Mutations in the Gene Encoding Filamin A as a Cause for Familial Cardiac Valvular Dystrophy

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Background—Myxomatous dystrophy of the cardiac valves affects ≈3% of the population and remains one of the most common indications for valvular surgery. Familial inheritance has been demonstrated with autosomal and X-linked transmission, but no specific molecular abnormalities have been documented in isolated nonsyndromic forms. We have investigated the genetic causes of X-linked myxomatous valvular dystrophy (XMVD) previously mapped to chromosome Xq28.

Methods and Results—A familial and genealogical survey led us to expand the size of a large, previously identified family affected by XMVD and to refine the XMVD locus to a 2.5-Mb region. A standard positional cloning approach identified a P637Q mutation in the filamin A (FLNA) gene in all affected members. Two other missense mutations (G288R and V711D) and a 1944-bp genomic deletion coding for exons 16 to 19 in the FLNA gene were identified in 3 additional, smaller, unrelated families affected by valvular dystrophy, which demonstrates the responsibility of FLNA as a cause of XMVD. Among carriers of FLNA mutation, the penetrance of the disease was complete in men and incomplete in women. Female carriers could be mildly affected, and the severity of the disease was highly variable among mutation carriers.

Conclusions—Our data demonstrate that FLNA is the first gene known to cause isolated nonsyndromic MVD. This is the first step to understanding the pathophysiological mechanisms of the disease and to defining pathways that may lead to valvular dystrophy. Screening for FLNA mutations could be important for families affected by XMVD to provide adequate follow-up and genetic counseling. (Circulation. 2007;115:40-49.)

Key Words: genetics • mitral valve • regurgitation • valves

Myxomatous dystrophy of the cardiac valves is a heterogeneous group of disorders that includes syndromic diseases, such as Marfan syndrome, and isolated valvular diseases. Mitral valve prolapse, the most common form of this disease, is presumed to affect ≈2% to 3% of the population and remains one of the most common indications for valvular surgery.1

Typical histopathologic features of myxomatous valvular dystrophy include fragmentation of collagenous bundles within the valve fibrosa and accumulation of proteoglycans, which produces excessive valve tissue that leads to billowing of the valve leaflets, with or without prolapse and regurgitation. The causes of these histological changes remain unknown, however.

The diagnosis of myxomatous valvular dystrophy is predominantly determined by echocardiography. There is no effective treatment to prevent the progressive alteration of the valves, and follow-up is limited to prevention of complications, which include endocarditis and periodic evaluation of the severity of the valvular defects until surgery is required. This situation is largely due to the limited understanding of the pathophysiology of the valvular defects.2

Familial inheritance has been demonstrated, and a familial study is recommended when a case of mitral valve prolapse is suspected.

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The online-only Data Supplement, consisting of a table and a figure, is available at http://circ.ahajournals.org/cgi/content/full/CIRCULATIONAHA.106.622621/DC1. The correspondence to Hervé Le Marec, MD, PhD; l’Institut du Thorax, Service de Cardiologie du CHU de Nantes, CHU de Nantes, Hôpital G and R Laennec, Bd Jacques Monod, 44093 Nantes Cedex, France. E-mail herve.lemarec@chu-nantes.fr
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identified.\textsuperscript{3} Autosomal dominant transmission is the usual inheritance, with reduced penetrance and variable expressivity.\textsuperscript{4} Three loci have been mapped to chromosomes 16p11–p12,\textsuperscript{5} 11p15.4\textsuperscript{e} and 13q31–32,\textsuperscript{7} but the underlying genetic defects are not currently known. An X-linked recessive form was originally described by Monteleone and Fagan\textsuperscript{8} in 1969 and by Newbury-Ecob et al\textsuperscript{9} in 1993.

We previously clinically and genetically characterized a large family (family 1a) affected by X-linked myxomatous valvular dystrophy (XMVD) (OMIM 314400) and used linkage analysis to map the gene to an 8-cM interval on chromosome Xq28.\textsuperscript{10,11} A large familial and genealogical survey led us to refine the locus of the XMVD gene, and a standard positional cloning approach identified a P637Q mutation in the filamin A (FLNA) gene in all affected members of this family. Screening of additional, smaller, unrelated families with severe forms of valvular dystrophy allowed us to find 3 other mutations in this gene. The present study demonstrates that FLNA is the first gene known to be responsible for isolated nonsyndromic valvulopathy.

Methods

Clinical Evaluation

The present study was conducted according to French guidelines for genetic research and approved by the ethics committee of Nantes University Hospital.

Written informed consent was obtained from all participants. Clinical investigation included a review of medical history and a physical examination, with particular attention given to the cardiovascular system and any connective tissue diseases. The phenotypic assignment of family members was based on echocardiographic examination. Family members were followed up with regular valvular surgery. Transthoracic echocardiograms were recorded at clinical evaluation. Family members were followed up with regular echocardiograms. Aortic regurgitation was considered to exist if an abnormal prolapse of the posterior mitral leaflet was observed. Mitral regurgitation was estimated by using standard methods, which include the proximal isovelocity surface area (PISA) method. The length of the leaflet was measured in the parasternal, long-axis, 2-dimensional images without second harmonic.\textsuperscript{12} The length of the leaflet was considered to be abnormal if it was greater than 2 mm without aortic regurgitation.

Genetic Analysis

For molecular studies, DNA was isolated from peripheral blood lymphocytes or paraffin-embedded valve tissue with standard methods.

Linkage Analysis, Refined Mapping, and Haplotypic Constructions

Family 1 was already linked to chromosome Xq28. To narrow the candidate region, we selected 8 microsatellite markers (DXS998, DXS8091, DXS8069, DXS8061, DXS15, DXS1073, F8, and DXS1108) from the candidate interval on Xq28 for use in linkage analysis in the expanded family. F8 is a dinucleotide marker located within factor VIII gene intron 13.\textsuperscript{13} We carried out 2-point linkage analysis with the FASTLINK program in the easyLINKAGE software package (version 4.0; Medical University Clinic at the University of Würzburg, Würzburg, Germany).\textsuperscript{14} For linkage calculations, we assumed X-linked inheritance with a disease-allele frequency of 0.0001 and phenocopies at 2%. Penetrance was set at 100% for male family members and 70% for female family members. All family members were included in the analysis. Patients were defined as affected if the thickness of the mitral leaflets was >4 mm and if mild to severe aortic regurgitation was present. Subjects with a mitral leaflet thickness <2 mm without aortic regurgitation were defined as unaffected, and subjects with a 2- to 4-mm thickness of mitral leaflets were considered phenotypically undetermined. To detect more recombination events and refine the locus, we carried out linkage analysis with the FASTLINK program in the easyLINKAGE software package (version 4.0; Medical University Clinic at the University of Würzburg, Würzburg, Germany).\textsuperscript{14} For linkage calculations, we assumed X-linked inheritance with a disease-allele frequency of 0.0001 and phenocopies at 2%. Penetrance was set at 100% for male family members and 70% for female family members. All family members were included in the analysis. Patients were defined as affected if the thickness of the mitral leaflets was >4 mm and if mild to severe aortic regurgitation was present. Subjects with a mitral leaflet thickness <2 mm without aortic regurgitation were defined as unaffected, and subjects with a 2- to 4-mm thickness of mitral leaflets were considered phenotypically undetermined. To detect more recombination events and refine the locus, we carried out linkage analysis with the FASTLINK program in the easyLINKAGE software package (version 4.0; Medical University Clinic at the University of Würzburg, Würzburg, Germany).\textsuperscript{14} For linkage calculations, we assumed X-linked inheritance with a disease-allele frequency of 0.0001 and phenocopies at 2%. Penetrance was set at 100% for male family members and 70% for female family members. All family members were included in the analysis. Patients were defined as affected if the thickness of the mitral leaflets was >4 mm and if mild to severe aortic regurgitation was present. Subjects with a mitral leaflet thickness <2 mm without aortic regurgitation were defined as unaffected, and subjects with a 2- to 4-mm thickness of mitral leaflets were considered phenotypically undetermined. To detect more recombination events and refine the locus, 11 microsatellite markers (DXS8103, DXS1684, DXS10052, DXS10053, DXS10054, Afm308yh1, DXS10051, DXS10049, Afm082xa5, GABRA3, and DXS10047) were genotyped in the recombinant individuals to determine the centromeric boundary.\textsuperscript{15} Two intragenic microsatellite markers, GAB3 and F8, were used to determine the telomeric boundary.

Analysis of Candidate Genes and FLNA Mutation Analysis

Candidate genes in the Xq28 interval were screened for mutations by sequence analysis in 1 affected patient of family 1a. Coding exons and short flanking intronic sequences were amplified by polymerase chain reaction (PCR), and excess primer was removed from the amplified fragments with the use of exoSAP (Amersham Biosciences, Piscataway, NJ) and sequenced with a dye-terminator cycle-sequencing system (ABI PRISM 377, Perkin-Elmer Applied Biosystems, Foster City, Calif). The coding sequence of FLNA (exons 2 to 48)\textsuperscript{16} was amplified from genomic DNA with the use of primers as previously described.\textsuperscript{18} Variants of FLNA identified by sequence analysis were confirmed by restriction-enzyme digestion with HaeIII (P637Q) and Sau96I (G288R). The required exons were amplified by PCR, digested with restriction enzyme, and size fractionated on an 8% acrylamide gel. In the 2 cases, the variant abolished 1 restriction site. The V711D mutation was confirmed by derived cleaved amplified polymorphic sequencing.\textsuperscript{17} To assess the frequency of the mutations, we used sequence analysis, restriction-enzyme digestion, or derived cleaved amplified polymorphic sequencing with DNA from family members and from 500 control chromosomes of European, African, or Asian origin.

Model Building

To study the structural consequences of the identified mutations, we constructed a 3-dimensional model of human filamin repeats with the crystal coordinates of Dictyostelium filamin deposited in Protein Data Bank (identification: 1WLH).\textsuperscript{18} The automated homology model construction was performed by the protein structure modeling program INSIGHTII (Accelrys, Cambridge, UK).

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.
of 91 living members. The proband (patient V-13) underwent aortic replacement for severe regurgitation at age 17. He was of normal size and morphology, and a physical examination found no connective tissue or joint abnormalities. Cardiac auscultation revealed aortic regurgitant murmur, and echocardiography showed severe aortic regurgitation. Aortic root dimensions were normal as confirmed by a nuclear magnetic resonance study of the thoracic aorta, without any aspect of Marfan or Ehlers-Danlos syndrome. Histological examination of the excised valve showed typical features of myxomatous valvular disease, with marked thickening of the free edge of the valve.

The proband’s cousin (patient V-10) underwent valvuloplasty for severe mitral regurgitation due to mitral valve dystrophy at age 20 (Figure 1B). Detection of a mild hemophilia A in these 2 patients and a familial study led to the identification of a very large family of >300 individuals. Among 44 male family members, 10 suffered from progressive mitral valve prolapse (Figure 1B), which was associated in 4 cases with moderate to severe aortic regurgitation, and 4 underwent valvular surgery (Table 1). Among 47 female family members, 10 were considered as affected with mitral or aortic valve abnormalities, although all were asymptomatic. One child (patient V-8), diagnosed at age 10, showed...
TABLE 1. Echocardiographic Characteristics of the Affected Male Family Members and Heterozygous Female Family Members of XMVD Family 1a

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at Diagnosis</th>
<th>Year of Examination</th>
<th>Mitral Prolapse</th>
<th>AML Thickness, mm</th>
<th>PML Thickness, mm</th>
<th>Mitral Regurgitation</th>
<th>Aortic Regurgitation</th>
<th>Tricuspid Regurgitation</th>
<th>Pulmonary Regurgitation</th>
<th>Age and Type of Valve Surgery</th>
<th>Phenotypic Status</th>
<th>Genotypic Status</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td>Severe</td>
<td>Mild</td>
<td>Mild</td>
<td>Ao replacement at 49</td>
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<td>Homozygous</td>
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<td>Ao and Mv replacement at 51</td>
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<td>ND</td>
<td>None</td>
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</tr>
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</tr>
</tbody>
</table>

AML indicates anterior mitral leaflet; PML, posterior mitral leaflet; ND, not determined; Ao, aortic valve; and Mv, mitral valve.

severe aortic regurgitation with aortic stenosis. In all affected family members, the valvular disease was associated with mild hemophilia A (factor VIII activity between 15% and 50%).11 To increase the size of this family and facilitate the identification of the gene responsible for the disease, we screened our clinical database for patients who both were affected by hemophilia A and had undergone surgery for valvulopathy. Identification of a new proband (patient III-14, family 1b) affected by valvular dystrophy and mild hemophilia A and originating from the same French geographic location, allowed us to identify a new branch of family 1a called family 1b (Figure 1C). Tracing family trees for families 1a and 1b identified a common ancestor born in the 18th century. The new proband (patient III-14, family 1b) underwent aortic valve replacement at the age of 52 for severe aortic regurgitation and was deceased at the time of this study. His mother had moderate mitral and aortic regurgitation. Three other male family members presented with mitral valvular dystrophy (Figure 1B) and variable aortic regurgitation. All of them had mild hemophilia A except patient IV-3, whose factor VIII activity was normal (>50%). Five female family members were considered as affected with variable mitral or aortic regurgitation (Table 2).

Family 2 (Figure 2A) was a British family with XMVD described by Newbury-Ecob et al10 in 1993. The proband (patient IV-1) was born with severe congenital valvular disease and died at 24 hours of age with severe cardiac failure. Autopsy showed dystrophy of all 4 valves and an atrial septal defect. His grandfather (patient II-2) underwent a triple valve replacement and closure of a persistent foramen ovale at age 41. At surgery, the grandfather’s mitral and aortic valves showed myxomatous dystrophy. The grandfather’s brother (patient II-1) was diagnosed as having mitral and aortic valvular disease at age 30.
TABLE 2. Echocardiographic Characteristics of the Affected Male Family Members and Heterozygous Female Family Members of XMVD Family 1b

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at Diagnosis</th>
<th>Examination Year (Subject Age, y)</th>
<th>Mitral Prolapse</th>
<th>Aortic Regurgitation</th>
<th>Tricuspid Regurgitation</th>
<th>Pulmonary Regurgitation</th>
<th>Age and Type of Valve Surgery</th>
<th>Phenotypic Status</th>
<th>Genotypic Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II-10</td>
<td>49</td>
<td>2005 (55)</td>
<td>AML/PML 5</td>
<td>Moderate</td>
<td>Mild</td>
<td>Mild</td>
<td>None</td>
<td>Affected</td>
<td>Homozygous</td>
</tr>
<tr>
<td>II-14</td>
<td>50</td>
<td>1982 (50)</td>
<td>None ND</td>
<td>Moderate</td>
<td>Severe</td>
<td>None</td>
<td>None</td>
<td>Affected</td>
<td>Unknown</td>
</tr>
<tr>
<td>IV-2</td>
<td>24</td>
<td>2005 (30)</td>
<td>None 5</td>
<td>Moderate</td>
<td>Mild</td>
<td>Mild</td>
<td>None</td>
<td>Affected</td>
<td>Homozygous</td>
</tr>
<tr>
<td>IV-3</td>
<td>21</td>
<td>2005 (27)</td>
<td>AML/PML 5</td>
<td>Moderate</td>
<td>Mild</td>
<td>None</td>
<td>None</td>
<td>Affected</td>
<td>Homozygous</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-7</td>
<td>86</td>
<td>1992 (60)</td>
<td>ND ND</td>
<td>Moderate</td>
<td>Moderate</td>
<td>ND ND</td>
<td>None</td>
<td>Affected</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>III-8</td>
<td>50</td>
<td>2005 (55)</td>
<td>None 3</td>
<td>Moderate</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Affected</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>IV-1</td>
<td>29</td>
<td>2005 (34)</td>
<td>None 3</td>
<td>Mild</td>
<td>None</td>
<td>Mild</td>
<td>None</td>
<td>Undetermined</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>IV-4</td>
<td>18</td>
<td>2005 (24)</td>
<td>None 4.5</td>
<td>Moderate</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Affected</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>IV-6</td>
<td>28</td>
<td>2005 (34)</td>
<td>None 4</td>
<td>Mild</td>
<td>Mild</td>
<td>Mild</td>
<td>ND</td>
<td>Affected</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>IV-7</td>
<td>26</td>
<td>2005 (32)</td>
<td>None 3</td>
<td>Mild</td>
<td>Mild</td>
<td>Mild</td>
<td>None</td>
<td>Affected</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>V-2</td>
<td>6</td>
<td>2005 (6)</td>
<td>None ND</td>
<td>Mild</td>
<td>None</td>
<td>Mild</td>
<td>None</td>
<td>Undetermined</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>V-3</td>
<td>7</td>
<td>2005 (7)</td>
<td>None 3</td>
<td>Mild</td>
<td>None</td>
<td>ND</td>
<td>None</td>
<td>Undetermined</td>
<td>Heterozygous</td>
</tr>
<tr>
<td>V-5</td>
<td>23</td>
<td>2005 (23)</td>
<td>None ND</td>
<td>Mild</td>
<td>None</td>
<td>ND</td>
<td>None</td>
<td>Unaffected</td>
<td>Heterozygous</td>
</tr>
</tbody>
</table>

Abbreviations as in Table 1.

A male infant was the first child of healthy black African parents (family 3) (Figure 2B). He was diagnosed antenatally with abnormally thick cardiac valves by ultrasound and fetal echocardiography and was born at 38 weeks in good health. Postnatal echocardiography confirmed moderate tricuspid incompetence, trivial mitral and pulmonary incompetence, and mild aortic incompetence. All valves were thickened and dystrophic. At 4 months of age, his growth and developmental assessment were within normal limits and he showed no signs of cardiac failure. An echocardiogram showed excellent ventricular function. The mitral valve remained dystrophic without evidence of regurgitation and with only very mild aortic regurgitation. His mother was examined clinically and showed no evidence of cardiac involvement.

Family 4 (Figure 2C) was of Hong Kong Chinese origin. The 2 boys, 12 and 4 years old, had both mitral and aortic dystrophy. A heart murmur was identified in patient II-1 at age 4 months on a routine check. Subsequent echocardiography revealed he had polyvalvular disease with myxomatous thickening of the mitral tricuspid and aortic valves. He had significant mitral and tricuspid regurgitation with mild aortic regurgitation. Patient II-2 was identified because of his brother’s history. He was shown to have polyvalvular disease with mitral incompetence and stenosis, tricuspid regurgitation, and mild aortic regurgitation. Their mother had an essentially normal echocardiogram at age 38 with mild aortic and pulmonary incompetence.

All 4 families presented no clinically apparent extracardiac abnormalities, no dysmorphic features, and no epileptic seizures.

Linkage Analysis and Refined Mapping to Narrow the Locus
A significant linkage (Zmax = 8.6 at θ = 0 for DXS1108) was obtained for family 1 (Data Supplement Table). To refine the XMVD locus, additional markers were genotyped. Recombination at DXS10049 for unaffected male patient IV-54 of family 1a (Data Supplement Figure) set the centromeric boundary for the locus. Analysis of markers from members of family 1b revealed the same disease haplotype, with the exception of patient IV-3. This person, affected by valvular dystrophy but with normal coagulation factor activity, harbored recombination at microsatellite marker GAB3 (Data Supplement Figure), which set the telomeric boundary for the linked region and showed that valvulopathy and hemophilia were transmitted as independent traits. Therefore, the disease gene was located between DXS10049 and GAB3, spanned 2.5 Mb, and excluded the factor VIII gene.

Mutation Identification
After exclusion of candidate genes in the disease interval, eg, BGN, G4.5, ZNF185, CETN2, DUSP9, STK23, SSR4, RENBP, MGC29729, and several predicted genes, we examined FLNA with a direct sequencing approach. The gene is composed of 48 exons that span ∼26 Kb immediately distal to the emerin gene. Mutational analysis of all coding regions of FLNA in affected members of family 1 (families 1a and 1b combined) revealed that all have a C-to-A transition at nucleotide 1910 in exon 13 (NM_001456: c.1910C > A), which predicts a missense mutation—substitution of a proline (P) for a glutamine (Q) at amino acid 637 (P637Q) (Figure 1D). In family 1, a total of 13 affected male family members and 30 female family members are mutation carriers. One clinically affected woman (patient IV-4) did not inherit the mutation and appeared to be a phenocopy. A G-to-A transition at nucleotide 862 in exon 5 (NM_001456: c.862G > A), which predicts a missense mutation of a glycine (G) for an arginine (R) (G288R), was found in the affected members of family 2 (Figure 2A). A T-to-A transition at nucleotide 2132 in exon 14 (NM_001456: c.2132T > A), which predicts a missense mutation of a valine (V) for an aspartic acid (D) at amino acid 711 (V711D), was found in the affected member of family 3 (Figure 2B). These sequence variations were not found in unaffected relatives of either family or in 500 control
chromosomes of white or African origin. The mutations were confirmed by testing for loss of a HaeIII (P637Q) and Sau96I (G288R) restriction sites and derived cleaved amplified polymorphic sequence (V711D), respectively (data not shown).

Finally, we identified a 1944-bp genomic deletion coding for exons 16 to 19, which corresponds to the 546-bp coding sequence (NT_025965.13:g.942144_944086del1944insTG) that predicts an in-frame deletion of 182 residues (from V761 to Q943) in 2 boys (family 4) diagnosed with plurivalvular dystrophy. The deletion was visualized by PCR analysis from intron 15 to intron 19 of the FLNA genomic sequence. The father (patient I-1) produced 1 fragment of 3216 bp, the heterozygous mother (patient I-2) produced 2 fragments of 3216 bp and 1272 bp, and the 2 affected boys (patients II-1 and II-2) produced 1 fragment of 1272 bp, which corresponds to the deletion of 1944 bp.

In addition to the mutations identified in FLNA, other known single-nucleotide polymorphisms were identified in the patients. These single-nucleotide polymorphisms occurred at frequencies comparable to those listed in the public National Center for Biotechnology Information (NCBI) database, some of them resulting in amino acid substitutions. No additional disease-associated variants were observed. Screening of 3 other families with potential X-linked valvular dystrophy did not find any mutation in FLNA.

Genotype-to-Phenotype Relations in Family 1

Genotype-to-Phenotype Relations in Male Family Members

In family 1, 13 male subjects were carriers of the P637Q and Sau96I (G288R) restriction sites and derived cleaved amplified polymorphic sequence (V711D), respectively (data not shown).
Genotype-to-Phenotype Relations in Female Family Members

None of the 30 heterozygous women was symptomatic, and none underwent valvular surgery. Among female carriers of the P637Q mutation, 14 were considered affected, 12 were considered undetermined because of minor valve disease, and 4 were considered unaffected. Mitral valve prolapse was found in 4 cases; these involved the anterior valve in 3 cases and the posterior valve in 1 case. Among heterozygous women, 19 had mild mitral regurgitation and 4 had moderate mitral regurgitation. Mitral valve thickness was $3.3 \pm 0.5$ mm for the anterior valve and $2.8 \pm 0.7$ mm for the posterior valve. The anterior mitral valve ($P<0.001$) and the posterior mitral valve ($P<0.001$) were thicker in male than in female family members. Eight heterozygous women had mild aortic valve regurgitation, 3 had moderate aortic valve regurgitation, and 1 had severe aortic valve regurgitation. Nine heterozygous women had mild tricuspid valve regurgitation, and 1 had moderate tricuspid valve regurgitation. Two heterozygous women had mild pulmonary valve regurgitation, and 1 had moderate pulmonary valve regurgitation.

Structural Aspects of FLNA Mutations in XMVD

Sequence comparisons revealed that all 3 missense mutations modify highly conserved residues and affect the repeat consensus sequence, which includes residues that are common to at least 10 repeats and are highly conserved across a wide range of vertebrate filamins, as compared with consensus sequences derived from the repeating backbones of chicken filamin and Dictyostelium gelation factor (Figure 3A).18–21

The G288R, P637Q, and V711D mutations are located within the first, the fourth, and the fifth repeat, respectively, and the truncated protein corresponds to a smaller protein that lacks repeats 5 to 7 (Figure 3B).

The predicted 3-dimensional model of human filamin repeats shows remarkable similarity to Dictyostelium gelation factor in the overall 3-dimensional fold. The complete structure of filamin repeat consists of 7 antiparallel $\beta$-strands arranged in 2 $\beta$-sheets of 3 and 4 $\beta$-strands (Figure 3C). Modeling the 3-dimensional structure of the filamin repeat showed that the Gly288 residue is located on the external face of the repeat, whereas the Pro637 and Val711 residues are internal. For all mutants, the replacement of a nonpolar residue with a polar residue (basic, uncharged, and acidic for G288R, P637Q, and V711D, respectively) could account for an increase in polarity. It can be expected, therefore, that these mutations would cause a significant change in the structural conformation of the $\beta$-strands.

All 3 substitutions, located in the same region and predicted to impact the antiparallel $\beta$-strands organization of the protein, could result in impaired partner binding.

Discussion

In this study, we describe the identification of FLNA as the first gene responsible for a nonsyndromic valvular dystrophy.

The identification of this genetic defect transmitted with a X-linked recessive pattern was facilitated by a genealogical and geographic approach to the disease. Screening of our hospital files coupled with genealogical analysis of patients affected with myxomatous valvular dystrophy led to the identification of 2 kindreds from the same geographic location who have inherited the same mutation in the FLNA gene from a common ancestor born in the 18th century.

We have several lines of evidence to show that the P637Q mutation in the FLNA gene is the gene responsible for the valvular defect in family 1. Linkage analysis that includes all male and female patients gave a significant positive lod score, and all affected males are carriers of the mutation. Furthermore, screening of additional unrelated families affected by valvular dystrophy potentially transmitted with an X-linked recessive pattern identified 3 other mutations (G288R, V711D, and a 182–amino acid deletion) in the FLNA gene. None of these mutations was found in 500 control chromosomes. All missense mutations are highly conserved across filamin repeats and species.

Within the families with X-linked valvular dystrophy, the disease was inherited with complete penetrance in male family members and incomplete penetrance in female family members, with variable degrees of expression, consistent with different X-inactivation patterns. Among 13 male carriers of the P637Q mutation, 12 had typical mitral valve prolapse characterized by thicker leaflets associated with mild to severe aortic regurgitation, and 11 also had tricuspid regurgitation. Among 30 female carriers, 14 were considered affected with aortic or mitral valve abnormalities. Female mutation carriers had thicker mitral leaflets than normal women but were less severely affected than male patients (no valvular surgery).

Age for valvular surgery ranged from 17 to 52 years in family 1. Age at diagnosis was also highly variable. Within family 1, male family members were diagnosed between age 11 and 55 years. Within family 2, 2 patients (II-1 and II-2) were diagnosed with progressive polyvalvular dys- trophy between age 25 and 30 years, and 1 patient (IV-1) was diagnosed at birth. In family 3, polyvalvular disorder was diagnosed antenatally, and it was diagnosed at age 4 and 12 years in family 4.

Filamin A is a ubiquitous phosphoprotein that X-links actin filaments and links the actin cytoskeleton to the plasma membrane by interacting with both actin and membrane proteins such as $\beta$-integrins.23,24 Filamin consists of an actin-binding domain at the N-terminus and 24 homologous repeats that correspond to the rod backbone of the protein.16,19 Each repeat consists of 7 antiparallel $\beta$-strands that are arranged in 2 $\beta$-sheets. Filamins exist in vivo as dimers mediated by interactions between C-terminal sequences.

In addition to its role as a structural component of the cytoskeleton, filamin A has also been implicated in regulating many cellular signaling pathways. Filamin A may contribute to the development of myxomatous changes of the cardiac valves by regulation of transforming growth factor-$\beta$ (TGF-$\beta$) signaling through its interaction with Smads activated by TGF-$\beta$ receptors.25,26 Defective signal- ing cascades that involve members of the TGF-$\beta$ superfam- ily have been described in impaired remodeling.
of cardiac valves during development. Mice that lack the TGF-β and BMP signaling inhibitor Smad6 show hyperplasia of the cardiac valves.27 On the other hand, BMP6:BMP7 double mutants have hypoplastic valves.

The weakened valves could be deformed by hemodynamic stresses that reveal or reinforce the underlying defect with age. The higher pressures that occur in the left cavities might explain why both the aortic and the mitral valves are more commonly affected. Pulsatile flow has a well-known impact on cell morphology and composition of valves are more commonly affected. Pulsatile flow has a well-known impact on cell morphology and composition of extracellular matrix proteins and signaling pathways that mediate transmission between extracellular matrix proteins and the cytoskeleton in syndromic valvular dystrophy. Identification of new genes that cause mitral valve prolapse will allow us to confirm this hypothesis.

Distinct mutations of filamin genes produce different phenotypes. Mutations in FLNB and FLNC genes were identified in skeletal and muscular disorders, respectively.28,29 Mutations in the FLNA gene had previously been described in human periventricular nodular heterotopia (OMIM 300049) and a broad range of congenital malformations: otopalatodigital syndromes (OMIM 311300 and 304120), frontometaphyseal dysplasia (FMD), Melnick-Needles syndrome (MNS), and X-linked myxoma-valvular dysplasia (XMDV). Numbers above symbols indicate total number of mutations in a given repeat identified in the 5 different FLNA diseases. Most mutations are localized within the ABD and repeat 10. All 4 XMVD mutations are clustered between repeats 1 and 7.

Remodeling of mitral valves might be caused by defects in proteins of the extracellular matrix, similar to cytoskeletal proteins and signaling pathways that mediate transmission between extracellular matrix proteins and the cytoskeleton in syndromic valvular dystrophy. Identification of new genes that cause mitral valve prolapse will allow us to confirm this hypothesis.

Distinct mutations of filamin genes produce different phenotypes. Mutations in FLNB and FLNC genes were identified in skeletal and muscular disorders, respectively.28,29 Mutations in the FLNA gene had previously been described in human periventricular nodular heterotopia (OMIM 300049) and a broad range of congenital malformations: otopalatodigital syndromes (OMIM 311300 and 304120), frontometaphyseal dysplasia (OMIM 305620),

Figure 3. Sequence comparison of filamin repeats and position of mutations. A, Amino acid sequence alignment of 24 repeats of human FLNA. Human, chicken, and Dictyostelium gelation factor (DGF) consensus sequences that include residues common to at least 10 repeats are listed at the bottom of the figure. Comparison showed that the 3 mutations modify highly conserved residues. Mutations are indicated in red, consensus residues are indicated in black, and the deletion is highlighted in red. B, Schematic representation of filamin’s actin-binding domain (ABD) and 24 repeats and summary of positions of mutations identified in periventricular heterotopia (PH), otopalatodigital syndromes (OPD), frontometaphyseal dysplasia (FMD), Melnick-Needles syndrome (MNS), and X-linked myxomatous valvular dysplasia (XMDV). Numbers above symbols indicate total number of mutations in a given repeat identified in the 5 different FLNA diseases. Most mutations are localized within the ABD and repeat 10. All 4 XMVD mutations are clustered between repeats 1 and 7. Modified from Feng and Walsh22 by permission from Macmillan Publishers Ltd. Copyright 2004. C, Homology model of human filamin repeats WT and mutants based on the crystal structure of Dictyostelium filamin repeat 4 (Protein Data Bank identification, 1WLH). Anti-parallel β-strands are shown in yellow. Amino acids are shown in CPK configuration scale to 0.8. In the right panel, Gly288 is present on the surface of the repeat, whereas the 2 other amino acids Pro637 and Val711 are present inside the repeat. In the left panel, the 3 mutants G288R, P637Q, and V711D are shown in orange, green, and red, respectively.
and Melnick-Needles syndrome (OMIM 309350).30 Valvular disease was reported in frontometaphyseal dysplasia31 and periventricular nodular heterotopia with Ehlers-Danlos syndrome.32 No signs of human periventricular nodular heterotopia, otopalatodigital syndromes, frontometaphyseal dysplasia, or Melnick-Needles or Ehlers-Danlos syndromes were found in these families. Because no extracardiac abnormalities were found, the valvular dystrophy reported in the present article is an isolated disease and not part of a syndrome.

The mechanisms by which different mutations in FLNA lead to variable expression of disease remain unknown. A large number of mutations identified in periventricular heterotopia are nonsense and splicing mutations, and all mutations identified in the other disorders are missense mutations that cluster in small regions of the protein with no clear genotype–phenotype correlation. The different phenotypes may highlight distinct interactions of filamin with protein partners, and so far >30 binding partners have been found for FLNA. Among >30 mutations identified in FLNA, most are localized in the actin-binding domain and the repeat 10 (Figure 3B), which suggests a functional importance of these regions of the protein.22 The 4 XMVD mutations are located within the same region of the protein (repeats 1 to 7) and could alter binding affinity for protein partners not yet identified. Screening of additional probes for FLNA mutations is required to determine how particular mutations are associated with different phenotypes.

In conclusion, our data have demonstrated that FLNA is the first gene to cause isolated nonsyndromic myxomatous valvular dystrophy. This is the first step to understand the underlying mechanisms of the disease and to define pathways that may lead to valvular dystrophy. In the future, the identification of the proteins involved in this cascade could provide therapeutic targets for the medical management of this disease. Screening of FLNA mutations could be important to provide adequate follow-up and genetic counseling for families affected by XMVD.

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Disclosures

None.

References


**CLINICAL PERSPECTIVE**

Familial inheritance has been demonstrated for mitral valve prolapse, the most common form of valvular dystrophy, and a familial study is recommended when a case is identified. Autosomal dominant transmission is the usual inheritance, with reduced penetrance and variable expressivity, and 3 loci have been mapped on chromosomes 11, 13, and 16. An X-linked myxomatous valvular dystrophy is a less frequent form of the disease, and we have previously mapped it to Xq28, but the underlying genetic defect was not previously known. Clinically affected patients are predominantly male with either mitral or aortic valve defects, whereas female patients present with minor forms of valvular dystrophy. The present study demonstrates that a defect in the filamin A (*FLNA*) gene is responsible for X-linked valvulopathy. These findings could have important clinical implications. For example, the identification of the genetic basis for nonsyndromic valvular dystrophy offers a potential molecular diagnostic tool to detect patients at risk. Early identification of presymptomatic patients might allow a better clinical follow-up to prevent complication of the disease.
Mutations in the Gene Encoding Filamin A as a Cause for Familial Cardiac Valvular Dystrophy


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