold increased risk of the disease.4 The mode of inheritance of AAA is unknown, although pedigree studies have suggested that it is probably polygenic.5 To date, most studies of candidate genes have proven to be disappointing.6

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.10 We investigated the association between this locus and the presence of an AAA to determine whether or not this was a genuine link.

Subjects

A case-control study was performed. Cases with AAA were recruited from 3 sources: an ongoing regional AAA screening program (which screens healthy 65-year-old men for AAA by ultrasonography), the vascular outpatient clinic, and the vascular admissions unit of a university teaching hospital (University Hospitals of Leicester National Health Service Trust). Unrelated white healthy controls were recruited from the same AAA screening program and the general surgical unit of the same hospital. Because of the fact that the controls were predominately recruited from a male-only AAA screening program, the majority of these controls were male. All cases and controls were screened for AAA by either ultrasonography or computed tomography. AAA was defined as abdominal aortic diameter \( > 3 \) cm. Controls were defined as those with maximal infrarenal aortic of \( 2.5 \) cm. Both male and female subjects were included in the study. Demographic and phenotypic data such as age, gender, smoking history, family history of AAA, history of hypertension (defined as blood pressure \( > 140/90 \) mm Hg or existing treatment), hyperlipidemia (defined as total cholesterol \( > 5.5 \) mmol/L or existing treatment), diabetes mellitus, and presence of CAD were recorded for each participant. CAD was defined as 1 or more of a history of myocardial infarction, previous coronary artery bypass surgery, or percutaneous coronary angioplasty. Those

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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

### Table 1. Distribution of Demographic and Other Phenotypes in AAA Cases and Controls

<table>
<thead>
<tr>
<th></th>
<th>Control (n=815)</th>
<th>AAA (n=899)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>798 (98)</td>
<td>814 (91)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tobacco use</td>
<td>556 (68)</td>
<td>799 (90)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AAA families</td>
<td>36 (4)</td>
<td>63 (9)</td>
<td>0.001</td>
</tr>
<tr>
<td>Hypertension</td>
<td>309 (38)</td>
<td>528 (60)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>294 (46)</td>
<td>401 (53)</td>
<td>0.01</td>
</tr>
<tr>
<td>CAD</td>
<td>94 (12)</td>
<td>262 (29)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>85 (11)</td>
<td>87 (10)</td>
<td>0.95</td>
</tr>
</tbody>
</table>

Numbers and percentages (in parentheses) of cases and controls with associated previously diagnosed pathology are shown. Data were missing or unknown for hyperlipidemia in 148 cases and 174 controls; for diabetes mellitus in 55 cases and 4 controls; for family history of AAA in 156 cases and 5 controls; and for hypertension in 14 cases and 6 controls. The P values are from univariate comparisons of cases and controls (all χ² test, 1 df).

### Table 2. rs1333049 Genotype Frequencies in the AAA and Control Groups

<table>
<thead>
<tr>
<th></th>
<th>GG</th>
<th>GC</th>
<th>CC</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>217</td>
<td>429</td>
<td>169</td>
<td>0.015</td>
</tr>
</tbody>
</table>

For each genotype, the absolute number (and percentage in parentheses) of subjects is shown. The P value is from the overall comparison of the distribution of genotypes in cases and controls.

### 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).
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; \( P = 0.054 \)) 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 (\( P = 0.723, 6 \text{ df, } \chi^2 \)) 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 aneurysms\(^12\) 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 ≈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.\(^13\) Furthermore, expression of ANRIL is coordinated with that of p14/ARF and possibly also the cyclin-dependent kinases p16/CDKN2A and

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**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>&lt;0.001</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th></th>
<th></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 (\( \chi^2 \) 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.

**References**

**CLINICAL PERSPECTIVE**
This study successfully replicates the association of a locus known to be associated with coronary artery disease and now also with abdominal aortic aneurysm (AAA) in addition. This has been achieved through the examination of a cohort of patients with AAA and a robust control group, all screened and found not to have AAA. The implications of this confirmed finding are that variations in the genomic features in this region (cyclin-dependent kinase and p14/ARF genes and the noncoding RNA ANRIL), all of which are involved in cell cycle regulation, are implicated in the development of AAA. These pathways represent a hitherto unsearched area in the field of aneurysm biology. If further work demonstrates a role for these pathways in aneurysm pathogenesis, they represent a potential therapeutic target to prevent the growth of small AAs and reduce the risk of rupture and/or the requirement for surgical intervention in this all too often physiologically compromised patient group.
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