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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 (N_{aff}) 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, N_{aff}, 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 AAA[10] were identified; details on the family collection have been reported previously[11] and are

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TABLE 1. Characteristics of AAA Families

Category	Group 1	Group 2	Total
No. of families	36	83	119
Average No. of affected individuals per family (range)	3.4(2–7)	3.1(2–7)	3.2(2–7)
Families with at least 4 affected first-degree relatives (%)	9 (25)	29 (35)	38 (32)
No. of affected individuals genotyped (male)	86 (75)	195 (155)	281 (230)
Status of AAA in			
Individuals genotyped (male)			
Elective surgery	67 (61)	131 (104)	198 (165)
Rupture	8 (7)	11 (11)	19 (18)
Detected by ultrasonography	11 (7)	53 (40)	64 (47)
Other affected individuals not genotyped (male)*			
Elective surgery	12 (11)	24 (20)	36 (31)
Rupture	10 (8)	17 (14)	27 (22)
Detected by ultrasonography	2 (1)	10 (6)	12 (7)
Unconfirmed	5 (3)	7 (4)	12 (7)
Unaffected individuals genotyped (male)	52 (20)	67 (26)	119 (46)
ASPs genotyped	62	151	213
Other ARPs genotyped	13	9	22
Total ARPs in study	75	160	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.

summarized in Table 1. An accepted definition of arterial aneurysm[10] was used.[12–14] Patients were identified from surgery records at vascular surgery units and were then contacted; consent was obtained, and family histories were collected. A certified vascular surgeon led the effort to identify AAA patients and affected family members. Family histories of any new patients presenting for surgical repair of AAAs at these sites were obtained in interviews conducted by a research nurse specifically trained for this work. In most cases, only a limited amount of information was available from relatives of second degree or greater. A specific questionnaire assessing skin and skeletal manifestations characteristic of Ehlers-Danlos syndrome type IV or Marfan syndrome was used to identify individuals with these disorders. Families with these disorders were excluded from the study. Whenever possible, the AAA diagnosis of a deceased family member was verified by requesting autopsy or medical records. Some family members, if aged ≥50 years, were examined by ultrasonography and were identified as affected if the infrarenal aortic diameter was ≥3.0 cm, a cutoff point used previously.[14] Occurrences of isolated iliac artery or other aneurysms (such as thoracic or thoracoabdominal) were noted but were not included in the study. All families were white: 42 Canadian, 36 Dutch, 23 Belgian, 10 American, 3 British, 3 Finnish, 1 Italian, and 1 Swedish. The study was approved by the institutional review boards of Wayne State University School of Medicine and each patient recruiting center,[11] and the subjects gave informed consent.

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 (Gentra 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.[6] 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 parameter[20] is specified that allows one to fit only a single additional parameter per covariate. The model is parameterized in

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

Group	Chromosome	Map Position,* cM	Flanking Markers	Multipoint LOD Score Baseline†	+Sex+N_aff‡
1	3	94	D3S3644–27666CA8.D§	0.94‖	2.60¶
2	3	98	27486CA1§	0.00	1.17
1	3	178	D3S3523–D3S1574	0.00	3.55¶
2	3	169	D3S3523–D3S1574	0.10	0.42
1	4	28	D4S403–D4S1567	0.10	2.86¶
2	4	16	D4S394	0.00	0.79
1	4	73	D4S3248–D4S2432	0.63‖	3.00¶
2	4	68	D4S3355–D4S2978	0.35	3.20¶
1	4	144	D4S1644–D4S1586	0.54	4.45¶
2	4	132	D4S2959	0.00	3.03¶
1	5	144	D5S1983–D5S2011	0.00	3.30¶
2	5	141	D5S1983	0.72‖	1.14
1	5	183	D5S211–D5S2008	0.01	2.86¶
2	5	190	D5S211–D5S2008	0.00	0.28
1	6	187	D6S1719–D6S1027	0.61‖	4.22¶
2	6	186	D6S1719–D6S1027	0.36	0.43
1	9	121	D9S930–D9S177	0.00	5.83¶
2	9	126	D9S177–D9S2145	0.00	0.84
1	14	72	D14S63–DG14S37§	0.00	2.66¶
2	14	75	DG14S37§–D14S588	0.00	0.68
1	19	50	D19S931–D19SS433	0.00	4.64¶
2	19	59	D19S245–D19S587	0.00	1.22
1	21	21	D21S1257–D21S2052	0.88‖	2.45¶
2	21	21	D21S1257–D21S2052	0.00	0.61

*Indicates position of peak LOD score based on Marshfield genetic map.[16]
Multipoint LOD score analyzed †without covariates or ‡with sex and N_{aff} as covariates.
§These markers are from deCODE marker set.
‖$P<0.05$.
¶$P<0.01$.

terms of offspring recurrence risk ratio (λ_1), conditional on K covariates x_k, as follows

$$(1) \qquad \lambda_1(x)=\exp(\beta+\Sigma_{k=1}^{K}\gamma_k x_k),$$

where β is a parameter that measures the "average" linkage in the sample, and the γ_k 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 (λ_2), conditional on K covariates x_k, as follows

$$(2) \qquad \lambda_2(x)=3.634\lambda_1(x)-2.634.$$

To simplify specification of constraints on parameter estimates, to improve numerical stability, and so that β reflects average allele sharing, all covariates are centered around their sample mean before inclusion. In general, the values of β and γ_k 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 β (which may equal zero).

Asymptotic distributions of the resulting likelihood ratio tests were used to obtain probability values.[6] We report as LOD scores the likelihood ratio statistics (LRSs) divided by 4.605 (ie, $2\log_e10$).

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 χ^2 distribution with 1 df. Addition of K covariates gives an LRS with a distribution that is a 50:50 mixture of a χ^2 with K df and a χ^2 with $K+1$ df. The difference in LRS between nested models that differ by J covariates has a χ^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_{aff}) 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 3. Multiple Regression Analysis of Chromosome 4 and 5 Regions

Model*	Group 1 (62 ASPs, 75 Total ARPs)				Group 2 (106 ASPs, 116 Total ARPs)				Total Sample (168 ASPs, 191 Total ARPs)			
	LOD Score	P†	β	$\gamma_1, \gamma_2, \ldots$	LOD Score	P†	β	$\gamma_1, \gamma_2, \ldots$	LOD Score	P†	β	$\gamma_1, \gamma_2, \ldots$
Chromosome 4 at 70 cM (*D4S2978*)												
Baseline	0.24	…	0.15	…	0.35	…	0.11	…	0.73	0.0334	0.13	…
+Sex	0.39	…	0.12	−0.31	0.85	…	0.17	0.29	0.96	…	0.16	0.17
+ N_{aff}	0.39	…	0.20	0.11	1.80§	0.0099	0.40	0.42	1.41	0.0249	0.18	0.12
+Sex+N_{aff}	0.82	…	0.35	−1.03, −0.23	3.20‡§	0.0013	0.71	0.21, 0.67	1.65	0.0387	0.21	0.17, 0.12
+Sex+N_{aff}+sex*N_{aff}	3.75‡§	0.0012	0.26	−0.27, 1.24, −0.73	3.74	0.0012	1.43	2.28, −1.39, 1.31	2.41‡§	0.018	1.17	2.64, −1.51, 1.35
Chromosome 4 at 140 cM (*D4S1644*)												
Baseline	0.63	0.0443	0.28	…	0.00	…	0.00	…	0.00	…	0.00	…
+Sex	0.81	…	0.28	−0.49	2.40‡§	0.0024	0.00	−0.40	3.04‡§	0.00055	0.04	−0.41
+N_{aff}	0.81	…	0.35	−0.12	0.01	…	0.00	0.01	0.12	…	0.00	−0.04
+Sex+N_{aff}	1.59‡§	0.0440	0.42	−0.85, −0.17	2.55	0.0056	0.00	−0.42, 0.02	3.35	0.00097	0.05	−0.43, −0.03
+Sex+N_{aff}+sex*N_{aff}	1.69	…	0.33	−0.09, 0.45, −0.35	2.74	0.0094	0.00	−0.46, −0.05, 0.03	3.73	0.0012	0.11	−0.54, −0.20, 0.09
Chromosome 5 at 140cM (*D5S816*)												
Baseline	0.00	…	0.00	…	0.72‡§	0.0343	0.17	…	0.23	…	0.08	…
+Sex	0.09	…	0.00	0.27	1.11	…	0.20	−0.18	0.35	…	0.09	−0.09
+N_{aff}	1.44‡§	0.0232	0.05	0.39	0.76	…	0.18	0.03	0.40	…	0.09	0.05
+Sex+N_{aff}	2.49‡§	0.0063	0.29	0.33, 0.60	1.14	…	0.20	−0.18, 0.03	0.50	…	0.09	−0.08, 0.05
+Sex+N_{aff}+sex*N_{aff}	2.36	0.203	0.20	0.21, 0.62, −0.05	1.45	…	0.23	−0.15, 0.24, −0.10	1.02	…	0.12	−0.06, 0.25, −0.11

*Linkage analysis models: baseline, without covariates; sex, sex as a covariate; N_{aff}, No. of affected first-degree relatives of probands as a covariate; sex+N_{aff}, sex and N_{aff} as a covariate; sex+N_{aff} + sex * N_{aff}, sex and N_{aff} and their interactions as a covariate.

†P for overall linkage effect (average and covariate-related), if $P<0.05$.

‡Most parsimonious model.

§Significant covariate effect compared with nearest nested model.

significant at the $\alpha=0.01$ 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 because (1) it had the second highest LOD score in the original genome scan (Table 2); (2) we have recently identified a putative locus for intracranial aneurysms on chromosome 19[17]; and (3) it contains a large number of biologically

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

Model	Group 1 (64 ASPs, 78 Total ARPs)*				Group 2 (149 ASPs, 157 Total ARPs)*				Total Sample (213 ASPs, 235 Total ARPs)*			
	LOD Score	P†	β	$\gamma_1, \gamma_2, \ldots$	LCD Score	P†	β	$\gamma_1, \gamma_2, \ldots$	LCD Score	P†	β	$\gamma_1, \gamma_2, \ldots$
Baseline	0.00	…	0.00	…	0.00	…	0.00	…	0.00	…	0.00	…
+Sex	0.45	…	0.00	−0.36	0.00	…	0.00	0.01	0.07	…	0.00	−0.06
+ N_{aff}	3.30‡§	0.00030	0.00	0.40	0.23	…	0.00	0.07	1.22‡	0.039	0.00	0.11
+Sex+N_{aff}	3.61	0.00054	0.00	0.08, 0.48	0.46	…	0.00	−0.18, 0.12	1.74	0.032	0.00	−0.18, 0.14
+Sex+N_{aff}+sex*N_{aff}	4.38	0.00031	0.06	0.41, −0.30, 0.46	4.12‡§	0.00054	0.00	0.14, −0.35, 0.30	4.75‡§	0.00014	0.00	0.14, −0.30, 0.29

*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.
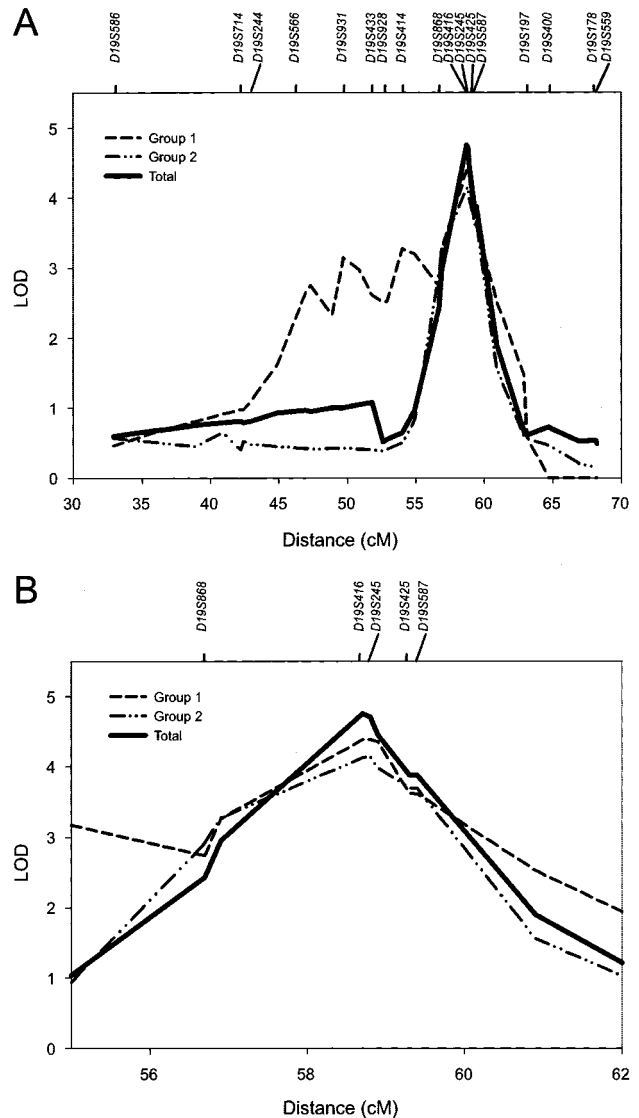
plausible candidate genes.[21] The highest LOD score on chromosome 19 for group 2 was 4.12 ($P=0.00054$) near *D19S416* and 58.69 cM from the p-terminus when sex, N_{aff}, and their interaction were used as covariates (Table 4). In the combined analysis with groups 1 and 2, including 213 ASPs and 22 other ARPs, the maximum LOD score was 4.75 ($P=0.00014$) at 58 cM, just proximal to *D19S416*, with sex, N_{aff}, and their interaction as covariates (Table 4). The interaction term (sex*N_{aff}) 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 N_{aff} as a covariate in group 1 and a model using sex, N_{aff}, 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[22] 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.[5–7] 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,[23] 11q,[24] and 3p24–25.[25] Because our collection of AAA families excluded patients with TAAD[11] 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 *LRP3* (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 *PEPD* (peptidase D; an Xaa-Pro dipeptidase important in collagen catabolism) on chromosome 19.[21,26] *LRP3* is particularly interesting because conditional knockout mice for *LRP1*, another member of the gene family, developed arterial aneurysms and atherosclerosis.[27]

A, Multipoint LOD score plot for AAA on chromosome 19. Sex and N_{aff} were used as covariates. The x axis shows distance in centimorgans on chromosome 19; y axis, LOD score. B, A higher-resolution plot for the region between 55 and 62 cM on chromosome 19, illustrating that several closely spaced markers support the peak shown in A.

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.

Acknowledgments

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