Screening of TGFBR1, TGFBR2 and FLNA in familial mitral valve prolapse

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ABSTRACT
So far only mutations in the filamin A gene (FLNA) have been identified as causing familial mitral valve prolapse (MVP). Previous studies have linked dysregulation of the transforming growth factor beta (TGF-β) cytokine family to MVP. We investigated whether mutations in the TGF-β receptors genes type I (TGFBR1) and II (TGFBR2) underlie isolated familial MVP cases. Eight families with isolated familial MVP were evaluated clinically and genetically. Ventricular arrhythmias were present in five of the eight families and sudden cardiac death occurred in 6 patients. Tissue obtained during mitral valve surgery or autopsy was available for histologic examination in 6 cases; all demonstrated myxomatous degeneration. A previously described FLNA missense mutation (p.G288R) was identified in one large family, but no mutations were discovered in TGFBR1 or TGFBR2. Our results suggest that TGFBR1 and TGFBR2 mutations do not play a major role in isolated myxomatous valve dystrophy. Screening for FLNA mutations is recommended in familial myxomatous valvular dystrophy, particularly if X-linked inheritance is suspected.
INTRODUCTION

Mitral valve prolapse (MVP) is a common cardiac abnormality with an estimated prevalence of 2-3%. It is characterized by single or bileaflet systolic billowing of the mitral valve into the left atrium. There is often leaflet thickening and redundancy, histologically characterized by myxomatous degeneration (Barlow disease). The natural history of MVP varies from asymptomatic with normal life expectancy to serious complications such as significant mitral regurgitation (MR), bacterial endocarditis, thromboembolism and even sudden cardiac death. Although these complications are uncommon, MVP is the most frequent cause of isolated MR requiring mitral valve surgery.

MVP can be part of a generalized connective tissue disorder, especially Marfan syndrome (MFS) and sub-diagnostic variants, but usually occurs as an isolated anomaly. Isolated MVP cases are mostly sporadic but autosomal dominant or X-linked forms have been described. The gene encoding filamin A (FLNA) is located on the X-chromosome and was the first gene discovered to cause isolated myxomatous valvular dystrophy, of which MVP is the most common form (OMIM 300017). Other studies have identified loci on chromosomes 11, 13, and 16 linked to familial MVP. Connective tissue disorders, besides MFS, in which MVP can occur are, for example, Loeys-Dietz syndrome and Ehlers-Danlos syndrome. Dysregulation of the transforming growth factor beta (TGF-β) cytokine family is a key factor in the pathogenesis of these syndromes. In addition, Ng et al. found a relationship between dysregulation of TGF-β and the development of MVP in a murine model of MFS. In the prolapsing valves of the Marfan-mice there was increased activity and signaling of TGF-β. A recent study also found TGF-β upregulation in the mitral valves of patients with isolated myxomatous MVP.

We therefore investigated whether mutations in the genes encoding the TGF-β receptors type I (TGFBR1) and II (TGFBR2) underlie non-syndromal familial MVP. As FLNA mutations have been previously shown to cause myxomatous valvular dystrophy, we also screened our patients for mutations in this gene.

METHODS

All families with isolated familial MVP known at our cardiogenetics outpatient clinics in two centers (University Medical Center Groningen and Antonius Hospital Sneek) were included in this study. Routine procedures at the cardiogenetics outpatient clinic include: complete evaluation of the index patient and constructing a pedigree. We provide letters for the index patients to give to family members at risk inviting them to our outpatient clinic for presymptomatic (“cascade”) screening. The clinical and genetic data of the index patients and family members were retrieved from clinical records. MVP was defined as echocardiographic single or bileaflet prolapse of at least 2 mm beyond the long-axis annular plane. Prolapse with thickening of the valve leaflets greater than 5 mm was termed classic prolapse. Familial MVP was considered present if there were at least two first- or second-degree relatives with MVP. As part of routine procedures, written informed consent was obtained from all patients for DNA-analysis, including DNA-analysis for scientific purposes.
DNA analysis

Genomic DNA was isolated from peripheral blood samples according to standard protocols. Bidirectional direct sequencing of the coding regions and flanking intronic sequences of \(\text{TGFBR1}\), \(\text{TGFBR2}\) and \(\text{FLNA}\) was performed in all index patients, using the BigDye Terminator DNA sequencing kit (version 2.0). Results were analyzed by standard software packages. Conditions and primers are available upon request.

RESULTS

Clinical evaluation

Eight families with familial isolated MVP were evaluated; seven families from the University Medical Center Groningen (family A to G) and one family from the Antonius Hospital Sneek (family H). Characteristics of the patients are summarized in Table I and see Figure 1. for pedigrees of all families. None of the patients had other anomalies and MFS or other generalized connective tissue diseases were excluded in all patients.

Table I. Characteristics of the patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Echocardiography</th>
<th>LV function</th>
<th>Pathologic examination</th>
<th>Arrhythmia</th>
<th>Mutation</th>
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<tr>
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<td>39</td>
<td>F</td>
<td>prolapse AML, early systolic MR</td>
<td>normal</td>
<td>NA</td>
<td>-</td>
<td>-</td>
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<tr>
<td>A-2</td>
<td>30</td>
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<td>prolapse AML, mild MR</td>
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<td>-</td>
<td>-</td>
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<td>NA</td>
<td>sustained VT (ICD)</td>
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<td>VF</td>
<td>-</td>
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<td>NA</td>
<td>-</td>
<td>-</td>
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<td>M</td>
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<td>NA</td>
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<td>VF</td>
<td>-</td>
</tr>
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<td>-</td>
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<td>VF</td>
<td>-</td>
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<tr>
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<td>-</td>
<td>-</td>
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<td></td>
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<td>classic prolapse AML and PML, mild MR, mild annulus calcification, bicuspid aortic valve</td>
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<td>SSS and 2nd degree AV-block (DDD pacemaker)</td>
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<td>F</td>
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<td>M</td>
<td></td>
<td>NA</td>
<td>NA</td>
<td>myxomatous degeneration mitral valve</td>
<td>VT (ICD) VF</td>
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<td>24</td>
<td>F</td>
<td></td>
<td>prolapse AML, mild MR</td>
<td>moderate</td>
<td>NA</td>
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<tr>
<td>G-1</td>
<td>38</td>
<td>F</td>
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<td>classic prolapse AML and PML, mild late systolic MR</td>
<td>normal</td>
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<td>33</td>
<td>F</td>
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<td>normal</td>
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<tr>
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<td></td>
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<td>moderate</td>
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<tr>
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<td>19*</td>
<td>M</td>
<td></td>
<td>classic prolapse AML and PML</td>
<td>normal</td>
<td>myxomatous degeneration mitral valve</td>
<td>VF</td>
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<td>H-3</td>
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<td>prolapsed AML</td>
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<td>F</td>
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<td>NA</td>
<td>3rd degree AV-block (DDD-R pm)</td>
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<td>F</td>
<td></td>
<td>normal</td>
<td>normal</td>
<td>NA</td>
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</table>

- not present
* sudden cardiac death
Patient A-1 was a 39-year-old woman who was referred to our MFS outpatient clinic because of a long and slender body habitus and a mid-systolic click on auscultation of the heart. Aortic dimensions were normal on echocardiography but a prolapse of the anterior mitral valve leaflet (AML) and late systolic MR were discovered. No other signs of MFS were present. She had a 30-year-old brother (patient A-2) with a prolapse of the AML accompanied by mild regurgitation. Aortic diameters were normal but he had a dilated left ventricle with a normal ejection fraction.

Patient B-1 was a 51-year-old woman who was also evaluated for a possible diagnosis of MFS. She had a classic prolapse of the AML and posterior mitral valve leaflet (PML) with moderate MR, aortic diameters were normal. Her brother (patient B-2) had died suddenly at the age of 34 years and autopsy had shown an abnormally formed mitral valve with myxomatous degeneration, aortic dimension were normal. Holter examination of patient B-1 showed frequent non-sustained ventricular tachycardias (VT). This, combined with her family history, was reason to implant a cardioverter defibrillator (ICD). Two months post implantation she experienced a sustained VT which was successfully terminated by the ICD.

Patient C-1 was a 45-year-old woman who was evaluated because her brother (patient C-2) had died suddenly at the age of 36 years. Autopsy had documented a floppy mitral valve and a rupture of one of the chordae (Fig. 2). Aortic dimensions were normal. On microscopic examination there was extensive myxomatous degeneration of the mitral valve. Echocardiography of patient C-1 showed a prolapse of the AML and PML with mild late systolic MR. Her 17-year-old daughter (patient C-3) had a prolapse of the PML and late systolic MR. Patient C-1’s younger brother (patient C-4) was also

Figure 1. Pedigrees of all families. Filled symbols: individuals with a MVP. Filled symbols family H: individuals with a MVP and the FLNA missense mutation (p.G288.R). Patients H-5 and H-6 have the FLNA missense mutation but do not have a MVP.

* denotes patients with (sustained) ventricular arrhythmias
evaluated. On echocardiography there was a prolapse of the AML and PML with mild late systolic MR. Aortic dimensions of patients C-1, C-3 and C-4 were normal.

Patient D-1 was an 18-year-old woman who collapsed due to ventricular fibrillation while giving an oral presentation at high school. Cardiopulmonary resuscitation was unsuccessful. Autopsy demonstrated an enlarged and thickened mitral valve showing extensive myxomatous degeneration. Her uncle (patient D-2) had a prolapse of the AML with mild late systolic MR, but in contrast to his niece he had no thickening of the mitral valve. In both patients aortic dimensions were normal. Patient D-1’s father (patient D-3) had died aged 41 years of a non-cardiac cause when she was 17 years old, cardiac analysis was never performed.

Patient E-1 was a 29-year-old woman who was evaluated because of familial occurrence of valvular heart disease. Echocardiography demonstrated a classic prolapse of the AML with moderate MR. Her brother (patient E-2) had a classic prolapse of the AML and PML with mild holosystolic MR. He had received a DDD-R pacemaker because of a “sick sinus syndrome” and a 2\textsuperscript{nd} degree atrioventricular block. They had a sister who had died neonatally of heart failure. The autopsy report mentioned a ‘glassy papillary thickening of all heart valves’. Their father (patient E-3) had a classic MVP, a tricuspid valve prolapse (TVP), and a bicuspid aortic valve. At the age of 44 years
he was operated on because of severe aortic stenosis and severe MR, both valves being replaced by mechanical prostheses. Postoperatively a VVI pacemaker was implanted because of 3rd degree AV-block. Four years later he died suddenly, but no autopsy was performed. All patients in this family had normal aortic diameters.

Patient F-1 was a 54-year-old woman who was evaluated because her son (patient F-2) had died suddenly due to ventricular fibrillation (VF) at the age of 29 years. Autopsy had shown myxomatous degeneration of the mitral valve. Echocardiography of patient F-1 revealed a classic prolapse of the AML with mild MR. She received an ICD because of non-sustained VT’s, unexplained syncope and her family history. Her daughter (patient F-3) was also screened and a prolapse of the AML was discovered. All patients in this family had normal aortic diameters.

Patient G-1 was a 38-year-old woman who was evaluated because of palpitations. On echocardiography a classic prolapse of the AML was visible with moderate MR. Her sister (patient G-2) had a classic prolapse with late-systolic moderate MR. Both patients had normal aortic diameters.

Patient H-1 was a 26-year-old man who was referred for evaluation of a possible genetic cause of his mitral valve pathology, aortic root dilatation (aortic root diameter: 39 mm; maximum predicted aortic root diameter: 36 mm) and dilated left ventricle.18 At the age of 3 years he had received a mechanical prosthetic valve (Björk-Shiley 23 mm) in the mitral valve position due to severe MR and

Figure 3. Microscopy (after hematoxylin and eosin staining) of the mitral valve leaflet of patient H-2 showing a superficial stromal nodule with surface fibrosis and extensive myxomatous change. Original magnification 200x.
stenosis. He had a brother (patient H-2), who had died suddenly at the age of 19 years, who also had mitral valve pathology. Since his youth he had a classic prolapse of the AML and PML with myxomatous degeneration. In addition, the other valves (aortic, pulmonary and tricuspid) were also abnormal, being thickened and having abundant tissue. Furthermore, he had a muscular ventricular septal defect. At the age of 18 years he was operated on because of severe MR. Pathologic examination of the resected valve-tissue showed myxomatous degeneration (Fig. 3). One year later he died suddenly. Besides the known valve pathology, autopsy revealed no other anomalies. Their mother (H-3) and grandmother (H-4) were also evaluated and both had a prolapse of the AML. No anomalies were discovered on the echocardiogram of their aunt (H-5) and her daughter (H-6).

**Genetic evaluation**

*TGFBR1, TGFBR2* and *FLNA* were analyzed by direct sequencing in all index-patients. This led to the identification of a previously described *FLNA* missense mutation (c.862G>A), leading to a change of amino acid glycine into arginine at position 288 (p.G288R) in family H. Family members were all shown to be carriers of the mutation. No mutations in *TGFBR1* and *TGFBR2* were identified and there were no *FLNA* mutations found in families A-G.

**DISCUSSION**

Until now, only mutations in the *FLNA* gene have been demonstrated to cause isolated MVP. In 2007 Kyndt et al. described 4 families having myxomatous valvular dystrophy, transmitted in an X-linked recessive pattern. They identified 4 different mutations in *FLNA* causing the valvular dystrophy; 3 missense mutations and one 1944-bp genomic deletion of exons 16 to 19.

*FLNA* is one of the 3 filamin genes expressed in humans and filamin A is a protein that crosslinks filamentous actin (an important component of the cytoskeleton) into orthogonal networks, contributing to both strength and flexibility of cells. The *FLNA* mutations lead to impaired binding abilities of filamin A which eventually influences the elastic properties of cells. As *FLNA* is located on the X-chromosome, women carrying *FLNA* mutations are usually unaffected or only mildly affected as they have another (normal) *FLNA* gene copy on their second X-chromosome. Besides a major component of the cytoskeleton, filamin A is also involved in the regulation of TGF-β signaling through interaction with SMADs (signal transducer- and transcriptional modulator-proteins). The TGF-β signaling pathway is one of the many regulatory pathways involved in controlling normal and abnormal cardiac valve development. Genetic deletion of *SMAD6*, which inhibits TGF-β signaling, causes atrioventricular canal and outflow tract valve primordial hyperplasia in mice. TGF-β signaling plays a role in endocardial cushion formation and endothelial to mesenchymal transdifferentiation (EMT) of endocardial endothelial cells, a crucial step in the development of the mitral valve. In addition, when EMT is completed, TGF-β activity and other signaling mechanisms induce mesenchymal cell proliferation, another important process in the development of the mitral valve. Furthermore, TGF-β plays an important role in the pathogenesis of MFS and Loeys-Dietz.
syndrome, in which MVP can be present.

Based on the above premises, and a recent study demonstrating upregulation of TGF-β in the mitral valves of patients with isolated myxomatous MVP, we argued that isolated familial MVP might be caused by mutations in the TGF-β receptors I and II. However, we found no mutations in TGFBR1 and TGFBR2 in our group of eight families, suggesting that mutations in these genes generally do not cause isolated familial MVP. This confirms earlier observations by Arrington et al. that TGFBR1 and TGFBR2 mutations are absent in isolated valve pathology. Although our patient group was relatively small, it was well defined and important disease was clearly present; MVP affected all index patients, ventricular arrhythmias were present in at least 5 of the 8 families, and 6 family members had died suddenly. The high prevalence of ventricular arrhythmias and sudden cardiac death in our patient group is striking, although there might be a referral-bias to our tertiary center. Indeed the association between MVP and sudden cardiac death has been established, still the exact cause of the malignant ventricular arrhythmias remains obscure. In sudden death survivors with solely a MVP, a high background rate of complex ventricular ectopy (ventricular bigeminy and/or non-sustained VT) has been reported. This frequent ventricular ectopy might be caused by mechanical reasons from the MVP, but why they deteriorate in to even more malignant arrhythmias remains unknown.

Although no mutations were identified in TGFBR1 and TGFBR2 in our patients, we did, however, identify a previously described p.G288R missense mutation in FLNA in family H. Phenotypic characteristics in this family varied from normal (patient H-5 and H-6) to asymptomatic, MVP (patient H-3 and H-4) in female carriers to early onset of polyvalvular pathology (patient H-2) and sudden death in affected males. Aortic root dilatation was also present in one of these patients (patient H-1). In the 4 families described by Kyndt et al., the cardiac phenotype was mainly characterized by dystrophy of the mitral and/or aortic valve. Involvement of the tricuspid and pulmonary valves occurred less frequently and aortic root dilatation was not reported. In Table II we summarize the clinical characteristics of all the patients having an p.G288R missense mutation in FLNA. Besides causing X-linked valvular dystrophy, FLNA mutations can also cause X-linked periventricular nodular heterotopia (OMIM 300017), X-linked chronic idiopathic intestinal pseudo-obstruction (OMIM 300048), otopalatodigital syndrome types I and II (OMIM 311300), Melnick-Needles syndrome (OMIM 309350), frontometaphyseal dysplasia (OMIM 305620) and FG syndrome-2 (OMIM 300321). Cardiac defects have been described in these diseases, for example aortic coarctation, aortic dilatation, aortic regurgitation, aortic stenosis, double outlet right ventricle, hypoplastic left ventricle, left ventricular noncompaction, mitral atresia, mitral valve prolapse, patent ductus arteriosus, and tricuspid valve prolapse. Although there are many (unknown) causes of these cardiac defects other than FLNA mutations, screening for FLNA mutations could be considered in unexplained cases, and in particular in the case of suspected X-linked inheritance.
We screened 8 families with familial MVP for mutations in TGFBR1, TGFBR2 and FLNA. Importantly, malignant arrhythmias were present in at least five of them and sudden death occurred in six patients. In one family we discovered a previously described FLNA missense mutation but we found no TGFBR1 and TGFBR2 mutations, suggesting that such mutations do not play a major role in isolated myxomatous valvular dystrophy. These latter findings need to be confirmed in larger cohort studies. Screening for FLNA mutations is recommended in familial mitral valve dystrophy, particularly if X-linked inheritance is suspected. In other unexplained cardiac defects, screening for FLNA mutations could also be considered, again particularly if inheritance is suspected to be X-linked.

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REFERENCES


