Case 1: Marfan syndrome: a family history of sudden death

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I. Background

James and Thomas are brought by their mother to their primary care physician for a routine annual examination. James is 12 years old, Thomas 15.

This has been a difficult year for the family, as their father, Henry, died suddenly and unexpectedly at age 45 of a ruptured aorta.

The physician is aware of this event and reviews the family history with the boys' mother. Henry's mother died in her early fifties of unknown causes, and Henry had two brothers and a sister. One brother is also dead of cardiovascular problems. The other brother and sister are alive, but the sister has severe visual problems due to dislocation of the lens in both eyes. Similar visual problems were present in Henry and his mother.

The family history suggests the possibility of autosomal dominant transmission of a cardiovascular disorder, perhaps associated with ocular problems.

The fact that Henry died of ruptured aorta is particularly suggestive of a disorder involving connective tissue. Ruptured aorta is characteristic of a number of disorders, including Ehlers-Danlos syndrome (type IV), Marfan syndrome, and can exist in isolation as a familial disorder.

Apparently, the genetic pattern has eluded detection in this family so far. The primary care physician can provide an important service to the family by recognizing that there is a familial disorder and evaluating James and Thomas for possible signs of the disorder.

This presents the possibility of altering the boys' medical management to prevent a disastrous outcome, such as occurred to their father, and allows for genetic counseling to be provided.
2. Cardiovascular examination

Although he has examined the boys many times, the physician devotes special attention this year to cardiovascular problems.

He hears a faint extra heart sound—a “click”—in Thomas.

Both boys are quite tall, exceeding the ninety-fifth percentile for their age. (This had been true since early childhood, but was not viewed as being remarkable because Henry was tall.)

Thomas is found to have mild scoliosis (lateral curvature of the spine).

Both boys also are found to have long fingers (Fig. 1) and loose joints.

Examination of James and Thomas provides further evidence for a connective tissue disorder. The boys are tall, which could be an incidental familial tendency or could be part of a disorder.

That they have long limbs and fingers and toes suggests the latter. Flat feet, scoliosis, and hyperextensible joints further indicate a laxity of connective tissue.

The click heard in Thomas's cardiac examination is indicative of a floppy mitral valve, referred to as mitral valve prolapse. All these features are suggestive of Marfan syndrome.

Marfan syndrome is an autosomal dominant disorder in which connective tissue is weakened. Skin may be soft, but, unlike in Ehlers-Danlos syndrome, is not hyperelastic. Skin striae (stretch marks) tend to develop with age.

People with Marfan syndrome tend to be tall and lanky, with especially long arms, legs, fingers, and toes. Elongation of digits is referred to as arachnodactyly. This growth pattern can be apparent even in the newborn period, although often it does not come to attention until later in childhood or adolescence.

Joints are hyperextensible and may be prone to dislocation. Flat feet and scoliosis occur commonly. In the eye, the ligaments that support the lens may become weak and lead to lens dislocation.

Myopia also is common. Cardiovascular manifestations include mitral valve prolapse and dilation of the root of the aorta, which may lead to aneurysm. Aortic dissection is the most life-threatening manifestation of Marfan syndrome.

3. The cardiologist
In view of the family history and the finding of a click in Thomas, both boys are referred to a cardiologist. An echocardiogram reveals mitral valve prolapse in both and mild dilation of the root of the aorta in Thomas but not in James (Fig. 2).

The cardiologist suggests that the boys might have Marfan syndrome. He also prescribes a beta-blocker medication, atenolol, for both brothers.

The cardiologist contacts the primary care physician to explain his findings and suggests that both boys should be followed with annual echocardiograms. Also, both will require antibiotic treatment at the time of any dental procedures.

It is the cardiologist who puts the features together and suggests the diagnosis of Marfan syndrome for this family. Diagnostic criteria are provided in Table 1.

Mitral valve prolapse is a relatively common problem, affecting nearly 5% of the general population. It consists of floppiness of the mitral valve, leading to regurgitation of blood into the left atrium during contraction of the left ventricle. It is characteristic of a wide variety of connective tissue dysplasias, including Marfan syndrome. It can also occur in isolation and can be a familial autosomal dominant trait. In isolation, it is most common in thin women. Mitral valve prolapse usually is asymptomatic, although it can be associated with fatigue, chest pain, or palpitations. Sudden death occurs rarely. The presence of a midsystolic click is the only physical sign on examination.

Usually mitral valve prolapse does not require treatment. Those who have an audible click are at risk of bacterial endocarditis due to seeding of the floppy valve during times when bacteria are transiently present in the bloodstream. This occurs most commonly during dental procedures, so it is recommended that such individuals receive antibiotic treatment with such procedures.

Dilation of the aortic root, which is detected by echocardiography, is highly characteristic of Marfan syndrome. This has been attributed to the force of blood during systolic contraction of the heart causing stretching of a weakened aortic wall. This can be a progressive problem, beginning in childhood or adolescence, and might be the harbinger of aortic dissection, which can lead to sudden death.

It is important to recognize this problem in people with Marfan syndrome, as careful surveillance can help prevent a catastrophic outcome. There is evidence that use of beta-blocker medications can reduce the likelihood of dissection. Such medications reduce the force of systolic contraction and, hence, stress on the aorta. They are commonly used to reduce blood pressure in those with hypertension.

Regular monitoring by echocardiography can lead to early detection of aneurysms, which, if present, can be treated surgically by replacement of the damaged vessel with a prosthesis.

4. The ophthalmologist
The boys are referred to an ophthalmologist. No significant problems are noted. An orthopedist prescribes inserts for their shoes because of flat feet. Both boys have some degree of scoliosis, but neither requires treatment at present. The primary care physician speaks with James, Thomas, and their mother about Marfan syndrome. The family members have been seen by a number of specialists, and the family is getting a bit confused. Their physician refers them to a geneticist, who further explains the disorder and provides literature from the National Marfan Foundation.

The primary care physician plays a pivotal role in helping the family deal with a genetic disorder such as Marfan syndrome. Anticipatory guidance amounts to educating the family about possible problems and providing appropriate medical surveillance.

Specialists may be called on to deal with particular problems. For Marfan syndrome, the major systems involved are cardiovascular, orthopedic, and ophthalmologic. Orthopedic problems include scoliosis, joint dislocations, and flat feet. Ophthalmologic problems include dislocation of the lens and myopia. Orthopedic and ophthalmologic complications of Marfan syndrome are not preventable by medical management, but prompt recognition of these problems is important to ensure the best outcome from symptomatic treatment.

Although Marfan syndrome can lead to lifelong medical problems, it is also compatible with a healthy productive life. Aside from educating the family about the potential complications, the physician should place the disorder in perspective. There is a wide range of expression of Marfan syndrome; some individuals are obviously affected even in infancy, whereas others are so mildly affected as to be unaware of having the disorder. Medical management should be customized to the needs of the individual and normal activities encouraged as much as possible.

5. The extended family

Medical review of the extended family reveals several others with Marfan syndrome (Fig. 3).

Recognition of a condition such as Marfan syndrome can have implications for many members of a family. Other members may be affected and should be offered medical evaluation and genetic counseling.

Usually this is communicated by family members themselves, who should encourage their at-risk relatives to seek medical evaluation. The process may be impeded, however, by emotional rifts within the family, or fear of medical problems or loss of insurance benefits on the part of some individuals.

In some instances, this can create an ethical dilemma for the physician, who may have information that can be important for the health of a relative yet who is constrained by the confidentiality of the physician-patient relationship from divulging such information without permission of the patient.
6. Genetic research

The family is contacted by a research geneticist (through the family doctor) who is studying the molecular basis of Marfan syndrome. Skin and blood samples are obtained from James and Thomas.

The skin samples are studied for fibrillin metabolism. Cultured cells are incubated in the presence of $[^{35}S]$ cysteine for 30 minutes, after which they are grown in non–radioactively labeled cysteine an additional 0 to 20 hours. Extracts from cells, culture medium, and the extracellular matrix then are subjected to electrophoresis, and the radioactively labeled protein is visualized by autoradiography.

Amounts of fibrillin in cellular, culture medium, and extracellular matrix fractions are determined by measuring the intensity of bands on the autoradiogram in James's and Thomas's samples as compared with controls.

Overall fibrillin synthesis values for both boys' fibroblasts are approximately 50% of control values, and the amount of fibrillin deposited into the extracellular matrix is less than 30% of control. These results are shown in Fig. 4. Immunofluorescence staining of fibrillin in skin fibroblasts is also performed (Fig. 5).

The gene responsible for Marfan syndrome was identified through a convergence of genetic and histochemical analyses. The genetic approach amounted to linkage analysis using random genetic markers, which implicated a locus on chromosome 15 as being responsible for the disorder.

Histochemical studies indicated a deficiency of the extracellular protein fibrillin in the tissues of individuals with Marfan syndrome. The cDNA for fibrillin was cloned, and the gene was found to map to chromosome 15 in the same region as the Marfan syndrome locus. Final proof that fibrillin represents the gene involved in Marfan syndrome was provided by identification of mutations in fibrillin in several individuals with Marfan syndrome.

Fibrillin is a 35-kDa glycoprotein found in connective tissue microfibrils. The protein contains 44 tandem domains homologous to a sequence found in epidermal growth factor. There are 65 exons encoded in a 9287–base pair transcript. There is a homologous gene on chromosome 5. This gene is referred to as fibrillin 2, and the gene responsible for Marfan syndrome on chromosome 15 as fibrillin 1.

Fibrillin 2 has been implicated in a separate genetic disorder, congenital contractural arachnodactyly. In addition to having a role in Marfan syndrome, mutations in the gene for fibrillin 1 have been found in a set of related disorders, including mitral valve prolapsed syndrome, MASS phenotype (myopia, mitral valve prolapsed, non-progressive aortic enlargement, and nonspecific skin and skeletal abnormalities), familial ectopia lentis, Shprintzen-Goldberg syndrome (heart and skeletal features of Marfan syndrome with
craniosynostosis and other developmental abnormalities), and Weill-Marchesani syndrome (short stature, cardiac, and ocular anomalies).

At the biochemical level, five types of fibrillin 1 abnormalities are seen. One type (type I) is characterized by decreased fibrillin synthesis, but normal proportions of fibrillin are secreted into the extracellular matrix. Total amounts of fibrillin in the extracellular matrix are approximately 50% of normal, proportional to the amount of fibrillin made. This biochemical phenotype is associated with a mild clinical phenotype.

Type II involves reduced synthesis and secretion, associated with a more severe form of Marfan syndrome. Types III and IV involve normal rates of synthesis but moderate or severe reductions of deposition in the extracellular matrix. The final biochemical phenotype is entirely normal fibrillin production and deposition at the biochemical level.

The mutation in this family results in a type II biochemical phenotype. As shown in Fig. 4, there is marked reduction of total amounts of fibrillin both in the cellular fraction and in the extracellular matrix. The immunofluorescence photograph (Fig. 5) shows reduction in fibrillin staining.

7. DNA

DNA is obtained from peripheral blood lymphocytes from James and the fibrillin 1 gene studied by denaturing high performance liquid chromatography. An altered pattern, indicative of probable mutation, is found. The DNA is sequenced, and reveals a substitution of T for G, changing a glutamic acid codon at position 745 to a stop codon.

Genetic linkage studies have indicated that all cases of Marfan syndrome are likely to be due to fibrillin mutations. These mutations, however, are highly diverse, and therefore locating an individual mutation can be challenging.

Mutations responsible for reduced synthesis but normal deposition of fibrillin tend to be chain-termination mutations. The normal allele produces normal fibrillin, but the quantity is reduced to 50%. Mutations responsible for reduced deposition of collagen, with or without reduced synthesis, tend to be compatible with production of some abnormal fibrillin, which interacts with the product of the normal allele. Many of these are missense mutations.

In this family, the mutation was identified first by denaturing high performance liquid chromatography (DHPLC) analysis and then was characterized by DNA sequencing. DHPLC involves denaturing the DNA by heating and then allowing reannealing. If a heterozygous mutation is present, some hybrid molecules will form consisting of one strand with the mutation and the other with the wildtype sequence. Such hybrid molecules are referred to as heteroduplexes.
The renatured DNA is applied to the DHPLC column and then DNA molecules are eluted from the column as single strands by chemical treatment to denature the DNA. Heteroduplex molecules tend to be unstable compared with homoduplexes, and therefore elute sooner.

The presence of a early-eluting fraction is suggestive of the presence of heteroduplexes, and identifies that a mutation is present in a specific part of the gene. This can then be subjected to sequencing to confirm the mutation.

The sequence analysis shows that the patient has a G-to-T transversion, which changes a glutamic acid codon (GAA) to a stop codon (TAA). This causes truncation of the protein at amino acid 745. The truncated protein interferes with fibrillin deposition in a dominant negative fashion, explaining the less than 30% of normal deposition seen in Fig. 4 and the low concentration of stained microfibrils in the immunofluorescence photograph (see Fig. 5).

Other genotype-phenotype correlations have been made. The type I biochemical phenotype, in which there is decreased fibrillin synthesis, tends to be associated with mutations that either reduce levels of transcription or cause frameshifts that lead to low levels of product.

Unlike the type II mutations, these type I mutations do not have a dominant negative effect, and the Marfan phenotype tends to be milder. Groups III and IV tend to be associated with missense mutations.

Type IV seems to be associated with mutations that inhibit microfibril assembly, also a dominant negative effect. Type III may be more heterogeneous pathogenetically. Both reduction in quantity of fibrillin produced and inhibition of assembly may occur in different cases.

8. Outcome

Three years have passed since the initial diagnosis was made, and both boys are doing well. Through a mailing from the National Marfan Foundation, their mother learns of a new clinical trial and asks the geneticist who has been following the boys about this trial.

Given the apparent structural weakness of connective tissue in Marfan syndrome and the fact that fibrillin-1 is a component of connective tissue, it seemed likely that the pathogenesis of the disorder would be related to weakened connective tissue. Further study at the molecular level, however, has revealed a more complex mechanism, and one that suggests an approach to treatment.

Fibrillin-1 is a member of a family of proteins that include a set of proteins referred to as latent TGF β-binding proteins (LTBP). TGF β is a cytokine that binds to a cell surface receptor, and is involved both in differentiation and inflammatory pathways. Proteins in the fibrillin-LTBP family bind TGF β in the extracellular matrix and thereby play a role in regulating the action of TGF β.
It is now believed that absence or reduction of fibrillin in the extracellular matrix in Marfan syndrome leads to excessive TGF β signaling, which causes progressive damage to connective tissue. This mechanism implies that the pathology of Marfan syndrome is due to accumulation of tissue injury, which suggests the possibility that inhibition of TGF β signaling might forestall the rate of damage.

The angiotensin II receptor antagonist Losartan, developed as an antihypertensive drug, has been found to reduce activity of this signaling pathway, and is currently being testing in clinical trials for patients with Marfan syndrome.

9. Further reading

Dietz HC. Marfan syndrome. GeneReviews


10. Tables

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<tr>
<th>System</th>
<th>Major Criteria</th>
<th>Minor Criteria</th>
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<tbody>
<tr>
<td>Skeletal</td>
<td>Presence of at least 4 of:</td>
<td>At least two of:</td>
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<td>• Pectus carinatum or pectus excavatum</td>
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<td>• Reduced upper/lower segment ratio</td>
<td>• Joint hypermobility</td>
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<td>• Wrist and thumb signs</td>
<td>• Highly arched palate</td>
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<td>• Scoliosis or spondyloolisthesis</td>
<td>• Facial features (dolichocephaly, malar hypoplasia,</td>
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<td>• Reduced elbow extension</td>
<td>retrognathia, down-slanting palpebral fissures</td>
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<td>• Deep acetabulum by X-ray</td>
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<td><strong>Ocular</strong></td>
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<td>• Hypoplastic iris or ciliary muscle</td>
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<td>• Dilation of ascending aorta</td>
<td>• Mitral valve prolapse</td>
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<td>• Calcification mitral annulus &lt; 40 years of age</td>
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<td>Lumbosacral dural ectasia</td>
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<td><strong>Genetic</strong></td>
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<td>• Parent, child, or sib who meet criteria</td>
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<td>• Presence of known FBN1 pathogenic mutation</td>
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<td>• Inheritance of haplotype associated with disease mutation</td>
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