Identification of a Chromosome 11q23.2-q24 Locus for Familial Aortic Aneurysm Disease, a Genetically Heterogeneous Disorder

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Background—Aortic aneurysms cause significant morbidity and mortality, and >20% relate to hereditary disorders. Familial aortic aneurysm (FAA) has been described in such conditions as the Marfan and Ehlers-Danlos type IV syndromes, due to defects in the fibrillin-1 and type III procollagen genes, respectively. Other gene defects that cause isolated aneurysms, however, have not thus far been described.

Methods and Results—We studied 3 families affected by FAA. No family met the diagnostic criteria for either Marfan or Ehlers-Danlos syndrome. Echocardiography defined involvement of both the thoracic and abdominal aorta. In family ANA, candidate gene analysis excluded linkage to loci associated with aneurysm formation, including fibrillin-1, fibrillin-2, and type III procollagen, and chromosome 3p24.2-p25. Genome-wide linkage analysis identified a 2.3-cM FAA locus (FAA1) on chromosome 11q23.3-q24 with a maximum multipoint logarithm of the odds score of 4.4. In family ANB, FAA was linked to fibrillin-1. In family ANF, however, FAA was not linked to any locus previously associated with aneurysm formation, including fibrillin-1 and FAA1.

Conclusions—FAA disease is genetically heterogeneous. We have identified a novel FAA locus at chromosome 11q23.3-q24, a critical step toward elucidating 1 gene defect responsible for aortic dilatation. Future characterization of the FAA1 gene will enhance our ability to achieve presymptomatic diagnosis of aortic aneurysms and will define molecular mechanisms to target therapeutics. (Circulation. 2001;103:2469-2475.)

Key Words: aneurysm ▪ aorta ▪ genetics ▪ mapping

Aortic aneurysms cause significant cardiovascular morbidity and mortality, and their rupture accounts for 1% to 2% of deaths. Although infectious, atherosclerotic, and hemodynamic etiologies may contribute, at least 20% of aneurysms result from inherited disorders. Aortic aneurysms can present along with bicuspid aortic valve and aortic coarctation and as components of rare mendelian disorders, including Turner syndrome, Noonan syndrome, osteogenesis imperfecta, and homocystinuria. Familial aortic aneurysm (FAA) has been related to systemic connective-tissue disorders, such as Ehlers-Danlos type IV and Marfan syndromes, caused by mutations in the type III procollagen (COL3A1) and fibrillin-1 (FBN-1) genes, respectively. The MFS2 locus for Marfan-like disease, including aortic aneurysms, maps to chromosome 3p24.2-p25. Mutations in fibrillin-2 (FBN-2) cause congenital contractural arachnodactyly, variably associated with aortic disease.

Many inherited aortic aneurysms, however, present as isolated cardiovascular disease with or without aneurysmal dilatation of other arterial beds. Isolated thoracic aortic aneurysms (TAAs) and abdominal aortic aneurysms (AAAs) can be transmitted as autosomal dominant traits. Genetic analysis of FAA has been hampered by a dearth of large affected families and by high mortality. Additional obstacles have included incomplete penetrance in some families and indeterminate phenotypes for young individuals. We have now applied age-specific, body surface area–indexed nomograms and linkage analysis to demonstrate that an FAA gene defect maps to chromosome 11q23.3-q24. Identification of specific gene(s) responsible for aneurysm development will enhance our understanding of the vulnerable aorta.
Methods

Clinical Evaluation
All family members in 3 northern European extraction kindreds provided informed consent as required by Weill Medical College's Committee on Human Rights in Research and were evaluated, without knowledge of genotypes, by history, physical examination, ECG, and 2D transthoracic echocardiography. Aortic measurements were made by echocardiography at end diastole perpendicular to the long axis of the aorta via leading-edge technique in views with maximal aortic diameters. Thoracic aortic measurements were made at the aortic annulus, sinuses of Valsalva, sinotubular junction, proximal ascending aorta, aortic arch, and descending aorta. In the abdominal aorta, measurements were made at the level of the celiac axis and the infrarenal aorta. All aortic dimensions, then, were indexed for body surface area. Previously validated age-appropriate nomograms defined aortic dilatation in thoracic segments (individuals of all ages) and abdominal segments (35-year-old adults only). To assign FAA affected status to any individual, we required a family history of aortic aneurysm/dissection, without hypertension or bicuspid aortic valve, as well as 1 of the following: (1) true aortic or other arterial dissection/aneurysm (≥4.5 cm TAA, ≥3.0 cm AAA) diagnosed by any imaging modality, including ultrasound, CT, MRI, or angiography; (2) quantified dilatation of the thoracic aorta of a person of any age; and (3) quantified dilatation of an adult's abdominal aorta. Marfan and Ehlers-Danlos syndromes were excluded by consensus clinical criteria.

Genetic Analyses
Genetic studies were performed using DNA extracted from blood as previously described and with QIAamp columns (Qiagen). Linkage analyses used polymorphic short tandem repeats (STRs) flanking candidate loci: FBN-1: MTS1, MTS2, MTS4; FBN-2: D5S644, D5S659, D5S2059, D5S615, D5S2115; COL3A1: D2S364, D2S389, D2S311, D2S117; MFS2: D3S1293, D3S3700, D3S2335, D3S1567, D3S2466; and 5q-TAA: D5S424, D5S253, D5S641. For genome-wide linkage analysis, STRs (ABI Prism
Mutational Analysis
Mutational analysis of protein-encoding segments of candidate genes [SM22α (accession No. AF01371122); HSP73 (accession No. Y0037123)] was performed by bidirectional sequence analysis of genomic DNA samples. Oligonucleotides were designed from intrinsic sequences. HSP73 exons 2 to 9 were individually amplified by polymerase chain reaction. SM22α exons 2 to 5 were amplified as a single product. Sense and antisense sequencing of amplicons was performed on an ABI-377 sequencer using Big-Dye Terminator Cycle Sequencing reagents (Perkin-Elmer).24

Results
Clinical Evaluations
In family ANA, the proband (II-2, Figures 1 and 2) presented at age 36 with a 5.7-cm ascending TAA and type I aortic dissection. Aortic pathology revealed cystic medial necrosis. Echocardiography revealed 13 of 20 additional family members at risk (ages 2.5 to 65 years) affected by aortic dilatation. Sinuses of Valsalva were enlarged in all but 1 affected person (Figure 3). That individual (III-11, age 15 years) was considered affected by FAA even though the aortic dimension at the sinuses of Valsalva was normal (29 mm, 95% of upper limits of normal), because there was aortic dilatation at the sinotubular junction (27 mm, 112% of upper limits of normal). Involvement of other aortic and arterial segments was present, eg, dilatation of the abdominal aorta (individual II-10, Figure 2) or the left subclavian artery (individual II-8). No valvular abnormalities and no anthropometric, skeletal, ocular, or cutaneous abnormalities suggestive of generalized connective-tissue disease were evident in any family member.

In family ANB (Figure 4), the proband (II-1) underwent repair of a type 1 aortic dissection at age 34 years and required subsequent repairs of abdominal and arch aortic aneurysms. Seven of 14 additional family members at risk exhibited aortic dilatation. All affected individuals had dilatation of the sinuses of Valsalva (Figure 3). Individual I-1 had mild pectus excavatum and II-1 pectus carinatum, but no other skeletal, anthropometric, ocular, or cutaneous abnormalities were noted. No individual met the criteria for Marfan or Ehlers-Danlos type IV syndromes.

In family ANF (Figure 4), the proband (II-4) underwent repair of an ascending TAA at age 33 years. Seven of 11 other family members at risk exhibited TAA and/or dissection. All living affected individuals exhibited aortic dilatation at the sinuses of Valsalva (Figure 3). Individual II-4 had a history of bilateral fifth digit flexion-contractures, retinal detachment, and cataracts. Individual III-5 had a pectus excavatum and bilateral fifth digit flexion-contractures. No member of family ANF, however, met criteria for any known connective-tissue disorder.

Figure 3. Aortic dilatation at sinuses of Valsalva in 3 families with FAA. Aortic dimensions in all FAA-affected individuals in families ANA, ANB, and ANF are plotted as percentage of maximum normal value derived from age- and body surface area-specific nomograms.23 Dotted line indicates 100% of maximum normal diameter. All FAA-affected individuals exhibited aortic root dilatation except 1 in family ANA, who had marked aortic dilatation at the sinotubular junction.

Figure 4. Pedigrees of families ANB and ANF. Generation/subject number and FAA disease status of each family member analyzed are indicated. Squares, male family members; circles, female. Affected and unaffected individuals are represented by solid and open symbols, respectively. Gray symbols represent unknown diagnosis. Slashes denote deceased. FAA is transmitted in these families in an autosomal dominant fashion.
Genetic Analyses

FAA was transmitted through 4 generations in family ANA as a highly penetrant autosomal dominant trait (Figure 1). We initially sought to exclude linkage of FAA in family ANA to chromosomal loci previously associated with aortic aneurysm formation: FBN-1, FBN-2, COL3A1, MFS2, and a recently described 25 chromosome 5q TAA locus. Studies with the FBN-1 intragenic STR MTS4 excluded linkage to FBN-1: Z = 3.75, theta = 0.0; Z = -2.0, theta = 0.042. Similar analyses excluded linkage to the FBN2, COL3A1, MFS2, and 5q-TAA loci (Figure 5). Therefore, we explored other genetic loci to account for FAA in family ANA.

Highly polymorphic STRs dispersed throughout the genome were analyzed. After excluding 35% of the genome, we observed evidence of linkage of FAA by 2-point LOD score to chromosome 11q microsatellites: D11S925 (Z = 3.08, theta = 0.06), and maximal 2-point LOD score with D11S1356 (Z = 3.35, theta = 0.06). Calculations were robust to changes in allele frequencies. Multipoint linkage analyses with other microsatellite markers in the region confirmed statistically significant linkage to the long arm of chromosome 11. They revealed a maximum multipoint LOD score of 4.4, 1.1 cM telomeric to D11S1356 (Figure 6A), consistent with odds of 25 000:1 that the FAA disease gene resides in this region of chromosome 11q. Analysis with only affected individuals confirmed linkage at this locus with a maximum multipoint LOD score of 3.0. Haplotype analyses (Figures 1 and 6B) further refined this locus. Genotyping of individual II-8 revealed recombination between the FAA disease gene and D11S1341. Similarly, we observed recombination between FAA and AFMB031WC9 in individual II-2. Collectively, these data mapped the FAA disease gene to a 2.3-cM locus (hereafter called FAA1) at chromosome 11q23.3-q24 between D11S1341 and AFMB031WC9 (Figure 7).

Analysis of family ANB revealed that FAA in this family was also inherited in a highly penetrant autosomal dominant manner (Figure 4). Genetic analyses excluded linkage to the FBN2, COL3A1, MFS2, and 5q-TAA loci as well as to FAA1 (Figure 8A). Analysis using intragenic FBN1 STRs MTS1 and MTS4, however, did suggest linkage of FAA in family ANB to FBN1, with a pairwise LOD score of 1.5 at theta = 0 for both STRs.

FAA in family ANF was also inherited as a highly penetrant autosomal dominant trait (Figure 4). Analysis with intragenic STRs MTS1, MTS2, and MTS4 distributed over the 200-kb FBN1 gene also excluded linkage to FBN1, with pairwise LOD scores of -3.7, -2.9, and -3.2, respectively, at theta = 0. Moreover, linkage was excluded to other aortic aneurysm loci FBN2, COL3A1, MFS2, 5q-TAA, and FAA1 (Figure 8B). Notably, among the several individuals in family

Figure 5. Multipoint LOD score analysis of candidate loci in family ANA. Multipoint linkage analysis [LOD score < -2.0 (dashed line)] excludes linkage of FAA in family ANA to COL3A1 (black), MFS2 (red), and chromosome 5 loci (green): FBN2 and 5q-TAA. Genetic interval corresponding to each locus is shown.

Figure 6. Multipoint LOD score and haplotype analyses of FAA in family ANA. A, Family ANA genotypes at STRs D11S1356, AFMB031WC9, and D11S925 were used to determine multipoint LOD scores at chromosome 11q2. D11S1356 is arbitrarily plotted at 0 cM on abscissa. Other STRs near FAA locus are indicated along abscissa from centromere to telomere. FAA1 locus interval, based on 95% CI, is shown. A maximum multipoint LOD score of 4.4 is observed at 1.1 cM. B, Haplotype analysis of FAA1. Designations of individuals affected by FAA are in bold italics. FAA1 STRs are ordered from centromere to telomere. Recombination events with FAA disease gene are black, concordant genotypes white, and uninformative genotypes gray. Boxed region shows minimal FAA1 interval.
ANF who were discordant at FAA1 were 2 individuals (II-6, III-3) affected by FAA.

**FAA1 Candidate Gene Analysis**

Matrix metalloproteinases (MMPs) have previously been associated with aortic aneurysm pathogenesis.\(^{26}\) A gene cluster encoding several MMPs (MMP1, MMP3, MMP8, and MMP10) maps to chromosome 11q between D11S1339 and D11S1167.\(^{27}\) We demonstrated, however, that FAA1 resides within the interval flanked by D11S1341 and AFMB031WC9, which maps \(-13\) cM telomeric to the MMP genes.\(^{27}\)

SM22α is a cytoskeletal protein widely expressed in vascular tissue, including aortic smooth muscle, and the SM22α gene\(^{28}\) cytogenetically maps to chromosome 11q23.2.

We therefore searched for mutations in SM22α in individuals from family ANA. Exons encoding protein were amplified from individuals in family ANA and subjected to bidirectional sequence analysis. However, we observed no SM22α sequence variants.

The gene encoding HSP73, a member of the heat-shock protein family that may contribute to vascular disease,\(^{29}\) maps cytogenetically\(^{30}\) to chromosome 11q23.3-q25. Exons encoding protein were analyzed in family ANA. Bidirectional sequence analysis revealed only an intron 5, bp 74, C insertion polymorphism that provided no information to refine FAA1 and no mutations.

**Discussion**

Our study demonstrates that a gene defect for FAA disease is located on chromosome 11q23.3-q24. The FAA1 gene defect can be inherited in an autosomal dominant fashion to result in aortic dilatation, aneurysm formation, and dissection. Several clinical and genetic features distinguish FAA related to the FAA1 locus from aortic aneurysms caused by defects at other genetic loci.

FAA due to the 11q defect in family ANA has no demonstrable extravascular manifestations. Skeletal, ocular, or cutaneous abnormalities typical of connective-tissue disorders that also affect the aorta, eg, Marfan and Ehlers-Danlos syndromes, are not evident in this family. Therefore, we suggest that FAA related to this gene defect is an isolated vascular disorder.

FAA1-related disease affects multiple aortic segments, with dilatation of the thoracic and abdominal aorta. Other large arterial vessels, eg, the subclavian artery in individual II-8 in family ANA, may also be affected by the FAA1 gene defect. Tilson and Dang\(^{31}\) similarly observed extra-aortic vascular dilatation in families with aortic aneurysms and hypothesized that FAA-like syndromes may represent syndromes of "generalized arteriomegaly."\(^{7}\) Notably, however, like several other monogenic forms of aortic aneurysm disease,\(^{25,32}\) FAA in

![Figure 7. Ideogram of chromosome 11 with Giemsa banding pattern and localization of FAA1 locus. Positive bands are black, and pericentromeric region hatched. Numbers indicate cytogenetic band designations. Genetic map with chromosome 11q STRs and MMP genes. FAA1 is within 2.3-cM interval between D11S1341 and AFMB031WC9. Bar=1 cM.](image)

![Figure 8. Multipoint LOD score analysis of candidate loci in families ANB and ANF. Multipoint linkage analysis [LOD score< -2.0 (dashed line)] excludes linkage of FAA to COL3A1 (black), MFS2 (red), FBN2 (green), FAA1 (blue), and 5q-TAA (purple) loci in (A) family ANB and (B) family ANF. Genetic interval corresponding to each locus is shown.](image)
family ANA typically affected the proximal aortic root. All but 1 affected individual in family ANA exhibited annuloaortic ectasia.

Unlike other aortic aneurysm disorders, FAA1-related aortic disease is highly penetrant. There is no evidence of incomplete penetrance in family ANA. Incomplete penetrance is noted in familial aortic disorders caused by genetic loci distinct from FAA1, eg, Marfan syndrome,12 TAA,31,25,32 and AAA.3,35 Other genetically heterogeneous cardiovascular syndromes also exhibit locus-specific degrees of penetrance, eg, cardiomyopathies and long-QT syndrome.4

The apparently higher penetrance of FAA1-related disease also may reflect, in part, differing techniques used to phenotype family members. Others2,3,32 have used the presence or absence of frank aortic aneurysms as a sole diagnostic criterion to determine affected status. We used age-specific, body surface area–indexed nomograms to assess aortic dilation even in the absence of frank aneurysms. Therefore, the clinical evaluations described here detect subtle aortic dilation and affected status with increased sensitivity. In our analyses, it still remains possible that we have underdiagnosed FAA. Although the nomograms we used for evaluation of the thoracic aorta are well validated not only for adults but also for children as young as 1 month old, such extensive data are not currently available for assessment of the abdominal aorta in children and young adults.

The long arm of chromosome 11 has previously been associated with aortic and conotruncal abnormalities. Jacobsen syndrome is a clinical syndrome associated with large associated with aortic and conotruncal abnormalities. Jacobson syndrome exhibits a broad spectrum of congenital anomalies, including dysmorphism, mental retardation, and hematologic abnormalities, left-sided cardiovascular abnormalities are also frequent manifestations.4 Cardiac defects include hypoplastic left heart, ventricular septal defect, coarctation of the aorta, and interruption of the aortic arch.3,33–35 Although Jacobsen syndrome patients exhibit a broad spectrum of congenital cardiovascular abnormalities with 11q23 deletions in Jacobsen syndrome further supports the findings of our linkage studies that as yet unidentified gene(s) that can contribute to aortic structure and homeostasis map to this chromosomal region.

The cluster of MMP genes at chromosome 11q2 might seem obvious candidates to cause aortic aneurysms. Our studies demonstrated, however, that FAA1 is telomeric to the MMP gene cluster. In the present study, we also explored as FAA candidates the chromosome 11q SM22α and HSP73 genes but observed no mutations. Future studies will explore other known and as yet unidentified genes that map to FAA1.

Tunnacliffe et al27 assembled a genomic clone contig of chromosome 11q23 to 11q24 that does include the interval between D11S1341 and AFMB031WC9. Their physical maps show that the FAA1 locus contains several known genes: **HMBS** (hydroxymethylbilane synthase gene associated with porphyria), **MLL** (transcription factor gene mutated in leukemias), **LARG** (guanine nucleotide exchange factor potential MLL translocation partner in acute myelogenous leukemia), **CD3D** (T-cell CD3-antigen δ-subunit gene), **HDLR** (RNA-helicase-2 gene), **CBL2** (oncogene), **H2AX** (histone gene), **THY1** (T-cell antigen gene), and **ATDC** (ataxia-telangiectasia-

group D gene). On the basis of proteins known4–6 to be associated with aneurysmogenesis (fibrillin-1, type III procollagen, and MMPs), one might hypothesize that the FAA1 disease gene would encode an extracellular matrix protein or a protein that modifies vascular extracellular matrix; none of the above FAA1 genes clearly fit that description. The **ATDC** gene has no known function, and further studies will explore its candidacy. Several expressed sequence tags mapped by the Human Genome Project to the FAA1 locus will also be targets of future investigation.

**FAA1** does not account for all hereditary isolated aortic aneurysms. Our analyses of family ANB confirm findings by others7,8 that **FBN1** mutations can cause aortic aneurysm disease without Marfan syndrome. Furthermore, a novel 5q locus for TAA has also been described,23 and we report that some FAA families, eg, ANF, map to no known locus. Ongoing investigation to elaborate additional loci for FAA and associated aortic aneurysm-causing genes may inform candidate- positional cloning strategies to identify the specific mutated gene at FAA1. Analysis of the FAA1 disease gene will ultimately delineate molecular mechanisms for maintenance of vascular wall integrity.

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