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Genome Scan for Familial Abdominal Aortic Aneurysm Using Sex and Family History as Covariates Suggests Genetic Heterogeneity and Identifies Linkage to Chromosome 19q13

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Background—Abdominal aortic aneurysm (AAA) is a relatively common disease, with 1% to 2% of the population harboring aneurysms. Genetic risk factors are likely to contribute to the development of AAAs, although no such risk factors have been identified.

Methods and Results—We performed a whole-genome scan of AAA using affected-relative-pair (ARP) linkage analysis that includes covariates to allow for genetic heterogeneity. We found strong evidence of linkage (logarithm of odds [LOD] score = 4.64) to a region near marker D19S433 at 51.88 centimorgans (cM) on chromosome 19 with 36 families (75 ARPs) when including sex and the number of affected first-degree relatives of the proband (Naff) as covariates. We then genotyped 83 additional families for the same markers and typed additional markers for all families and obtained a LOD score of 4.75 (P=0.00014) with sex, Naff, and their interaction as covariates near marker D19S416 (58.69 cM). We also identified a region on chromosome 4 with a LOD score of 3.73 (P=0.0012) near marker D4S1644 using the same covariate model as for chromosome 19.

Conclusions—Our results provide evidence for genetic heterogeneity and the presence of susceptibility loci for AAA on chromosomes 19q13 and 4q31. (Circulation. 2004;109:2103-2108.)

Key Words: aorta ■ aneurysm ■ genetics ■ mapping

Approximately 15% of patients with abdominal aortic aneurysms (AAAs) and without any recognizable connective tissue disorder, such as Ehlers-Danlos syndrome or Marfan syndrome, have a positive family history for AAA.1 Two segregation studies favored a genetic model in explaining the familial aggregation of AAA and suggested the presence of a major gene effect.2,3 Finding a susceptibility gene for AAA could lead to a simple DNA test to identify individuals at risk for developing an AAA. Such a test could be extremely useful because surgery for unruptured AAA is highly successful, with low mortality and morbidity.4 However, diagnosing AAAs is difficult because most AAAs are asymptomatic before their rupture, and population-based ultrasonography screening to detect AAAs is not used routinely.

The aim of the present study was to find susceptibility loci for AAA with the use of linkage analysis with covariates to allow for locus heterogeneity.5–7 We used affected-relative-pair (ARP) linkage analyses, methods recognized as useful for identifying genes in complex genetic diseases.8 Additionally, we chose the 2-phase/2-stage design for cost-effectiveness and for minimizing the effort required in genotyping while maintaining statistical power to detect linkage.9

Methods

Subjects and Phenotyping

Families with at least 2 members with AAA10 were identified; details on the family collection have been reported previously11 and are available from the Center for Molecular Medicine and Genetics (H.S., G.T., T.O., M.S., H.K.) and Department of Surgery (H.K.), Wayne State University School of Medicine, Detroit, Mich; Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, Ohio (J.M.O., S.G.B., D.M.D.); Departments of Vascular Surgery (C.v.V.–v.K.) and Clinical Genetics (G.P.), Free University Medical Center, Amsterdam, the Netherlands; Departments of Cardiovascular Surgery (N.S., R.L., O.D.) and Human Genetics (A.V.), University Hospital of Liège, Liège, Belgium; Department of Surgery, Dalhousie University, Halifax, Nova Scotia, Canada (G.L.M., C.A.); Department of Surgery, University of Toronto, Toronto, Ontario, Canada (A.G.L., M.B.); and Department of Surgery, Hiroshima University, Hiroshima, Japan (T.S.). Drs Buxbaum and Shibamura are also affiliated with the University of Pittsburgh, Pennsylvania.

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TABLE 1. Characteristics of AAA Families

<table>
<thead>
<tr>
<th>Category</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of families</td>
<td>36</td>
<td>83</td>
<td>119</td>
</tr>
<tr>
<td>Average No. of affected individuals per family (range)</td>
<td>3.4(2–7)</td>
<td>3.1(2–7)</td>
<td>3.2(2–7)</td>
</tr>
<tr>
<td>Families with at least 4 affected first-degree relatives (%)</td>
<td>9 (25)</td>
<td>29 (35)</td>
<td>38 (32)</td>
</tr>
<tr>
<td>No. of affected individuals genotyped (male)</td>
<td>86 (75)</td>
<td>195 (155)</td>
<td>281 (230)</td>
</tr>
</tbody>
</table>

*Individuals genotyped (male)*
- Elective surgery: 67 (61) vs. 131 (104) vs. 198 (165)
- Rupture: 8 (7) vs. 11 (11) vs. 19 (18)
- Detected by ultrasonography: 11 (7) vs. 53 (40) vs. 64 (47)
- Unconfirmed: 12 (11) vs. 24 (20) vs. 36 (31)
- Elective surgery: 10 (8) vs. 17 (14) vs. 27 (22)
- Detected by ultrasonography: 2 (1) vs. 10 (6) vs. 12 (7)
- Unconfirmed: 5 (3) vs. 7 (4) vs. 12 (7)

*Other affected individuals not genotyped (male)*
- Elective surgery: 52 (20) vs. 67 (26) vs. 119 (46)
- ASPs genotyped: 62 vs. 151 vs. 213
- Other ARPs genotyped: 13 vs. 9 vs. 22

Total ARPs in study: 75 vs. 160 vs. 235

*There were a total of 87 (29 in group 1 and 58 in group 2) affected first-degree relatives who were known to have AAA but from whom no sample was available because of death before the start of the study (n=73) or unwillingness to provide a sample (n=14). In 5 and 7 such cases in groups 1 and 2, respectively, information about details of AAA was not available.*

Design for DNA Linkage Study
We used an ARP design because the mode of inheritance of AAA is unknown and because an unaffected individual may develop an AAA subsequently or carry the susceptibility gene with incomplete penetrance. A 2-phase/2-stage design for DNA linkage analysis was chosen,9 in which a 10- to 15-centimorgan (cM) genome scan is performed on a relatively small number of ARPs (stage 1 of phase I), followed by typing of additional markers in regions detected in stage 1 (stage 2 of phase I), and finally followed by additional typing of new ARPs (phase II) in all positive regions obtained in the first phase. For the combined data set of 213 affected sibling pairs (ASPs) and 22 other ARPs from 119 families (groups 1 and 2; Table 1), we had at least 95% power to detect “significant linkage” (logarithm of odds [LOD] score of 3.6)15 for a locus with a locus-specific relative risk of 2.3 in the absence of locus heterogeneity.

Genotyping
We isolated genomic DNA from peripheral blood using a Puregene kit (Genentra Systems, Inc). A whole-genome scan was performed by the Mammalian Genotyping Service with the use of screening set 10 with 405 highly polymorphic microsatellite markers and an average marker-to-marker distance of 10 cM.16 Additional microsatellite markers on chromosome 19 were genotyped as described previously.17 Before genotyping polymerase chain reactions were performed, a whole-genome amplification was carried out to increase the amount of template DNA available for genotyping and to ensure that limited resources were used cost-effectively.18 Additional genotyping on chromosomes 3, 4, 5, 6, 9, 14, and 21 after the whole-genome scan was performed by deCODE Genetics Inc. A slightly smaller number (116 ARPs) of samples were genotyped in group 2 for these chromosomes compared with the number of samples genotyped for chromosome 19 (157 ARPs) in our own laboratory, where new ARPs were included into the study continuously. In addition, 2 new ASPs and 1 other new ARP were identified in group 1 families while the study was in progress, and they were included in chromosome 19 analyses.

Statistical Analyses
The genotype data were analyzed for genetic linkage with the multipoint model-free ARP LOD score analysis with the use of the computer program LODPAL from S.A.G.E. (version 4.2).19 To allow for covariate-related locus heterogeneity, we applied a covariate-based ARP LOD score method.8 The model is a 1-parameter modification of the conditional logistic parameterization of the ASP LOD score introduced by Olson.6 An optimal mode of inheritance parameter20 is specified that allows one to fit only a single additional parameter per covariate. The model is parameterized in...
terms of offspring recurrence risk ratio ($\lambda_1$), conditional on $K$ covariates $x_k$, as follows

$$\lambda_1(x) = \exp(\beta + \sum y_i x_i)$$

where $\beta$ is a parameter that measures the “average” linkage in the sample, and the $y_i$ are covariate-specific parameters that measure the change in linkage as a function of the covariates and in terms of the recurrence risk ratio for monozygotic twins ($\lambda_2$), conditional on $K$ covariates $x_k$, as follows

$$\lambda_2(x) = 3.634\lambda_1(x) - 2.634.$$  

To simplify specification of constraints on parameter estimates, to improve numerical stability, and so that $\lambda_2$ reflects average allele sharing, all covariates are centered around their sample mean before inclusion. In general, the values of $\beta$ and $y_i$ depend on the choice of “coding scheme” for the covariates; a linear transformation of the covariate changes neither the LOD score nor the estimates of covariate-specific recurrence risk ratios. More importantly, conclusions about the existence of locus heterogeneity and the extent or nature of locus heterogeneity do not depend on the estimated value of $\beta$ (which may equal zero).

Asymptotic distributions of the resulting likelihood ratio tests were used to obtain probability values. Critical values for the LRSs were obtained as follows. The distribution of the LRS for the basic 1-parameter model is a 50:50 mixture of a point mass at zero and a $\chi^2$ distribution with 1 df. Addition of $K$ covariates gives an LRS with a distribution that is a 50:50 mixture of a $\chi^2$ with $K$ df and a $\chi^2$ with $K - 1$ df. The difference in LRS between nested models that differ by $J$ covariates has a $\chi^2$ distribution with $J$ df. One can therefore test both the significance of the contribution of a covariate and the overall evidence for linkage. The overall evidence for linkage includes information about both the “average” linkage for the sample and the change in linkage as a function of the covariate.

### Results

A whole-genome scan was performed with 36 AAA families, including 62 ASPs and 13 other ARPs (group 1; Table 1). We performed a model-free multipoint linkage analysis and identified 4 regions, on chromosomes 3, 4, 6, and 21, as significant at the $\alpha = 0.05$ level (baseline values in Table 2). We then extended the analyses to include sex and number of affected first-degree relatives of the proband ($N_{af}$) as covariates, and a total of 12 regions on chromosomes 3, 4, 5, 6, 9, 14, 19, and 21 were identified with a covariate effect.

### Table 2. Group 1 and 2 LOD Scores for Baseline and Covariate Models for Regions With Largest LOD Scores for Group 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Chromosome</th>
<th>Map Position, cM</th>
<th>Flanking Markers</th>
<th>Multipoint LOD Score</th>
<th>Baseline† + Sex + N_{af}‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>94</td>
<td>D3S3644–27666CA8</td>
<td>0.94</td>
<td>2.06¶</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>98</td>
<td>27486CA1§</td>
<td>0.00</td>
<td>1.17</td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>178</td>
<td>D3S523–D3S1574</td>
<td>0.00</td>
<td>3.55¶</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>169</td>
<td>D3S523–D3S1574</td>
<td>0.10</td>
<td>0.42</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>28</td>
<td>D4S403–D4S1567</td>
<td>0.10</td>
<td>2.86¶</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>16</td>
<td>D4S394</td>
<td>0.00</td>
<td>0.79</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>73</td>
<td>D4S248–D4S2432</td>
<td>0.63¶</td>
<td>3.00¶</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>68</td>
<td>D4S355–D4S2978</td>
<td>0.35</td>
<td>3.20¶</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>144</td>
<td>D4S1644–D4S1567</td>
<td>0.54</td>
<td>4.45¶</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>132</td>
<td>D4S2959</td>
<td>0.00</td>
<td>3.03¶</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>144</td>
<td>D5S1983–D5S2011</td>
<td>0.00</td>
<td>3.30¶</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>141</td>
<td>D5S1983</td>
<td>0.72¶</td>
<td>1.14</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>183</td>
<td>D5S211–D5S2008</td>
<td>0.01</td>
<td>2.86¶</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>190</td>
<td>D5S211–D5S2008</td>
<td>0.00</td>
<td>0.28</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>187</td>
<td>D6S1719–D6S1027</td>
<td>0.61¶</td>
<td>4.22¶</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>186</td>
<td>D6S1719–D6S1027</td>
<td>0.36</td>
<td>0.43</td>
</tr>
<tr>
<td>1</td>
<td>9</td>
<td>121</td>
<td>D9S930–D9S177</td>
<td>0.00</td>
<td>5.83¶</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>126</td>
<td>D9S177–D9S2145</td>
<td>0.00</td>
<td>0.84</td>
</tr>
<tr>
<td>1</td>
<td>14</td>
<td>72</td>
<td>D14S63–D14S37§</td>
<td>0.00</td>
<td>2.66¶</td>
</tr>
<tr>
<td>2</td>
<td>14</td>
<td>75</td>
<td>D14S37§–D14S588</td>
<td>0.00</td>
<td>0.68</td>
</tr>
<tr>
<td>1</td>
<td>19</td>
<td>50</td>
<td>D19S931–D19S433</td>
<td>0.00</td>
<td>4.64¶</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>59</td>
<td>D19S245–D19S587</td>
<td>0.00</td>
<td>1.22</td>
</tr>
<tr>
<td>1</td>
<td>21</td>
<td>21</td>
<td>D21S1257–D21S2052</td>
<td>0.88¶</td>
<td>2.45¶</td>
</tr>
<tr>
<td>2</td>
<td>21</td>
<td>21</td>
<td>D21S1257–D21S2052</td>
<td>0.00</td>
<td>0.61</td>
</tr>
</tbody>
</table>

*Indicates position of peak LOD score based on Marshfield genetic map.†Without covariates or ‡with sex and $N_{af}$ as covariates. §These markers are from deCODE marker set. ¶$P<0.05$. £$P<0.01$.  

Critical values for the LRSs were obtained as follows. The distribution of the LRS for the basic 1-parameter model is a 50:50 mixture of a point mass at zero and a $\chi^2$ distribution with 1 df. Addition of $K$ covariates gives an LRS with a distribution that is a 50:50 mixture of a $\chi^2$ with $K$ df and a $\chi^2$ with $K + 1$ df. The difference in LRS between nested models that differ by $J$ covariates has a $\chi^2$ distribution with $J$ df. One can therefore test both the significance of the contribution of a covariate and the overall evidence for linkage. The overall evidence for linkage includes information about both the “average” linkage for the sample and the change in linkage as a function of the covariate.
significant at the α=0.01 level, suggesting the presence of genetic heterogeneity (Table 2).

Twelve regions that were significant in the whole-genome scan were selected for a follow-up study, and additional microsatellite markers were genotyped in the 36 families and in 83 new AAA families that included 151 ASPs and 9 other ARPs (groups 1 and 2; Table 1). Three loci (68 and 132 cM on chromosome 4, and 141 cM on chromosome 5) showed some evidence of linkage in group 2 (Table 2), and these regions were selected for detailed analyses (Table 3). Table 3 shows the LOD scores and parameter estimates for groups 1 and 2 as well as the total sample at the location that gave the highest LOD score for the total sample. In the combined analysis with groups 1 and 2 together, the locus on chromosome 5 did not appear significant (Table 3). The region at 140 cM on chromosome 4 had a LOD score of 3.73 (P=0.0012) (Table 3). The 70-cM region had a peak LOD score of 3.13 (P=0.0042), although the parameter estimates were unstable (not shown), and we therefore report the LOD score of 2.41, which was 4 cM away from the peak, to be able to give more accurate parameter estimates (Table 3).

The chromosome 19 region was also analyzed further which was 4 cM away from the peak, to be able to give more

### TABLE 4. Multiple Regression Analysis of Chromosome 19 at 58.69 cM (D19S416)

<table>
<thead>
<tr>
<th>Model</th>
<th>LOD Score</th>
<th>Parameter Estimates</th>
<th>LOD Score</th>
<th>Parameter Estimates</th>
<th>LOD Score</th>
<th>Parameter Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>+ Sex</td>
<td>0.45</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>+ N</td>
<td>3.30†‡§</td>
<td>0.00030</td>
<td>0.00</td>
<td>0.00</td>
<td>1.22‡‡</td>
<td>0.0039</td>
</tr>
<tr>
<td>+ Sex + N</td>
<td>3.61</td>
<td>0.00054</td>
<td>0.00</td>
<td>0.00</td>
<td>1.74</td>
<td>0.0032</td>
</tr>
<tr>
<td>+ Sex + N + sex*N</td>
<td>4.38</td>
<td>0.00031</td>
<td>0.00</td>
<td>0.00</td>
<td>4.12‡‡</td>
<td>0.00054</td>
</tr>
</tbody>
</table>

*No. of ARPs genotyped for chromosome 19 was larger than that analyzed for the other chromosomes (for details, see Methods). For definition of other footnotes, see Table 3.
plausible candidate genes. The highest LOD score on chromosome 19 for group 2 was 4.12 (P=0.00054) near D19S416 and 58.69 cM from the p-terminus when sex, Naff, and their interaction were used as covariates (Table 4). In the combined analysis with groups 1 and 2, including 213 ASPs and 22 other ARPs, the maximum LOD score was 4.75 (P=0.00014) at 58 cM, just proximal to D19S416, with sex, Naff, and their interaction as covariates (Table 4). The interaction term (sex*Naff) was significant in the total sample (P=0.00317) as well as in the 2 subsamples. These results suggested that female-female pairs from families with larger numbers of affected persons are most at risk from this locus, although this locus also gives substantial risk to male-male pairs from families with fewer affected persons. Both groups 1 and 2 had the peak LOD score at same location on chromosome 19 (Figure). The best, most parsimonious model was the one with Naff as a covariate in group 1 and a model using sex, Naff, and their interaction as covariates in group 2 (Table 4).

Discussion

We found no evidence of linkage unless sex and number of affected persons were included as covariates in the linkage model. How then should our results be interpreted? As Dizier and coworkers have shown, absence of a linkage signal can be due to a factor on which the siblings differ, such as a characteristic of the disease (eg, severity), or an environmental factor. For common diseases that are genetically complex, such situations may be the rule rather than the exception. By allowing for heterogeneity in the analysis by including covariates chosen a priori, we avoid these concerns and are able to detect linkage signals obscured by the presence of heterogeneity.

No prior DNA linkage studies with AAA exist, although 3 studies investigated familial thoracic aortic aneurysms and dissections (TAAD) and identified linked loci on 5q, 11q, and 3p24–25. Because our collection of AAA families excluded patients with TAAD and the AAA loci do not overlap with the TAAD loci, different genetic risk factors are probably involved in the development of TAAD and AAA.

There are several plausible candidate genes in the 2 regions with the highest LOD scores, such as IL15 (interleukin 15; a plausible candidate gene with respect to inflammation in AAA), GAB1 (GRB2-associated binding protein 1; an important mediator of branching tubulogenesis and a central protein in cellular growth response, transformation, and apoptosis), and EDNRA (endothelin receptor type A; an endothelin-1 receptor expressed in many human tissues with the highest level in the aorta) around 140 cM on chromosome 4, as well as LR3 (LDL receptor-related protein 3), HPN (transmembrane protease, serine 1; a serine-type peptidase involved in cell growth and maintenance), PDCD5 (programmed cell death 5; a protein expressed in tumor cells during apoptosis independent of the apoptosis-inducing stimuli), and PEKD (peptidase D; an Xaa-Pro dipeptidase important in collagen catabolism) on chromosome 19. LR3 is particularly interesting because conditional knockout mice for LR3, another member of the gene family, developed arterial aneurysms and atherosclerosis.

It is likely that additional AAA loci will be identified by testing other possible covariates, such as smoking, hypertension, and coronary artery disease, which was not possible in this study because these risk factors are so common both in the general population and in patients with AAA that the relatively small number of families in this study did not provide enough power to study them.

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References


