The Familial Non-Syndromic Thoracic Aortic Aneurysms and Dissections maps to Marfan Disease Gene (Fibrillin 1) locus

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Abstract

Background

Thoracic aortic aneurysms and dissections (TAAD) is a critical condition that often goes undiagnosed with fatal consequences. While majority of the cases are sporadic, more than 20% are inherited as a single gene disorder. The most common familial TAA is Marfan syndrome, which is primarily caused by mutations in fibrillin 1 (FBN1) gene. Patients with fibrillin 1 mutations have higher risk for dissection compared to other patients with similar size aneurysms.

Methods

Fifteen family members were genotyped by Affymetrix-10K genechips. A genome-wide association study was carried using an autosomal dominant model of inheritance with incomplete penetrance.

Results

The index case presented with agonizing substernal pain and was found to have TAAD by transthoracic echocardiogram. The family history was significant for 3 first degree relatives with TAA. Ten additional family members were diagnosed as having TAA by echocardiography examinations alone. The affected individual had no syndromic features. A genome-wide analysis of linkage mapped the disease gene to a single locus on chromosome 15q21 with the peak lod score of 3.6 at fibrillin 1 gene locus (odds ratio >4000:1 In favour of linkage). Haplotype analysis identified additional mutation carriers had unknown status due to borderline echocardiographic findings.
Conclusions

FBN1 mutation careers may lack syndromic features of the Marfan syndrome, yet have an equally malignant disease course. Our finding indicates importance of obtaining detailed family history and echocardiographic screening of extended relatives of patients with nonsyndromic TAAD to prevent unanticipated events. Moreover, association of nonsyndromic TAAD with the Marfan disease gene raises the question to whether secondary prevention strategies employed for Marfan syndrome patient should also be applied to all patients with familial TAAD.

Background

Thoracic aortic aneurysms and dissection (TAAD) is a lethal condition with rising incidence\(^1,2\). Although this condition occurs mostly sporadic, it is estimated that one out of every 5 cases are familial. This includes syndromic forms of the disease, mainly Marfan syndrome (MFS) and the less commonly seen Loeys-Dietz syndrome as well as the nonsyndromic thoracic aortic aneurysm. The mode of inheritance is often consistent with autosomal dominant with incomplete penetrance\(^3\). It is a heterogeneous disorder for which at more than 4 loci for TAAD have been mapped \(^4,5,6\).

Marfan syndrome is a connective tissue disorder that affects multiple organs including cardiovascular and skeletal systems\(^7\). It is often inherited as an autosomal dominant disorder caused by FBN1 gene mutation \(^8,9,10,11,12\). The most lethal complication is aortic aneurysm which frequently has a malignant course with early onset of sudden dissection necessitating operative repair early in the disease course \(^13\). Whether FBN1 mutation can cause nonsyndromic familial aneurysm and more importantly if the course is equally malignant is not known. Indeed recognition of the phenomenon that
the FBN1 mutation carriers may not have physical findings of Marfan syndrome has serious implications for disease screening. This may implicate that not only extensive family history of these patients should be obtained, but also extended family members may need to undergo echocardiographic screening. In addition, in familial cases the disease course should be carefully studied for planning the time of surgery. With the advent of cost effective high throughput sequencing techniques mutation screening of patients with thoracic aortic aneurysms may soon become part of the clinical workup. In the current study we identified a large three-generation family with multiple members affected by early onset TAAD in absence of syndromic features of MFS. A genome-wide screen was carried out to identify the disease gene.

**Methods**

**Family Characterization**

Family history revealed that 3 other member of the family had already suffered from ascending thoracic aneurysm dissection or undergone surgical repair of the thoracic aorta. Extended family members were thoroughly examined, including ophthalmologic and echocardiographic examinations. For assessment of the size of the aneurysm the diameters of the aorta at the sinus of Valsalva, the supra-aortic ridge, and the aortic root were measured from cross-sectional echocardiography images in the parasternal long-axis. Individuals were identified as affected if they had an aneurysm (diameter ≥3.6 cm at the aortic root or supra-aortic ridge level, fig.1) or dissection of the ascending thoracic aorta; Family members were examined for other skeletal features of MFS. Study was approved by the Institutional Review Board at both institutions. After obtaining informed consent, blood samples were collected from family members. Genomic DNA was isolated from 15 family members.

**Genotyping and Analysis of Linkage**
Thirteen family members were called affected, 2 unaffected and 2 unknown. Genomic DNA was obtained from eleven living affecteds, two unaffected and two family members with unknown affectation status. Affymetrix 10K DNA genechip arrays were used to genotype >10,000 SNPs across the genome in all 15 family members. Allele frequencies for each SNP were mean allele frequencies of 20 unrelated unaffected Iranian (ethnically matched) and penetrance was set at 90%. Multipoint analysis of linkage with Genehunter was used for the genome scan analysis.

**Results**

**Clinical Evaluations**

We studied a 3-generation Iranian family. The Index case (individual 6) was a 55 years old woman who presented with acute substernal chest pain. The echocardiographic examination revealed normal left ventricular wall motion but dilated aorta with a proximal ascending aorta diameter of 5.5 cm with evidence for dissection (Figure 1). Patient was only 170 cm tall, had no disproportionately long extremities compared with the trunk (dolichostenomelia), and no arachnodactyly. Wrist (Walker) and thumb (Steinberg) signs were absent and no other phenotypic abnormalities such as hypertelorism were identified. Immediate surgical repair was planned, but patient deceased on the way to the operating room. Her older sister had presented at the age of 51 years with dissection of a 5 cm ascending aortic aneurysm and the younger sister had been diagnosed with proximal ascending aortic dilation involving the aortic root with moderate aortic regurgitation and both had undergone Bentall procedures. Interestingly, the daughter of the index case presented later with TAAD and had to undergo Bentall procedure. Ten additional immediate family members were classified as affected and 2 as unaffected by echocardiographic
examinations (aortic root or ascending aortic diameter ≥ 3.6 cm). Two other individuals (14 and 17) had aortic diameters (3.2 and 3.1 cm) that were considered relatively large for their age but did not exceed the cut off for diagnosis and were assigned the unknown status.

Mapping of the Locus

A genome-wide screen with 10 K genechips was completed using 13 of the original 15 DNA samples collected (11 affecteds and 2 unaffecteds) (Figure 3). The analysis of linkage was carried out with Genehunter 2.0, assuming an autosomal dominant model with 90% penetrance 1% phenocopies, and disease allele frequency of 0.001. The disease gene for TAAD in this kindred was mapped to a single locus with the peak lod score of 3.5 (θ=0) on FBN1 locus. No other locus had lod score >1. Construction of the 15q haplotype with SNPS revealed recombinants immediately prior to rs668842 and after rs1072974, refining the linked interval to less than 500 kb. Haplotype analysis indicated the segregation of the disease haplotype in two family members who were considered as borderline for aortic disease, thus identifying two individuals with unknown status as mutation carriers.

Discussion

Thoracic aortic aneurysms and dissection caused by fibrillin mutations can have malignant course. It is therefore of great importance to identify individuals at risk prior to the onset of serious complications. In current study genome-wide analysis in a family with nonsyndromic TAAD has mapped the disease gene to FBN1 disease locus. Although mutation careers in this family did not have syndromic features of the Marfan syndrome, the aortic disease in many of the affected family members had a malignant course. This finding suggests that autosomal dominant nonsyndromic
TAAD may be caused by mutations in FBN-1 gene(s) and implicates the importance of echocardiographic examination of the extended family of patients with familial TAAD.

Acute aortic aneurysm is an uncommon but potentially catastrophic illness with an incidence of approximately 2.9/100,000/yr\textsuperscript{14}. Predisposing factors are either acquired such as trauma, caused by complex traits like, hypertension, and bicuspid aortic valve or inherited such as in Marfan syndrome. Among these patients with Marfan syndrome are especially at high risk for aortic dissection of the ascending aorta at a relatively young age\textsuperscript{13}. Patients with familial aneurysms also tend to be younger and have higher rates of rupture than those with sporadic aneurysms\textsuperscript{15}, suggesting the importance of early recognition and familial screening in extended relatives of patients. In an analysis of a large database of patients treated at Yale-New Haven Hospital\textsuperscript{16}, probands with a familial pattern of thoracic aortic aneurysms were ascertained. The major mode of inheritance in these kindred was autosomal dominant.

Compared to sporadic cases (mean age 64 years) mean age for TAAD in patients with familial aneurysm was significantly lower (mean 57 years).

Although genes for nonsyndromic TAA are vastly unknown, there is significant evidence that nonsyndromic TAA have major genetic components. An investigation of the families of 158 patients referred for surgical repair of thoracic aortic aneurysms or dissections found that first-degree relatives of probands had a higher risk (risk ratio [RR] 1.8 for fathers and sisters, RR 10.9 for brothers) of thoracic aortic aneurysms or sudden death compared with control subjects\textsuperscript{1}.

At least four different loci have been thus far mapped for familial nonsyndromic thoracic aortic aneurysms. Mutations in transforming growth factor-beta receptor type II have been associated with some cases of familial thoracic aortic aneurysms\textsuperscript{5,6}.
Mutations in myosin heavy chain (MYH11), a smooth muscle cell-specific contractile protein, have been identified in familial TAAD associated with patent ductus arteriosus (PDA) linked to 16p12.2-12.13\textsuperscript{17}. However, some families have mapped to neither of these loci suggesting additional loci. We provide a strong evidence for linkage of nonsyndromic thoracic aneurysm and dissection to fibrillin 1 locus. Although a unique phenotype of aortic dissections involving both the ascending and descending aorta along has been mapped to chromosome 15q in a single kindred\textsuperscript{18} we report here for the first time linkage a large family with isolated TAAD to Marfan disease gene locus. Interestingly studies of the molecular pathology of aneurysm have suggested common pathways for both syndromic and nonsyndromic thoracic aneurysms. Dysregulations of TGF-β retention and Smad2 signaling has shown to contribute to both syndromic and non-syndromic aneurysms of the ascending aorta\textsuperscript{19}. In this study we do not perform mutation analysis of the fibrillin1 gene in the affected individuals. It is a general consensus that absence or presence of FBN1 mutation does not exclude or include diagnosis because of false negative or positive results. FBN1 mutations can be found in many disorders that have some clinical overlap with Marfan syndrome such as aortic enlargement, skin and skeletal findings (Shprintzen-Goldberg syndrome)\textsuperscript{20}, syndrome of mitral valve prolapse, myopia, borderline aortic enlargement, skin and skeletal findings (MASS phenotype), Marfanoid habitus and isolated ectopia lentis. There is at this time increasing controversy about significance of many identified mutations. In contrast analysis of linkage remains one of the most reliable molecular methods for diagnosis of Marfan syndrome. In addition, the refined linked interval in this kindred contains only two other genes (SLC12A1, DYUT) which because of their tissue distributions and functions cannot be causative gene for TAAD.
Conclusions

We demonstrate here for the first time in a study of a large kindred that nonsyndromic TAAD links to fibrillin 1 gene. This finding indicates that in genetic study of TAAD one has to consider mutations in fibrillin 1 gene in even in absence of syndromic features. Moreover, the malignant course of the disease in this kindred underscores the importance of echocardiographic screening in extended relatives of patients with familial nonsyndromic TAAD. Finally, it raises an important question to whether secondary prevention strategies employed for Marfan syndrome patient should be also examined in patients with early onset familial TAAD.

Competing interests

None

Authors' contributions

Anita Sadeghpour and Maryam Moshkani Farahani identified the kindred, conducted the clinical examination and phenotyping, obtained IRB approval and collected DNA.

Ali Reza Keramati and Gurangad Chandok did the bench work and helped with manuscript preparation.

Arya Mani, designed and supervised the study and wrote the manuscript.

Acknowledgement

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References


**Figures**

**Figure 1 - Ascending aortic dissection**

Transthoracic echocardiogram in index case depicting dissection in proximal ascending aorta.

**Figure 2 - Echocardiography image-aneurysm**

A left parasternal view of the ascending aorta in echocardiography examination of an affected family member with an aneurysm radius of 3.8.

**Figure 3 - TAAD pedigree**

Relationships of members of kindred with TAAD are shown. The index case is indicated by the arrow. Numbered individuals correspond to those in table1. Individuals with TAA are indicated by black symbols; individuals without TAA are
shown as unfilled symbols; and individuals who have unknown status are shown as half-gray symbols. Circles represent females; squares represent males. Symbols with a slash through them indicate deceased subjects. Genotypes of informative d SNP markers are shown in their chromosomal order below the symbol for each individual and their distance in megabases from 15pter is indicated.

**Figure 4 - Lod Score**

Multipoint lod scores for linkage of TAAD to the *Marfan* locus on chromosome 15q. SNPs tightly linked to the location of the maximum lod score are indicated and the location of FBN-1 is shown. The lod score peak occurs at zero recombination with marker rs1876207. y-Axis, LOD score; x-axis, position of the markers on chromosome 15q, based on their relative chromosomal distance. Position of the markers is given in centimorgans (cM).

**Tables**

**Table 1 - Echocardiography Measurements**

Measurements of the ascending aorta and age of onset/diagnosis of the family member diagnosed with TAA by transthoracic echocardiograms
<table>
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