Practical Genetics of Thoracic Aortic Aneurysm

John A. Elefteriades, MD, PhD (hon)
William W.L. Glenn Professor of Surgery
Director, Aortic Institute at Yale-New Haven
Yale University School of Medicine
New Haven, Connecticut, USA

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Genetics in Thoracic Aneurysm

• Historical glimpse
• Manifestation of Familial Patterns
• Mendelian Genetics
• Molecular Genetics (DNA)
• Molecular Genetics (RNA)
  – Investigational “RNA Signature” Test
• Whole Exome Sequencing
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“The Nomads and Scythians have *lax joints and easy bruising*.”

Hippocrates  400 B.C.

Describing *Ehlers-Danlos Syndrome*
Human genetics of the abdominal aortic aneurysm.
Tilson MD, Seashore MR. Surg Gynecol Obstet 1984 Feb;158(2):129-32

The results of recent studies suggest that genetic factors may be important in the pathogenesis of abdominal aortic aneurysms. In the present report, the apparent mechanisms of inheritance in 16 families with a total of 41 affected individuals are summarized. The results suggest that there may be both X-linked and autosomal dominant forms of the disease, with the X-linked variant as the more common type. A multifactorial mechanism cannot be ruled out from the results of the present data.
Fifty Families With Abdominal Aortic Aneurysms in Two or More First-Order Relatives

M. David Tilson, MD, New Haven, Connecticut
Margretta R. Seashore, MD, New Haven, Connecticut

We have previously described 16 families with two or more first-order relatives affected with abdominal aortic aneurysms [1]. The present report extends this collected series to a total of 50 families.

Patients
About a third of the families (16) are from our nearby geographic region in Southern Connecticut, 13 other families are from other New England states, and the remaining families are widely scattered across the United States.

Results
The patterns of affected first-order relatives are summarized in Figures 1 through 3. Twenty-eight families had patterns in which the only known affected members were siblings (Figure 1). Two of these were identical twins. One additional pair of identical twin brothers had an affected mother. The most common observation was an association of brothers; 22 of the total of 50 families fit this pattern. There were three sibships with sisters only, including one set of identical twins, and there were three mixed sibships. Figure 2 illustrates the families with affected members in two generations. In eight of these families, the fathers apparently transmitted to sons, and in six the mothers apparently transmitted to sons. In one case, a father had two affected daughters. Figure 3 illustrates three families with three generations in whom all affected persons were male and three “complex” families. In one complex family, husband and wife both had aneurysms, along with three affected sons. In the second complex family, we had initially presumed the proband and his father fit the pattern in Figure 2, but ascertainment of a wider pedigree disclosed a maternal great uncle with an aneurysm. The third complex family had a proband linked to his paternal uncle.

Comments
When we first became interested in the human genetics of aneurysmal disease, we were intrigued with the hypothesis of an X-linked gene for two reasons. First, the number of men with abdominal aortic aneurysms exceeded the number of women by ratios as high as 8:1 [2]. Second, a laboratory mouse that is prone to the development of spontaneous aortic aneurysms has a known mutation on the X chromosome [3]. However, we found it necessary to postulate an autosomal dominant pattern in man when we found that 4 of our first 16 collected families had apparent inheritance from fathers to sons. This pattern is confirmed in the present study in 12 of the 50 families.

The hypothesis of a single autosomal mutation to account for these familial patterns leaves the mechanism for sex limitation unexplained. For example, in the 14 families in which fathers apparently transmitted to offspring, there were totals of 39 affected male subjects to 2 affected female subjects. As we do not yet know the ratio of affected to unaffected persons, a multigene mechanism cannot be excluded.

Ascertainment of wider pedigrees, a search for biochemical phenotypic markers, and determination of possible polymorphisms at candidate gene loci are some of the methods that may be used to clarify the mechanisms of transmission in these families.
Thoracic aortic aneurysm is a genetic disease

Marfan’s is just the tip of the genetic iceberg
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Three Generations of Type A Dissection in One Single Family

59 year old Female

Type A Dissection

Well

Well

Well

Well
Three Generations of Type A Dissection in One Single Family

78 year old Female
Type A Dissection

59 year old Female
Type A Dissection

Well

Well

Well
Three Generations of Type A Dissection in One Single Family

78 year old Female
Type A Dissection

59 year old Female
Type A Dissection

Well

12 year old Female
Type A Dissection

Well

Well

Well
Thumb-Palm Sign
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Genetic Patterns in 300 Yale Family Pedigrees

- Autosomal dominant: 38.5%
- Autosomal dominant or X-linked: 23.1%
- Recessive: 26.9%
- Other: 11.5%
Familial Patterns of Thoracic Aortic Aneurysms

Michael A. Coady, MD, MPH; Ryan R. Davies, BA; Michele Roberts, MD, PhD; Lee J. Goldstein, BA; Matthew J. Rogalski, BS; John A. Rizzo, MD; Graeme L. Hammond, MD; Gary S. Kopf, MD; John A. Elefteriades, MD

Hypothesis: To provide evidence that genetic factors contribute to the development of thoracic aortic aneurysms (TAA) by demonstrating familial patterns of the disease.

Design: Retrospective review.

Setting: University hospital.

Patients and Methods: We sought to identify familial patterns of TAA from a database of 398 patients evaluated or treated for TAA at the Yale Center for Thoracic Aortic Disease, New Haven, Conn, from January 1985 to August 1998. Of the 398 patients, 45 patients had a diagnosis of Marfan syndrome and 533 patients had no known history of any collagen vascular disorder. Of the 333 patients in the latter category, 398 patients had confirmed TAA, 66 had TAA with concomitant aortic dissections, and 98 had aortic dissections. From a group of 464 patients with TAA with or without concomitant aortic dissections, 2 interviewers attempted to contact 15 randomly selected patients for telephone screening to determine the presence of familial patterns of aortic disease. Fifteen of these patients were lost to follow-up. Complete medical and family histories of the remaining 135 patients (85 men, 50 women) were reviewed. Of the 135 individuals screened, 26 (18 men, 8 women) (19.3%) were found to belong to multiplex pedigrees. These 26 patients with familial nonsyndromic TAA were compared with the remaining 109 patients with sporadic TAA and the 45 patients with Marfan syndrome—associated TAA.

Main Outcome Measures: Groups were examined for statistical differences in age and aortic size at the time of diagnosis, growth rates of TAA, and rates of concomitant diseases. Nonsyndromic family pedigrees were analyzed and potential modes of inheritance were determined.

Results: The mean age at presentation for patients with familial nonsyndromic TAA (56.8 years) was significantly younger than the mean age of presentation in sporadic cases (64.3 years, P < .03), and significantly older than that of patients with Marfan syndrome (24.8 years, P < .001). Patients with a family history of aortic aneurysms had faster growth rates (0.22 cm/year) compared with patients with sporadic TAA (0.03 cm/year) (P < .001) and patients with Marfan syndrome (0.10 cm/year) (P < .04). Familial nonsyndromic TAA in patients with a concomitant aortic dissection had a growth rate of 0.33 cm/year, which was greater than that of patients with sporadic TAA (0.10 cm/year) and patients with Marfan syndrome (0.08 cm/year) with associated aortic dissection. This growth of 0.33 cm/year was significantly faster than the overall growth rate estimate of aneurysms in patients with aortic dissection (0.14 cm/year) (P < .05). Ten pedigrees (38.9%) showed direct father-to-child transmission, consistent with an autosomal dominant mode of inheritance. Seven family pedigrees (23.1%) suggested an autosomal dominant or X-linked mode of inheritance. Seven pedigrees (26.9%) suggested a recessive mode of inheritance; 2 an autosomal recessive mode, and 5 an X-linked recessive or autosomal recessive mode. The remaining 3 pedigrees displayed more complex modes of inheritance.

Conclusions: This study supports the role of genetic factors influencing familial aggregation of TAA. Thoracic aortic aneurysms in association with multiplex pedigrees represent a new risk factor for aneurysm growth. Pedigree analysis suggests genetic heterogeneity. The primary mode of inheritance appears to be autosomal dominant, but X-linked dominant and recessive modes are also evident.


1999

Familial thoracic aortic dilations and dissections: A case control study

Alan Biddinger, MSE; Marnie Rocklin, MS; Joseph Coselli, MD, and Dianna M. Milewicz, MD, PhD, Houston, Tex.

Purpose: Evidence suggesting that genetic factors contribute to the development of common disorders can be obtained by demonstrating familial aggregation of the disease. This study investigated whether thoracic aortic dilations and dissections aggregate in families by comparing the prevalence of thoracic aortic aneurysms, thoracic aortic dissections, and sudden death in first-degree relatives of patients referred for thoracic aortic surgery.

Methods: Families were ascertained through 158 nonsyndromic patients referred for surgical correction of either thoracic aortic aneurysms or dissections (probands) and their 843 first-degree relatives. A control group of 547 first-degree relatives was derived from 114 proband spouses. Groups were examined for statistical differences in the prevalence of thoracic aneurysms, thoracic aortic dissections, abdominal aortic aneurysms, sudden death, and myocardial infarctions.

Results: First-degree relatives of probands demonstrated a higher prevalence of thoracic aortic aneurysms and sudden death when compared with the control group. Relative risks of thoracic aortic aneurysm development in proband fathers, brothers, and sisters were 1.8, 10.9, and 1.8, respectively. A pattern of inheritance of the thoracic aortic aneurysms could not be determined.

Conclusions: This study indicates proband first-degree relatives are at a higher risk for thoracic aortic aneurysms and sudden death compared with a control group. This study supports the role of genetic factors in the cause of thoracic aortic aneurysms and provides important information for identifying individuals at risk. (J Vasc Surg 1997;25:506-11.)

Aneurysms and dissections of the aorta are associated with a high degree of morbidity, mortality, and medical expenditure despite continued improvements in diagnostic and surgical techniques. Prevention of these disorders through early identification of predisposed individuals and the modification of contributing environmental and genetic factors is a potential cost-effective method for addressing these diseases. Establishing familial aggregation and estimating relative risks of these disorders for family members is useful not only for identifying susceptible individuals but also for implicating a genetic contribution to these disorders. Although abdominal aortic aneurysms have been well characterized in regard to familial aggregation, risk factors, possible causes, and potential modes of inheritance, less is known regarding thoracic aortic aneurysms (TAA) and thoracic aortic dissections. For this reason this study was primarily designed to compare first-degree relatives of patients referred for surgical
Distribution of Arterial Aneurysm and Dissection Sites in Kindred of Familial Probands

Distribution of Arterial Aneurysm and Dissection Sites in Kindred of Familial Probands

Ascending and Descending Thoracic aortic aneurysm are two different diseases.
Familial Age Clustering in Aortic Dissection

Aortic Dissection – Role of Positive Family History

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# Genetics of Thoracic Aortic Aneurysm

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<th>Chromosome</th>
<th>Gene</th>
<th>Protein</th>
<th>Location</th>
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<td></td>
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<tr>
<td>Marfan</td>
<td>15q21.1</td>
<td>FBN1</td>
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## Features of Specific Familial Etiologies of Thoracic Aortic Aneurysm

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<th>Affected Gene</th>
<th>% of Familial Aortic Aneurysm Patients</th>
<th>Special considerations</th>
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| ACTA2         | 10-15%                                | • Present with dissection  
|               |                                       | • Can dissect @ <5cm  
|               |                                       | • Risk stroke, MI |
| MYH11         | 2%                                    | • Involved in SMC contraction |
| MYLK          | 1%                                    | • Involved in SMC contraction  
|               |                                       | • Exclusively involved with dissections (not aneurysms), so hard to counsel regarding best time for intervention |

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“RNA Signature”

• DNA is blueprint
• RNA tells us what rooms (systems) are actively being worked on
Hierarchical Clustering Diagrams

Each vertical line represents a patient

Each horizontal line represents an RNA (One of 30,000 tested)
Timeline

Detectable Biomarkers:
- D-dimer
- Plasmin
- Fibrinogen
- MMPs
- Cytokines
- CD4+ CD28- T cells
- C-reactive protein
- Elastin peptide

Aortic Aneurysm

Aortic Dissection

Post Aortic Dissection

Biomarker Needed

Pre-Aortic Dissection
Genes **IL-10** and **IL-18** were expressed 4-fold higher in patients with Acute Aortic Dissection, compared with patients without dissection.
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Schematic of Exome Sequencing

1. **Construct shotgun library**
   - Genomic DNA
   - Fragments

2. **Hybridization**
   - Washing
   - Pulldown

3. **Captured DNA**

4. **Mapping, alignment, variant calling**

5. **DNA sequencing**
Advantages of Whole Exome Sequencing

• Comprehensive testing of all known aneurysm-causing genes simultaneously;

• Opportunity to reanalyze these data in the future if and when new TAD genes are discovered;

• Whole exome sequencing is becoming less expensive and more cost-effective;

• Opportunity to mine the data for potential new genes and variants.
Oragene Saliva Kits – Sample Collection for Whole Exome Sequencing

Images from: www.dnagenotek.com
Schematic of Whole Exome Sequencing

Overall Frequency Distribution of Detected Variants

- **NOTCH1 (n=10)**: 12%
- **MYH11 (n=12)**: 14%
- **FBN1 (n=13)**: 16%
- **FBN2 (n=3)**: 4%
- **FLNA (n=4)**: 5%
- **MYLK (n=4)**: 5%
- **PRKG1 (n=1)**: 1%
- **SMAD3 (n=2)**: 2%
- **TGFBR1 (n=2)**: 2%
- **TGFBR2 (n=3)**: 4%
- **TGFBR3 (n=1)**: 1%
- **ACTA2 (n=2)**: 2%
- **BGN (n=1)**: 1%
- **COL1A1 (n=4)**: 5%
- **COL1A2 (n=3)**: 4%
- **COL3A1 (n=5)**: 6%
- **COL5A2 (n=4)**: 5%
- **COL5A1 (n=7)**: 8%
Mapping mutations on genes

- **FBN1**
- **NOTCH1**
- **MYH11**

The diagrams show the distribution of mutations across the indicated amino acid ranges for each gene.
1000 Aortic wall specimens genotyped

Yale Aortic Institute Tissue Bank
Dictionary of Genetic Defects in TAA

Future Directions

Methods for determining if a variant of unknown significance (VUS) is Real

1. Extreme rarity in human genome (<1/10,000)
2. Animal model with knock-out replicates phenotype
3. Family studies: phenotype segregates with genotype
Testing Family Members

• Can be conducted via single site (Sanger) testing
• Cost-effective
• Substantial benefit in identifying TAAD and preventing related deaths
• Non-mutation carrying family members can be spared from repeated imaging studies and emotional burden of the disease
• 3 Family members suffered aortic dissection at 4.0 cm – all MYLK carriers

• 1 Family member (also MYLK carrier) operated on prophylactically at 3.9 cm to prevent aortic dissection
EMILIN1 gene

- Elastin-microfibril interfacer 1
- Chromosome 2p23
- 8 exons, 8.0 kb

- Variant causing TAA has **Zero** incidence in genetics databases
Genes Associated with Thoracic Aortic Aneurysm and Dissection
An Update and Clinical Implications

Adam J. Brownstein, BA¹, Bulat A. Ziganshin, MD¹, Helena Kuivaniemi, MD, PhD², Simon C. Body, MD, MPH³, Allen E. Bale, MD⁴, John A. Elefteriades, MD¹*

¹Aortic Institute at Yale-New Haven Hospital, Yale University School of Medicine, New Haven, Connecticut, USA
²Division of Molecular Biology and Human Genetics, Department of Biomedical Sciences, and Department of Psychiatry, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg, South Africa
³Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts, USA
⁴Department of Genetics, Yale School of Medicine, New Haven, Connecticut, USA
Intervention recommendations with specific genetic mutations

Conclusion:
Genetics of Thoracic Aortic Aneurysm

We are clearly headed toward a new era of:
1) Personalized strategic management of TAA patients.
2) Molecular identification of individuals at risk for TAA.