Familial Thoracic Aortic Aneurysms and Dissections
Genetic Heterogeneity With a Major Locus Mapping to 5q13-14

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Background—Aneurysms and dissections affecting the ascending aorta are associated primarily with degeneration of the aortic media, called medial necrosis. Families identified with dominant inheritance of thoracic aortic aneurysms and dissections (TAA/dissections) indicate that single gene mutations can cause medial necrosis in the absence of an associated syndrome.

Methods and Results—Fifteen families were identified with multiple members with TAAs/dissections. DNA from affected members from 2 of the families was used for a genome-wide search for the location of the defective gene by use of random polymorphic markers. The data were analyzed by the affected-pedigree-member method of linkage analysis. This analysis revealed 3 chromosomal loci with multiple markers demonstrating evidence of linkage to the phenotype. Linkage analysis using further markers in these regions and DNA from 15 families confirmed linkage of some of the families to 5q13-14. Genetic heterogeneity for the condition was confirmed by a heterogeneity test. Data from 9 families with the highest conditional probability of being linked to 5q were used to calculate the pairwise and multipoint logarithm of the odds (LOD) scores, with a maximum LOD of 4.74, with no recombination being obtained for the marker D5S2029. In 6 families, the phenotype was not linked to the 5q locus.

Conclusions—A major locus for familial TAAs and dissections maps to 5q13-14, with the majority (9 of 15) of the families identified demonstrating evidence of linkage to this locus. The condition is genetically heterogeneous, with 6 families not demonstrating evidence of linkage to any loci previously associated with aneurysm formation. (Circulation. 2001;103:2461-2468.)

Key Words: aorta ■ aneurysm ■ genetics

Aneurysms and dissections of the aorta result primarily from degenerative changes in the aortic wall. Prominent among the factors that lead to this degeneration are aging, arteriosclerosis, hypertension, and specific infectious, inflammatory, or autoimmune diseases that involve the aorta focally or diffusely. Arteriosclerotic lesions are associated with aneurysms and dissections that affect primarily the descending thoracic and abdominal aorta (thoracoabdominal aortic aneurysms, abdominal aortic aneurysms, and type III dissections). In contrast, aneurysms and dissections that affect the ascending aorta are primarily due to lesions that cause degeneration of the aortic media, a poorly understood pathological process called medial necrosis (also called cystic medial necrosis by Erdheim1). Medial necrosis is characterized by degeneration and fragmentation of elastic fibers, loss of smooth muscle cells, and interstitial collections of basophilic-staining ground substance.

Although the pathogenesis of medial necrosis is not understood, it is almost certainly not a single disease entity. Medial necrosis occurs with normal aging of the aorta but can be accelerated by such conditions as hypertension. Medial necrosis of the aorta also occurs in association with genetic syndromes, such as Marfan syndrome (MFS), but is more frequently found in the absence of an associated phenotypic syndrome. Descriptions of families with autosomal dominant inheritance of aortic aneurysms/dissections with medial necrosis on pathological examination indicate that single gene mutations can cause medial necrosis in the absence of an associated syndrome.2,3 In addition, medial necrosis of the proximal aorta with aneurysms/dissections is associated with

Received November 28, 2000; revision received February 23, 2001; accepted March 3, 2001.
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A list of primer sequences used in this study can be found Online at http://www.circulationaha.org.
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Figure 1. Pedigrees of TAA/dissections families that participated in this study. A, TAA/dissections families used for genome-wide scan for defective gene. B, TAA/dissections families in whom phenotype is linked to markers on chromosome 5q. C, TAA/dissections families in whom phenotype is not linked to markers on chromosome 5q. DNA samples used for linkage analysis are indicated below individual symbol on pedigree.
TABLE 1. Data From the Genome-Wide Scan for TAA: Results From Positive Markers

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<th>Chromosome</th>
<th>Locus or Marker</th>
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from a number of other conditions, including Turner syndrome (45,X0), Noonan syndrome, Ehlers-Danlos syndrome type IV, bicuspid aortic valve, coarctation of the aorta, and adult polycystic kidney disease. 4–7

MFS is an autosomal dominant condition characterized by skeletal, ocular, and cardiovascular complications. 8,9 The cardiovascular complications are thoracic aortic aneurysms and dissections (TAA/dissections), along with valvular abnormalities. Medial necrosis observed in MFS patients is the result of mutations in a specific component of the elastic fiber, fibrillin-1. The elastic fiber is composed both morphologically and biochemically of a core made of the protein elastin surrounded by a peripheral mantle of 10-nm microfibrils. Microfibrils contain several proteins, with the major component being fibrillin-1, encoded by FBN1 on chromosome 15.10 Numerous FBN1 mutations have been identified in MFS patients, the vast majority of which are unique to the affected family or individual.11 A second locus for MFS has been identified at 3p24.2-p25 on the basis of linkage analysis of a large French family with skeletal and cardiovascular features of the disorder, but the findings in this family have been controversial.12,13

Fifteen families with autosomal dominant inheritance of TAA/dissections have been identified, and linkage to FBN1 was excluded. The aortic disease in these families is characterized by aneurysms involving the ascending aorta leading to type I and II aortic dissections in the absence of hypertension. The age of onset of the aortic disease is variable, and there is decreased penetrance of the disorder in the families. This article presents data mapping a locus for this condition to 5q. Genetic heterogeneity for the disorder is demonstrated, but the majority of the families (9 of 15) demonstrate evidence of linkage to the 5q locus. A second locus for TAA/dissections has been reported separately,14 and we have determined that 5 of 15 of our families are not linked to either locus, indicating that there is at least 1 more locus for this condition.

Methods

Families and Sample Collection

Clinical descriptions of 4 of the families participating in these studies have been published.2 Another 11 families with multiple members with TAs and/or dissections were identified and clinically characterized. Individuals were identified as affected if they had a true aneurysm or dissection of the thoracic or abdominal aorta. Patients were diagnosed by any imaging modality, including ultrasound, 2D echocardiography, CT scan, MRI, and angiography, or preaneurysmal dilatation of the patient’s proximal aortic root measured echocardiographically at the sinuses of Valsalva and plotted against nomograms derived from normal values.15 The affected individuals in these families did not have any features of MFS or any other connective-tissue disorder. The majority of the families are Caucasian, except TAA003 (Iranian) and TAA014 (Japanese). There is no known consanguinity in the families.

The Institutional Review Committee at the University of Texas Health Science Center at Houston approved this study. After the appropriate consent was obtained, buccal cells, blood, autopsy samples, and/or skin biopsies were collected from the family members, and genomic DNA was isolated. Cultured fibroblast cell strains were established from the 3-mm skin biopsies by mincing the tissues and placing them into 60-mm culture dishes secured by glass coverslips. The primary cultures were maintained in DMEM with 10% FCS and supplemented with antibiotics and antimycotics. Aortic smooth muscle cells were purchased from American Type Culture Collection.

Genotyping

Genomic DNA was extracted from fibroblasts and blood samples with a PureGene genomic DNA isolation kit (Gentra Systems). Fluorescence-tagged primers (CHLC Human Screening Set, version 8.0, Research Genetics) were used to amplify polymorphic sequences spaced ~10 cM throughout the genome. The DNA fragments were analyzed on an ABI Prism 377 Genetic Analyzer. Primers to analyze other polymorphic microsatellite markers were designed according to the information from the Cooperative Human Linkage Center16 and the Genome Database.17 Twelve markers located at 3 chromosome regions were used to further define and verify putative TAA loci, including D3S1279, D3S523, D3S744, D3S753, D5S2500, D5S1725, D3S1453, D3S2501, D16S253, D16S3596, and D16S2624. Twenty-one markers span-
ng 52 cM of chromosome 5q were chosen for fine genetic mapping and identifying the critical interval, including D5S2029, D5S2500, D5S253, D5S626, D5S641, D5S806, D5S1453, and D5S2501.

The following markers were used to study linkage to FBN1 on chromosome 15q: MTS2, MTS4, D15S119, D15S126, and D15S209.18

**Sequencing**

Polymerase chain reaction (PCR) amplification from genomic DNA was performed with fluorescence-labeled (6-FAM, HEX) and tailed primers. The reactions were carried out in 12.5 μL with 10 to 50 ng genomic DNA by use of HotStarTaq DNA polymerase (Qiagen Inc) with a 2-step amplification program (PE, Applied Biosystems), and the PCR products were analyzed on an ABI Prism 310 DNA sequencer (Applied Biosystems). Primer sequences used in this study are available in an online data supplement available at http://www.circulationaha.org.

Total cellular RNA was isolated from dermal fibroblast cell culture by Trizol reagents (Gibco-BRL). The first-strand cDNAs were synthesized by SuperScript II reverse transcriptase (RT; Gibco-BRL), and the PCRs were performed in 12.5 μL containing 52 cM of chromosome 5q: MTS2, MTS4, D15S119, D15S126, and D15S209.18

**Results**

**Statistical Analysis**

The genome-wide scan marker data were analyzed by the affected-pedigree-member method of linkage analysis.19 The test for heterogeneity was performed as previously reported.20

Pairwise and multipoint logarithm of the odds (LOD) scores were calculated with MLINK and LINKMAP programs of the computer software FASTLINK, version 3.P.21,22 Allele frequencies for each marker were obtained by use of the founders in the pedigrees. Penetration values were deduced from the proportion of affected individuals in the pedigrees. The penetrance for the homozygous and heterozygous carriers of the rare disease allele was found to be age dependent, with 10% for individuals age <30 years, 30% between 30 and 40 years, 70% between 40 and 60 years, and 90% for individuals >60 years of age.

**Sequence Alignment**

**D3S1763**

<table>
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<tr>
<th>Marker</th>
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<th>LOD Score at θ = 0.01</th>
<th>LOD Score at θ = 0.05</th>
<th>LOD Score at θ = 0.1</th>
<th>LOD Score at θ = 0.2</th>
<th>LOD Score at θ = 0.3</th>
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<td>0.02</td>
<td>0.07 at 0.3</td>
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<td>0.18</td>
<td>2.74 at 0.0</td>
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<td>0.07</td>
<td>0.02</td>
<td>0.07 at 0.3</td>
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<tr>
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<td>0.74</td>
<td>0.23</td>
<td>2.41 at 0.05</td>
</tr>
</tbody>
</table>

**Total LOD Scores Obtained Between Disease and 7 Polymorphic Loci in the Chromosome 5q Region**

![Figure 2](image_url)

**Figure 2.** Three-point analysis of marker data of TAA/dissection pedigrees. y axis is LOD score; x axis is markers based on their relative distance (see Figure 3B). A maximum LOD score of 4.76 is obtained for marker DSS2029. Solid line shows total multipoint LOD score of all families; dotted lines show multipoint LOD score of individual families. Family TAA013 has a recombination in the marker DSS641; therefore, LOD score for this family is below —3.

As an initial step toward defining the genetic basis of the TAA/dissection in 15 families identified, linkage to FBN1 was excluded as the cause of TAA in these families by use of DNA from the families and markers within and surrounding FBN1. We then proceeded with a genome-wide scan for the genetic defect causing this syndrome using only affected members from TAA002 and TAA003 (Figure 1A), the 2 families with the most affected members. This approach was used in an attempt to avoid problems in mapping the gene due to the decreased penetrance and possible genetic heterogeneity. Multilocus analysis revealed 3 loci with multiple linked markers showing highly statistically significant regions of linkage for the TAA/dissection gene in these families (Table 1). Three loci, a 38-cM region of chromosome 3q, a 52-cM region of chromosome 5q, and a 26-cM region of chromosome 16q, showed multiple linked markers significantly associated with the disease.

Microsatellite markers were used to further analyze chromosomal regions on 3q, 5q, and 16q and verify the observed linkage. Linkage analysis was performed with DNA from 5 families (Figure 1, TAA001, 002, 003, 005, and 010, which included 18 affected, 33 status unknown, and 14 unaffected individuals) and the following markers: D3S1279, D3S4523, D3S1744, D3S1763, D5S2500, D5S1265, D5S1725, D5S1462, D5S1453, D5S2501, D16S3253, D16S396, and D16S2624.

This analysis excluded the 3q and 16q loci as the location of the defective gene causing TAAs/dissections. Three of the 5
families studied, however, showed evidence of linkage to 5q markers.

Analysis of more families confirmed linkage to markers on 5q. Fifteen TAA/dissection families in which linkage of the phenotype to FBN1 had been excluded were genotyped by use of 21 markers on 5q. In the following families, a 5q haplotype segregated with the disease: TAA002, 001, 009, 010, 012, 013, 014, 015, and 034. Some unaffected individuals in some of these families also had the affected haplotype, which is consistent with the decreased penetrance of the disorder.

Linkage analysis of all 15 families showed that some of the families had significant positive LOD scores, whereas some had significant negative LOD scores. Therefore, a heterogeneity test was performed to determine whether the families with positive LOD scores are linked to the marker and the families with negative LOD scores are unlinked to the marker locus. This analysis supported genetic heterogeneity for the condition (\( P = 0.004 \)). The estimate of \( \alpha \), the proportion of families that are linked, was found to be 0.45. Nine families with the highest conditional probability of being linked to 5q were used for the multipoint analyses (families in Figure 1A [TAA002 only] and Figure 1B).

Table 2 indicates the contributions of the markers on chromosome 5q that yielded positive LOD scores. A maximum LOD score (\( Z_{\text{max}} \)) of 4.74 at no recombination (\( \theta = 0 \)) was obtained for the marker D5S2029. Family TAA002 has the highest LOD score of 1.75 for this marker. Figure 2 shows 3-point analysis results assuming the following order between the markers: DSS1464 to 1.5 cM–DSS2029 to 0.6 cM–DSS2029 to 0.4 cM–DSS626 to 1.0 cM–DSS641. The 3-point and 4-point results also showed significant linkage and localized the TAA/dissection gene near DSS2029. Table 3 indicates the contributions of the individual families to the total LOD score for the 4 markers demonstrating the highest LOD scores. Note that FBN2, the gene that is altered in the Marfan-related syndrome called congenital contractual arachnodactyly, maps distal to this location. An intragenic FBN2 marker demonstrated a number of recombinants between the phenotype and the marker in these families.

The 5q haplotype constructed by use of the 21 polymorphic markers on 5q revealed 2 recombinants in affected individuals in TAA002 and TAA013 (Figure 3A). These results suggest that the critical interval containing the defective gene lies in the 7.8-cM region, bordered distally by D5S806 and proximally by D5S641 (Figure 3B).

One of the TAA families demonstrated segregation of the disease with chromosome 11q markers (TAA011), where another locus for familial aortic aneurysms and dissections has been mapped. Therefore, 5 families were not linked to markers on either 5q or 11q (families in Figure 1A [TAA003 only] and Figure 1C). Because linkage to FBN1 and 3p24 has already been excluded as the cause of aortic disease in these families, these data suggest that there is another locus for TAA/dissection.

### Table 3. LOD Scores for the Individual Families for the Markers Demonstrating the Highest LOD Scores

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<tr>
<th>Markers</th>
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### Sequencing of Candidate Genes in the Critical Interval

Versican, a large chondroitin sulfate proteoglycan that is found in the extracellular matrix of many tissues, including the aorta, mapped into the critical interval. Intron-based, exon-specific primers based on the genomic sequence were designed to amplify exons 3 through 13 of the versican gene. The 2 largest exons, exons 7 and 8, were amplified by use of overlapping primers spaced throughout the exons. Translation begins in exon 2, and exon 2 and part of exon 1 were sequenced with RNA from fibroblasts and RT-PCR. Versican was sequenced by use of genomic DNA from TAA002/675, TAA014/1755, and TAA007/1076; the versican cDNA was sequenced by use of fibroblasts from TAA002/675 and TAA014/1755. Eleven single nucleotide alterations were identified (Table 4). All of these alterations were identified in the largest exon of the gene, exon 8, which encodes the protein domain that attaches the large chondroitin side chains. Six of the 15 nucleotide changes did not alter an amino acid; the other alterations did alter an amino acid, with 3 of these alterations being nonconservative changes. Analysis of the segregation of the nucleotide changes within the families indicated that all the identified alterations were present in unaffected individuals and also in control DNA.

Thrombospondins are a family of extracellular calcium-binding proteins that are involved in cell proliferation, adhesion, and migration. Thrombospondin 4 also mapped into the 5q interval and is expressed in cardiac and skeletal muscle, including by aortic smooth muscle cells. The thrombospondin 4 cDNAs from TAA014/1755 and TAA002/784 fibroblasts were sequenced. Six expressed polymorphisms were identified in both cell strains, confirming that both alleles were expressed (Table 4).

CRTL1 encodes cartilage link protein, and expression of this gene in aortic smooth muscle cells was confirmed through RT-PCR using mRNA isolated from aortic smooth muscle cells. CRTL1 has only 5 exons, which were sequenced from genomic DNA from individuals TAA001/765 and TAA002/675. Four polymorphisms were identified (Table 4).
Discussion

This article describes the mapping of a locus that causes TAAs and dissections inherited in an autosomal dominant manner with variable expression and decreased penetrance. A genome-wide linkage scan was completed with only affected members of 2 families because of the decreased penetrance of the disorder and to avoid possible problems encountered as a result of genetic heterogeneity. Linkage analysis using polymorphic markers at 3 genomic regions identified in the original scan and 6 families with TAA/dissections indicated evidence of linkage of some of the families to a 5q locus. A test for genetic heterogeneity using the 15 families with TAA/dissection supported genetic heterogeneity for this condition. Linkage to the 5q locus results in an LOD score of 3.0 with 3 markers, D5S253, D5S2029, and D5S626. The highest LOD score obtained was with D5S2029, which gave an LOD score of 4.74 at a θ of 0.

Genetic heterogeneity for TAA/dissections was confirmed by genetic analysis of an unrelated family with autosomal dominant inheritance of TAAs and dissections. The clinical
features in this family differed from the families in this study; the disorder demonstrated complete penetrance at a young age, along with aneurysms and dissections involving both the ascending and descending aorta. A genome-wide scan for the defective gene in this family determined that the phenotype was linked to markers at a distinct locus on 11q23. Analysis of 6 families not linked to 5q determined that 1 family demonstrated evidence of linkage to 11q (TAA011, Figure 1C), although the limited number of affected individuals in this family prevented confirmation of this linkage.

Five families did not demonstrate evidence of linkage to markers on either 5q or 11q and were not linked to markers within and closely linked to FBN1. On that basis, we hypothesize that there is a third locus of this condition. Review of the clinical manifestations of the aortic disease in these families that were not linked to either locus failed to reveal any clinical features to differentiate these families from those linked to 5q.

The cardiovascular complications of the families with TAA/dissection that are linked to 5q are similar to those observed in MPS patients. In addition, the condition is inherited in an autosomal dominant manner, which raises the possibility that the defective gene encodes a connective-tissue protein. On that basis, we hypothesized that the defective gene was a connective-tissue protein. Three genes in the critical interval that encoded for the connective tissue proteins versican, thrombospondin 4, and cartilage-linked protein were sequenced with DNA from affected individuals. Single nucleotide polymorphisms were identified in all 3 genes. The following genes map into the region and are thought to be poor candidates for the defective gene involved in TAA/dissections because of their function: ribosomal protein S23, secretory carrier membrane proteins (membrane components of recycling vesicles), RAS-p21 protein activator, creatine kinase (mitochondrial 2), HMG-CoA reductase, arylsulfatase B, betaine-homocysteine methyltransferase, hydroxyacetyl glutathione hydrolase, dihydrofolate reductase, x-ray repair complementing defective repair gene, corticotropin-releasing hormone-binding protein, and antiquitin 1 (regulates turgor pressure in plants).

Identification of the defective gene at 5q13-14 causing TAAs and dissections will allow for the timely identification of individuals at risk for this life-threatening condition. The disorder demonstrates decreased penetrance in families, which raises the possibility that sporadic cases of TAAs and dissections result from inherited mutations, putting future generations at risk for aortic aneurysms and dissections. Alternatively, sporadic cases of aneurysms and dissections could result from new mutations in the gene, which could also increase the risk of aortic disease in the offspring. Identification of the defective gene will also allow for new insight into the proteins that are important for maintaining structural integrity of the aortic wall throughout a lifetime.

Acknowledgments

This work was supported by the following funds: an American Heart Association Established Investigator Award (Dr Milewicz), the Roderick Duncan McDonald Foundation at St Luke Episcopal Hospital (Dr Milewicz), NIH grant R01-AR-41135 (Dr Dietz), Howard Hughes Medical Institute (Dr Dietz), and a University of Texas–Houston Medical School Clinical Research Grant (M01-RR-02558). We would like to thank the families for participating in this study, Madeline Ottosen for her excellent nursing assistance, Carlos Infante for his technical assistance, and Dr Elizabeth Petty for recruiting families.

References


16. Laboratory for Population Genetics, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health. The Cooperative Human Linkage Center. Available at: http://lpg.nci.nih.gov/CHLC.


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Circulation. 2001;103:2461-2468
doi: 10.1161/01.CIR.103.20.2461

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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