Ghent University
Faculty of Medicine and Health Sciences
Centre for Medical Genetics

Cardiovascular characteristics in Marfan syndrome and their relation to the genotype

Julie De Backer

Thesis submitted in fulfilment of the requirements for the degree of doctor in medical sciences

Promotor: Prof. Dr. A. De Paepe
Co-promotor: Prof. Dr. J. De Sutter
2007

ISBN

Cover: Walking Woman by Alberto Giacometti (1932-1934)

Promotor:

Prof. Dr. A. De Paepe	Ghent University

Co-Promotor:

Prof. Dr. J. De Sutter	Ghent University

Members of the examination committee:

Prof. Dr. T. Gillebert	Ghent University
Prof. Dr. G. Jondeau	Centre de référence Marfan, service de Cardiologie, Hôpital Bichat - Paris
Dr. B. Loeys	Ghent University
Prof. Dr. D. Matthys	Ghent University
Prof. Dr. L. Van Bortel	Ghent University
Prof. Dr. G. Van Camp	Free University Brussels
Prof. Dr. J. Vandewalle	Ghent University
Prof. Dr. G. Vannootten	Ghent University

Julie De Backer
Centre for Medical Genetics
University Hospital Ghent
De Pintelaan 185
9000 Gent, België
Tel +3292405627
Fax +3292404970
Julie.debacker@UGent.be

This work is supported by a research mandate from the Ghent University (BOF 011D4701) to J. De Backer and by a research grant from the Fund for Scientific Research Belgium (FWO G029002) (Promotor: A. De Paepe)

“Soms is wat onmogelijk lijkt, alleen maar moeilijk”
Stefan Brijs, De Engelenmaker 2005

Dankzij Jan en mijn ouders
Voor Jules en Louise

Table of contents

List of abbreviations

A_a	Late mitral annular velocity
AAD	Aortic Aneurysms and Dissections
AIx	Augmentation Index
A wave	Late transmitral velocity wave
BSA	Body Surface Area
CSGE	Conformation Sensitive Gel Electrophoresis
dHPLC	denaturing High Performance Liquid Chromatography
DNA	Deoxyribonucleic acid
DT_E	Deceleration time of the E wave
EDS	Ehlers-Danlos Syndrome
EF	Ejection Fraction
E wave	Early transmitral velocity wave
E_a	Early mitral annular velocity
FBN1	fibrillin-1 gene
Kb	Kilobases (one thousand basepairs)
LV	Left Ventricular
MFS	Marfan syndrome
MPA	Main Pulmonary Artery
MRI	Magnetic Resonance Imaging
MVP	Mitral Valve Prolapse
PCR	Polymerase Chain Reaction
PWV	Pulse Wave Velocity
S_m	Peak systolic myocardial velocity
SSCP	Single Stranded Conformation Polymorphism
TDI	Tissue Doppler Imaging
TGFβ	Transforming Growth Factor beta
TGFBR	Transforming Growth Factor beta Receptor
VNTR	Variable Number Tandem Repeat

I. Introduction

I.1 The role of Marfan syndrome as a model for cardiovascular disease

Over the past decade, the link between cardiovascular medicine and genetics has evolved from nearly inexistent to a major interactive field, both for researchers and for clinicians.

Our growing understanding of the mechanisms by which single genes can cause disease in monogenic disorders has led to a better insight into the pathophysiological basis of more common, genetically complex cardiovascular diseases such as hypertension, hypercholesterolemia, hypertrophic cardiomyopathy, arrhythmias and aortic aneurysms/dissections (1).

Marfan syndrome (MFS) is a monogenic disorder, caused by mutations in the fibrillin-1 gene (*FBN1*) (2). Marfan syndrome is part of a spectrum of Aortic Aneursysm and Dissection disorders (AAD) and has served as a major paradigm for the study of the pathogenesis of aortic aneurysms.

Studying cardiovascular aspects of MFS and their relationship to the genotype will not only improve our knowledge on mechanisms underlying aortic aneurysms, but also other cardiovascular problems such as mitral valve prolapse or even left ventricular dysfunction. In this respect, we performed a detailed characterisation of cardiovascular manifestations in MFS and studied their relationship to the *FBN1* genotype. The results of these studies are provided in this thesis. As an introduction, a brief overview on AAD in general is provided.

I.2 Aortic aneurysms and dissections

I.2.1 Definitions and epidemiology

Aortic aneurysm is an abnormal widening or dilatation of the aorta with a tendency to progressive expansion. The risk for dissection and/or rupture rises substantially with increasing diameters. Thoracic aortic aneurysms can involve the ascending aorta, the aortic arch or descending aorta. Abdominal aortic aneurysms affect the infrarenal aorta. Thoraco-abdominal aortic aneurysms originate in the descending aorta and extend to the abdominal aorta.

Aortic dissection is a sudden event in which blood penetrates the aortic wall through an intimal tear and creates a false channel by dissection of the media (3). Aortic dissections are classified according to Stanford (fig 1).Type A is defined by the involvement of the ascending aorta irrespective of the location of the tear. In type B the dissection does not extend into the ascending aorta.

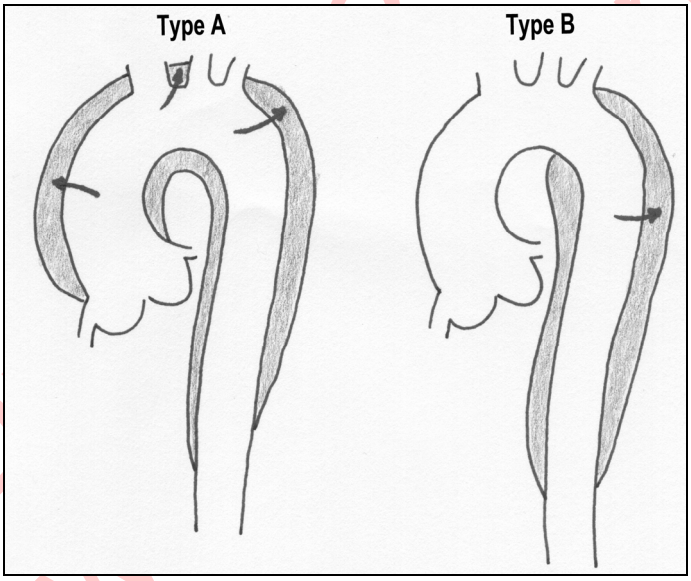


Figure 1: Stanford classification of aortic dissection. See text for explanation. Note the distribution of the entry point for each type

Aortic aneurysms cause significant cardiovascular morbidity and mortality and their rupture or dissection accounts for nearly 15,000 deaths annually in the United States (4).

Prevention of these disorders through the early identification of predisposed individuals and the modification of contributing genetic and environmental factors is a potential life- and cost-saving approach for managing these diseases of the aorta.

I.2.2 Pathophysiology

The normal aortic wall comprises a tunica intima, tunica media and tunica adventitia. In normal aorta, the intima and adventitia are thin and the main tensile strength of the wall is provided by the media. The media consists of smooth muscle cells in a well-organized extracellular matrix consisting of many different components. Among these, elastin, collagen and microfibrils are of interest for this work.

One of the major constituents of the 10-12 extracellular matrix microfibrils is fibrillin1, which serves as a scaffold for the deposition of amorphous elastin. Elastin plays an important role in mediating elastic recoil and allows the aorta to expand during systole (5). Microfibrils appear to subserve several global functions including scaffolding for tropoelastin deposition and elastic fibre formation and anchoring endothelial and epithelial cells to elastic fibres. Microfibrils are extensible themselves and may contribute to the mechanical properties of mature elastic tissues by means of load redistribution between individual elastic fibres(6). Fibrillin1 contains calcium binding sites that are important in stabilising the microfibril against proteolytic degradation by serine proteases and matrix metalloproteinases. Whether mutations in the gene encoding fibrillin 1 – which lead to MFS – influence aortic elasticity is unclear and will be discussed in this thesis.

Fibrillar collagens (types I and II) are the main providers of tensile strength in the aortic wall. As the aorta is distended the initial load is taken by elastin; as further expansion occurs, the coiled collagen fibres are stretched and further expansion is prevented (7).

In the descending thoracic and abdominal aortic aneurysm, atherosclerotic lesions are frequently found on the luminal surface. The media is thinned with loss of smooth muscle cells, decreased elastin content and a relative increase in collagen. A variable degree of chronic inflammation is usually seen. These findings are commonly observed in older people. Hypertension has been identified as a major predisposing factor in these patients.

Histological changes seen in the ascending aorta are slightly different and are characterized by varying degrees of medial necrosis (or cystic medial necrosis by Erdheim). Cystic medial necrosis is characterized by the degeneration of elastic fibers and collagen in the medial layer of the aorta and subsequent accumulation with mucoid material (8, 9) (fig 2).

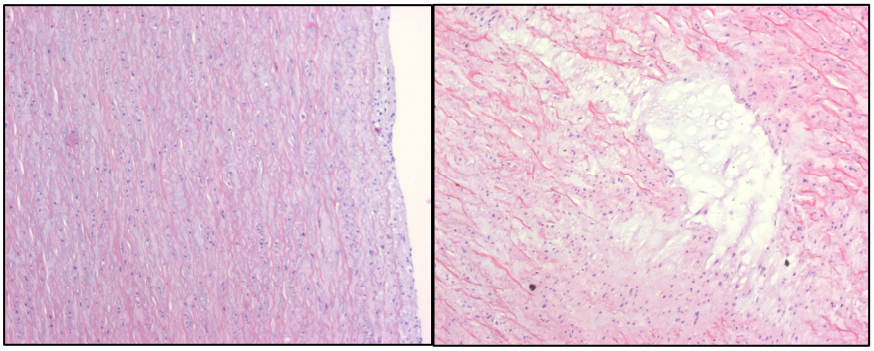


Figure 2: Haematoxylin and Eosin staining of aortic medial layer in a normal control (A) and a MFS patient (B). Note the fragmented elastic fibres and the mucoid material forming cysts in the specimen of the MFS patient

Cystic medial necrosis is typically observed in patients with MFS, but it is also encountered in aging aorta (10), in patients with hypertension (11) bicuspid aortic valves(12) and Fallot's tetralogy (13).

The subdivision in ascending and descending aortic aneurysms is arbitrary. It is now clear that substantial overlap exists in the pathophysiological process underlying aortic aneurysm formation, irrespective of the location or underlying histology of the aneurysm. The underlying triggers that cause the aorta to dilate are far from clear. It is now recognized that multiple factors play a role, including age, hypertension, inflammation and underlying connective tissue anomalies.

The observation that aortic disease in MFS patients is not limited to the ascending aorta is a clear example of the fact that extracellular matrix integrity is important in the entire aorta.

Over the years, increasing evidence has emerged for the contribution of genetic factors into the process of aneurysm formation.

First of all, it has been recognized for several years that thoracic AAD occur in the context of specific genetic syndromes, in particular MFS

Secondly, familial aggregation studies performed as well in families with abdominal as with thoracic aortic aneurysms have indicated that there is a higher prevalence of AAD in first-degree relatives of aortic aneurysm patients than in control groups (14-18). It is estimated that 20% of patients with AAD have other affected family members, supporting the hypothesis that genetic factors influence the formation of aortic aneurysms. Understanding the structural and functional role

of the genes involved in this process may help to elucidate at least part of this complex process.

Most syndromes associated with AAD as well as isolated familial AAD are transmitted in an autosomal dominant fashion, meaning that every child from an affected subject has a 50% chance for developing the disease with an equal distribution in both sexes.

Correct identification of an underlying genetic syndrome in a patient presenting with AAD is important, not only for the purpose of management and follow-up of affected individuals, but also for providing accurate genetic counselling to the family. To this end, detailed family history taking and careful clinical examination in each patient as well as in his first degree relatives is mandatory, as will be illustrated in one of the papers in this thesis.

I.3 Marfan syndrome

I.3.1 Definition and diagnosis

Marfan syndrome (MFS) (MIM #154700) is a connective tissue disorder with pleiotropic manifestations, mainly affecting the ocular, cardiovascular and skeletal organ systems (19). Marfan syndrome is inherited in an autosomal dominant manner and is caused by mutations in the *fibrillin-1* gene (*FBN1*). Up to one quarter of patients have sporadic mutations (20).

The estimated prevalence of MFS is 2-3 per 10.000 (21).

The diagnosis of MFS is based on the identification of major and minor criteria in different organ systems, as defined in the Ghent nosology (22). Major manifestations are highly specific and include the presence of 4 out of 8 skeletal findings, ectopia lentis, dilatation/dissection of the ascending aorta at the level of the sinuses of Valsalva, and dural ectasia (figure 3).

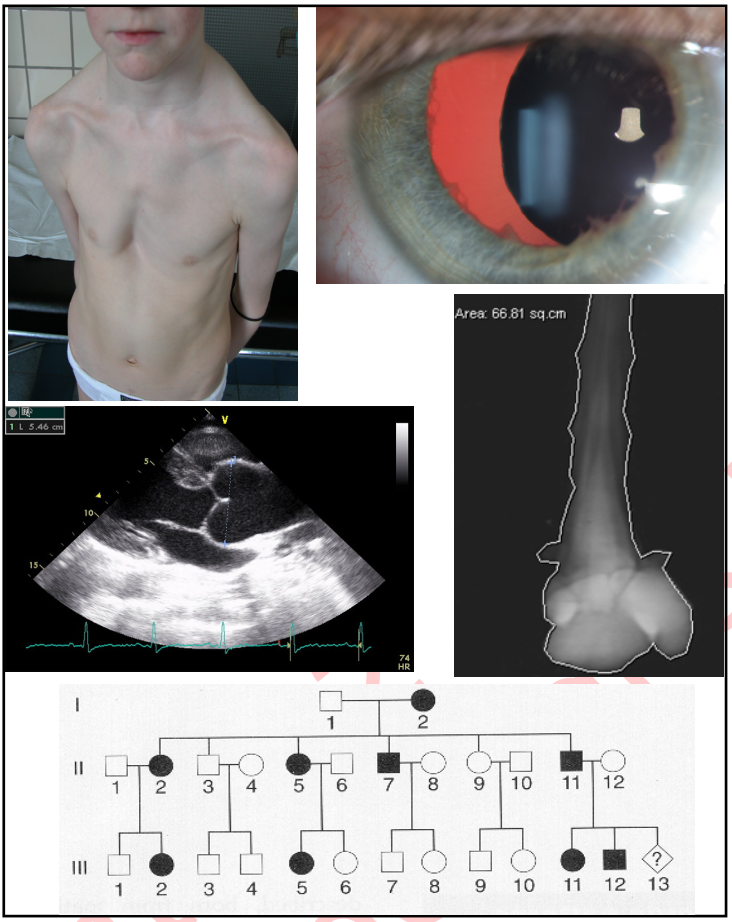


Figure 3: Major manifestations of MFS (clockwise from upper left): a combination of 4 out of 8 skeletal features (note the pectus excavatum; this patient also had arachnodactyly, flat feet and reduced elbow extension); ectopia lentis; dural ectasia and dilatation of the proximal aorta at the level of the sinuses of Valsalva. At the bottom is an illustration of autosomal dominant transmission.

Minor manifestations are less specific and occur frequently in the general population; these include, among others, mitral valve prolapse, striae distensae, pneumothorax and joint hypermobility. The complete list of major and minor manifestations for each organ system can be found in the appendix.

The diagnosis in an index patient is confirmed in the presence of major manifestations in at least 2 different organ systems, in association with involvement of a third organ system. For family members of the proband, the diagnosis is confirmed in the presence of a major manifestation in one organ system with involvement of a second organ system.

Although establishing a clinical diagnosis of MFS is usually straightforward in adult patients, it can be more problematic in certain instances for several reasons. First, many clinical manifestations of MFS, especially those in the cardiovascular system, are age-dependent, making the diagnosis in children particularly challenging. Second, substantial overlap can occur with other connective tissue disorders, related to MFS. Third, significant variability in clinical expression, both within as between families exists and may hamper the diagnosis or exclusion of MFS in family members of affected subjects.

The role of additional molecular testing in the diagnostic decision making in these challenging cases of MFS is not well established at present and will be discussed in this thesis. The underlying mechanisms for the intra-familial variability are also largely unknown. One study indicates a possible role for the expression level of the wild-type allele as a modifier for intrafamilial variability (23).

I.3.2 Molecular Aspects

Marfan syndrome is caused by mutations in the gene encoding fibrillin1, located on chromosome 15 (15q21.1) (2). To date, over 550 different mutations have been identified, located along the entire length of the gene (24). Most families have unique or private mutations.

The fibrillin1 gene (*FBN1*) contains 65 exons spanning 235kb of genomic DNA (25). It encodes a 350 kDa glycoprotein which is highly conserved among different species. The fibrillin 1 gene has a modular structure consisting of repetitive domains. The majority are calcium binding Epidermal Growth Factor like domains (Fig 4)

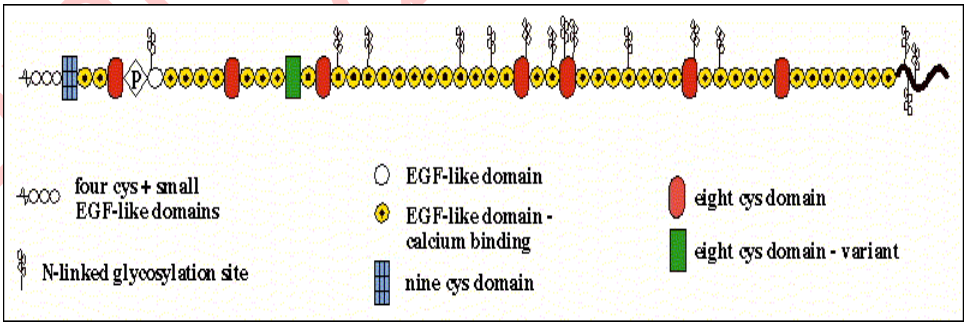


Figure 4: schematic representation of the fibrillin1 gene (EGF: epidermal growth factor)

Most mutations occur within one of the 47 tandemly repeated epidermal growth factor-like domains, and cause the substitution of one of the six predictably spaced cystein residues which influence domain folding via disulfide bonding or

affect residues that are involved in calcium binding to fibrillin1. Such perturbations lead to enhanced cleavage and proteolytic degradation (26).

With the current techniques for molecular analysis, the mutation detection rate reaches over 90% in patients fulfilling the clinical diagnostic criteria for MFS (27). About two-thirds of all *FBN1* mutations are missense mutations, approximately 20% are premature termination codons (PTC) and 12% are splice site mutations (28).

To date, both comprehensive linkage and mutational analyses strongly favour locus homogeneity for the classic MFS phenotype(29-31).

Based on the suggested structural role of fibrillin 1, the classification of mutations has been similar to other connective tissue disorders such as Osteogenesis Imperfecta where missense mutations are related to worse phenotypes due to a dominant negative effect of these mutations (32). In accordance with these findings, it was suggested that missense mutations in the *FBN1* gene would result in worse phenotypes when compared to premature stop codons. Several attempts have been made to prove this hypothesis (33, 34), but none has been convincing so far, except for the higher incidence of lens luxation in patients with cysteine substitutions(35). Even the association between the severe neonatal form of the disease with a cluster of mutations in the regions corresponding to exons 24-32 does not seem to be absolute since milder phenotypes have been found in this region (36-38).

Genotype-phenotype studies focussing on the cardiovascular manifestations of MFS are scarce in the literature.

In patients with premature termination codon mutations, aortic dissection occurred more frequently in the group described by Schrijver and co-workers (34). Mutations in the terminal region of the gene were thought to be associated with milder cardiovascular phenotypes (39).

The underlying mechanisms for the intrafamilial variability are still poorly understood. It is suggested that both genetic and environmental factors are involved. An interesting mechanism, proposed by Hutchinson and co-workers, is that the expression level of the non-mutated allele may play a role in the determination of intrafamilial variability (23).

In this work, we have searched for possible genotype/phenotype correlations in MFS, focussed on one specific cardiovascular characteristic, aortic stiffness. We also studied the role of the expression level of both alleles on clinic al variability. (see results section, chapter 4).

I.3.3 Cardiovascular manifestations

Seventy to 100 % of the overall mortality in MFS patients is related to cardiovascular problems. Eighty to 100% of these are the result of aortic dissection (40).

Cardiovascular manifestations according to the Ghent nosology are listed in appendix1.

I.3.3.1 Dilatation of the ascending aorta

The primary cardiovascular manifestation in MFS is a progressive dilatation of the ascending aorta eventually leading to aortic dissection or rupture. It is estimated that aortic root dilatation is present in >80% of adult MFS patients(41).

Evaluation of ascending aortic dilatation requires careful echocardiographic measurement and obtained values should be compared to normal values in control subjects matched for age and body-surface area (42). This can be done using nomograms (figure 4) or using Z-scores derived from the regression equation on the nomogram

$$\text{Z-score} = \frac{X - M}{SD}$$

X=obtained value; M=mean calculated value; SD=Standard Deviation
The mean calculated value is calculated with the regression equation provided in the graph in fig 4.
The standard deviation is calculated according to the following formula:
$SD = SEE / (\sqrt{(1-r^2)} \times \sqrt{(n/n-2)})$.
With r= correlation coefficient; n= study population

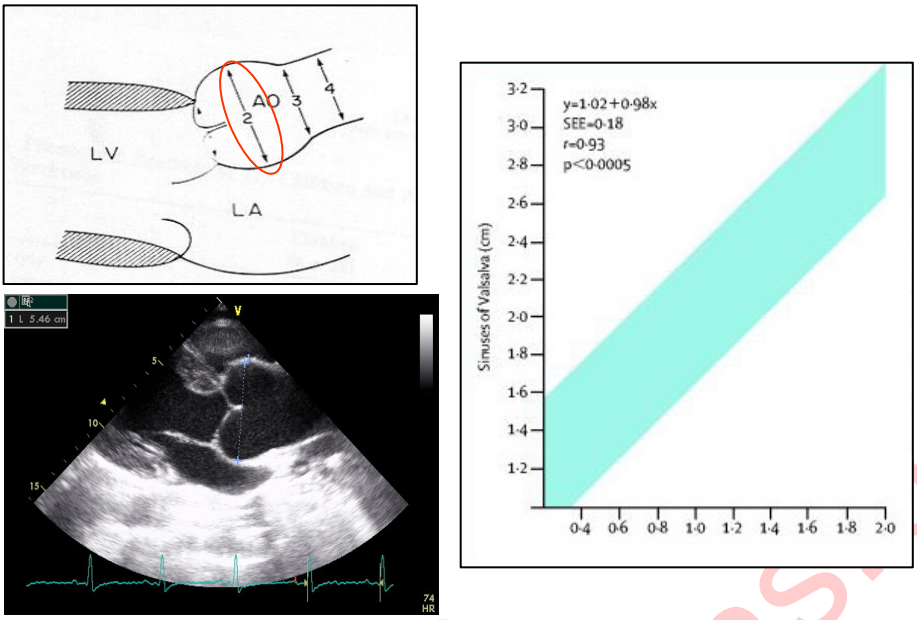


Figure 4: illustration and echocardiographic image of ascending aortic aneurysm at the level of the sinus of Valsalva in MFS. Measurement has to be done at end diastole using the "leading edge to leading edge" principle. LA: Left Atrium; LV: Left Ventricle. The graph shows a nomogram with the according regression equation for the age range <18yrs (from Roman et al, Am J Cardiol 1989).

Calculation of the body surface area is based on the formula by Dubois and Dubois, published in 1916 and based on measurements obtained in eight adults and one child (43). Values for infants and small children were obtained by extrapolation. Correction for body-surface area is certainly useful for the standardisation of cardiac output and intracardiac chambers, but is less useful for indexing linear cardiac chamber measurements, such as the aortic root, because the relation is nonlinear(44). Despite these inconveniencies, we preferred the use of this formula because both length and weight are considered and because most international publications on aortic root dimensions use the same formula, which facilitates comparison of the obtained measurements. One should however keep in mind that this formula might overestimate the importance of body weight – thus leading to false low values in obese subjects.

Rosendaal and co-workers performed an interesting study assessing the relationship between body-surface area and aortic root dimensions in a large group of normal "tall" children (Dutch population). They demonstrated a larger scatter of normal values in this tall population when compared to the normal population published by Roman and co-workers(42). These findings should also be considered in the standardisation procedure (45).

The predilection for the ascending aorta to dilate is a result of both structural and local hemodynamic factors. It has been demonstrated that elastic fibres are relatively more common in the ascending aorta than in any other region of the arterial tree (46). Diseases such as MFS affecting elastic fibre integrity will therefore manifest more easily in this region. Furthermore, it is primarily the ascending portion of the aorta which is subjected to the repetitive stress of left ventricular ejection, eventually resulting in progressive dilatation (47, 48).

In patients with aortic aneurysms not associated with MFS, the degree of aortic dilation has been well correlated with the risk of aortic rupture (49). The risk rises substantially when the diameter exceeds 55mm.

By contrast, in patients with MFS, risk of aortic rupture or dissection and degree of aortic dilation does not appear to depend solely on the degree of aortic dilation(50). Some patients develop aortic dissection at diameters below 55mm (40, 51). Silverman and co-workers demonstrated that a family history of severe cardiovascular disease in MFS is associated with increased aortic diameter and decreased survival (52).

Other independent risk factors that have been described in the literature are central pulse pressure (pressure difference between systolic and diastolic pressure in the ascending aorta) (53) and aortic stiffness(54-57).

Aortic stiffness has been investigated as an additional potential predictor of progressive aortic dilatation and dissection in patients with MFS. Several studies have demonstrated the presence of increased aortic stiffness, characterized by a decrease of local distensibility and an increase in flow wave velocity, assessed with magnetic resonance imaging or echocardiography in patients with MFS (54, 55, 57). More recently, it has been demonstrated that aortic stiffness is an independent predictor of progressive abdominal aortic dilatation(58). Furthermore, aortic stiffness appears to have a diagnostic value in young patients (59).

In daily clinical practice however, these parameters are not easy to apply since no accurate cut-off values are available. In this thesis, we have tried to assess the correlation between local and global parameters of aortic wave reflections and aortic dilatation in MFS patients (chapter 3).

Aortic stiffness is also associated with increased cardiovascular risk in a number of non-Marfan populations (60-63). A relationship between aortic stiffness and the *FBN1* genotype has been suggested, both in healthy middle aged men (64) as in patients with coronary artery disease(65). However, a larger scale trial could not reproduce these findings, questioning the value of the findings of the first studies (66, 67)

In contrast to the large amount of data on ascending aortic dilatation in MFS, data regarding the prevalence and guidelines for the diagnosis of minor cardiovascular manifestations are scarce.

I.3.3.2 Mitral valve prolapse

Mitral Valve Prolapse (MVP) is the only manifestation for which the investigational technique and definition are well delineated. 2D-echocardiography is the currently recommended tool for the identification of MVP(68) (69). Classic prolapse is defined as leaflet displacement in systole exceeding the mitral valve annular plane by ≥ 2mm with leaflet thickening exceeding 5 mm. Non-classic prolapse refers to leaflet displacement without valve thickening. An example is illustrated in figure 5.

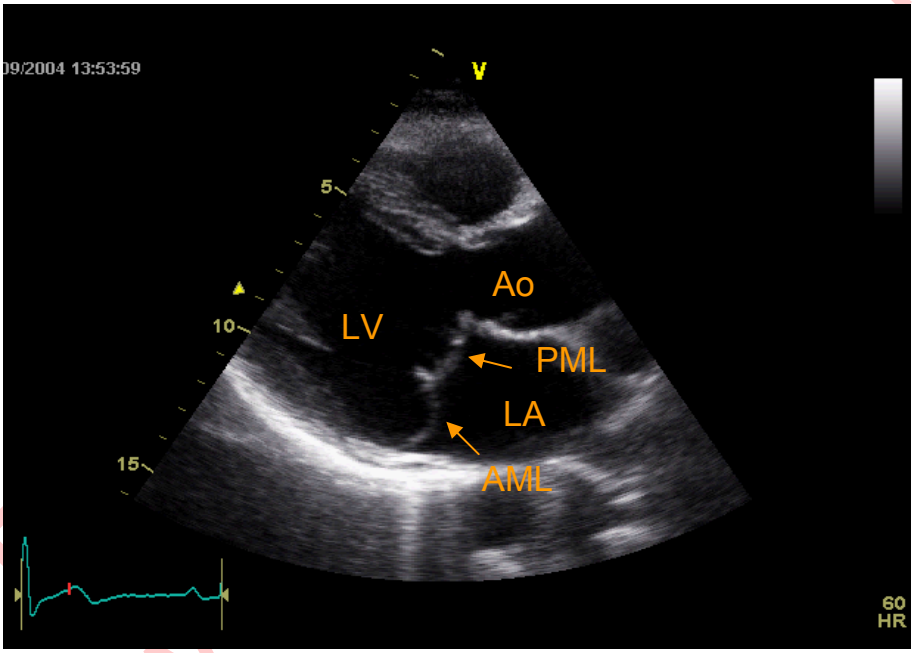


Figure 5: echocardiographic picture of mitral valve prolapse. AML: anterior mitral valve leaflet; PML: posterior Mitral Valve leaflet; LA: left atrium; LV: left ventricle; Ao: aorta

In a survey on 166 MFS patients, more than 50% were identified with auscultatory or echocardiographic evidence of MVP (70). These findings were confirmed in more recent studies (71, 72).

I.3.3.3 Main pulmonary artery dilatation

Guidelines for the assessment of main pulmonary artery (MPA) dilatation are scarce in the literature. Nollen and colleagues clearly demonstrated increased diameters assessed with Magnetic Resonance Imaging (MRI) (73). Using a cut-off value of 28 mm at the level of the MPA root, they report a prevalence of MPA dilatation of 74%. Since MRI is not recommended in a screening setting, we have attempted to set up guidelines for echocardiographic evaluation of MPA dilatation in MFS patients.

Complications arising from MPA dilatation are mild. Pulmonary regurgitation is reported in many MFS patients (74). Pulmonary artery dissection however is very rare. With increasing life expectancy in MFS patients, complications arising from MPA dilatation in a later stage cannot be excluded.

I.3.3.4 Dilatation or dissection of the descending aorta

Complications in the descending aorta occur in a minority of MFS patients (75, 76). Marfan syndrome patients presenting with thoraco-abdominal aortic aneurysm/dissection are reported in a few case reports (77, 78). Other reports on the descending aorta in MFS patients are mainly limited to surgical data describing the occurrence of primary or secondary complications in the descending aorta necessitating surgical intervention. Finkbohner and colleagues report that 15% of their patients had a first surgical intervention involving portions of the descending aorta (79). Nollen and colleagues report on increased growth (defined as >1mm/y) in a small subset of patients (6% in the descending thoracic aorta and 7% in the abdominal aorta) (58). Kawamoto and colleagues studied the progression of thoraco-abdominal aortic diameters in MFS patients after surgical repair and defined a subgroup of patients showing progressive dilatation of the distal aorta (>3mm yr) (80).

Guidelines for the assessment of descending aortic dilatation are lacking in the literature. In this work, we performed a MRI study comparing aortic diameters in MFS patients to controls in an attempt to provide cut-off values (chapter 1).

Interestingly, abnormal elastic properties of the aorta are not confined to the ascending aorta, but are also detected in the normal sized, more distal parts of the vessel (55) and this as well in patients having previously undergone aortic root surgery as in unoperated MFS patients (81). Local distensibility of the descending thoracic aorta appeared to be an independent predictor of progressive descending aortic dilatation (58).

I.3.3.5 Left ventricular dysfunction

Although not included in the diagnostic criteria for MFS, dilated cardiomyopathy, beyond that explained by aortic or mitral valve regurgitation, seems to occur with higher prevalence in patients with MFS, perhaps implicating a role of the extracellular matrix protein fibrillin1 in the cardiac ventricles (41).

Left ventricular (LV) dysfunction in MFS may occur as a consequence of valvular heart disease. However, there is recent evidence suggesting that LV function may be impaired irrespective of the presence of valvular heart disease, as indicated by increased LV dimensions in a subset of patients (82). In addition it has been demonstrated in a few small studies that left ventricular diastolic function is also impaired in MFS (83-85).

In this work, we present results of a detailed study of LV systolic and diastolic function in MFS patients using dedicated techniques (chapter 2).

I.3.4 Cardiovascular morbidity and mortality in Marfan syndrome

Life expectancy in patients with MFS is mainly determined by aortic complications (dissection or rupture), especially at the aortic root (40, 86-89). In a prospective study of 72 patients in 1972, the median life expectancy was about 45 years (87). A more recent re-evaluation of life expectancy in MFS suggested that early diagnosis and refined medical and surgical management have increased median life expectancy to about 70 years (90). Nevertheless, MFS continues to be associated with significant morbidity and selected subgroups are refractory to therapy and continue to show early mortality. In the classic form of MFS diagnosed after infancy, it is estimated that up to 90% of affected individuals will have a cardiovascular 'event' during their lifetime, including surgical repair of the aortic root, fatal or non-fatal aortic dissection or mitral valve surgery (47, 91, 92).

I.3.5 Management and treatment of cardiovascular manifestations in Marfan syndrome

I.3.5.1 Follow-up

Once the diagnosis of MFS is established, every patient should undergo regular echocardiographic follow-up which should be every year in case of an aortic root diameter <45mm, and every six months when diameters exceed 45mm or in cases of fast growing aneurysms (defined as an increase ≥0.5cm/yr). Imaging of the entire aorta, preferentially using MRI is recommended in every adult patient with MFS at the time of diagnosis and should be repeated yearly in patients with a documented aortic dilatation/ dissection and/or in patients who previously had aortic root surgery (93). Computed tomography is a possible alternative for MRI, although we would not recommend this in a serial setting in view of the important radiation dose.

I.3.5.2 Medical treatment

Medical treatment is mainly aimed at modifying the progression of aortic dilatation.

The standard of care for medical treatment in MFS consists of β-blockers. The rationale for this treatment strategy is primarily to decrease proximal aortic tensile wall stress, or dP/dT. β-Blockers are likely beneficial both through negative inotropic and negative chronotropic effects. The only randomized trial assessing the effect of β-blockade in patients with MFS was published in 1994 (94). It was demonstrated that the rate of progression of aortic growth was diminished in MFS patients treated with β-blockers. The use of β-blockers does not prevent attainment of other important clinical endpoints including aortic valve dysfunction, surgery, dissection and death in all patients.

Studies of aortic stiffness measured by echocardiography, MRI, and cardiac catheterization have shown a heterogeneous response to β-blockers in patients with MFS (95-97). Advanced aortic root size correlated with lack of response (97). It is therefore recommended that these drugs be initiated at the earliest age possible(98).

It is currently unclear whether the subgroup of patients not responding to β-blockers has a worse outcome and whether they should be given an alternative treatment.

Possible alternatives to β-blockers are calcium-channel blockers(99) and Angiotensin Converting Enzyme-inhibitors (100). Since the trials evaluating the effect of these drugs are non-randomized and uncontrolled, it is recommended to reserve them for cases with proved intolerance to β-blockers.

I.3.5.3 Surgical treatment

Surgical treatment for MFS has definitely contributed to the improved survival over the past decades (89).

Elective surgery to repair the aortic root in patients with MFS is recommended when the maximal aortic diameter in adults or older children reaches 5.0 cm (91, 101). Additional considerations include the rate of aortic growth and family history of aortic dissection at a size less than 5.0 cm. Earlier surgical intervention is recommended for individuals with an increase in aortic diameter exceeding 1 cm per year.

The initial surgical procedure implemented by Bentall and de Bono consisted of replacement of the aortic root by a Dacron tube and replacement of the aortic valve with a mechanical prosthesis (the so- called composite graft procedure) in 1968 (102).

In a recent report by Gott and co-workers on the results of aortic root surgery using the composite graft procedure in 675 patients with MFS from multiple institutions, the operative mortality was 1.5% for elective operations and 11.7% for emergency operations (91). The actuarial survival rates were 84% at 5 years, and 75% at 10 years.

Because of the risks of thromboembolism and the lifetime requirement for oral anticoagulation associated with mechanical prosthetic aortic valves, recent surgical techniques have been developed (pioneered by David and colleagues) with the purpose to maintain the native aortic valve (so called valve-sparing procedures) (103, 104). To date, no randomized clinical trials of valve replacement versus valve-sparing aortic root surgery have been performed, and long-term data on the outcomes with valve-sparing surgery are not yet available. Nevertheless, the short-term data are encouraging, with an extremely low rate of operative mortality (equivalent to that seen with composite graft repair) (105-107). The valve-sparing approach is particularly attractive for young women with MFS who anticipate pregnancy since it precludes the need for oral anticoagulation, which are known teratogens. Given the current preference for the valve-sparing procedure, the occurrence of significant aortic regurgitation is now widely accepted as an additional criterion to proceed with prophylactic surgery. Valve function can often be improved surgically if this operation is performed before development of severe regurgitation.

I.3.6 Differential diagnosis

In the differential diagnosis of MFS, other genetic syndromes associated with aortic aneurysm should be considered. For some of these disorders, the correct diagnosis may be easily established on clinical grounds, whereas in others, overlapping features with MFS may hamper confirmation of the diagnosis. Diagnosing a patient with MFS requires careful physical examination and assessment of other organ system involvement such as lens dislocation or dural ectasia. Additional molecular studies may be helpful in specific instances, as will be illustrated in this thesis (chapter 4).

I.3.6.1 Loeys-Dietz syndrome

Loeys-Dietz syndrome is a recently identified autosomal dominant aortic aneurysm syndrome (108) . In 2002, several patients were identified presenting with early onset aortic aneurysms with an aggressive course. Clinical examination revealed the presence of hypertelorism, cleft palate and/or bifid uvula and arterial tortuosity. Although some patients presented skeletal features of MFS such as arachnodactyly, pectus deformities and scoliosis, none of them fulfilled the clinical diagnostic criteria for MFS. None of these patients presented lens dislocation. The disease has an autosomal dominant transmission. The underlying genetic defects in Loeys-Dietz syndrome are mutations in the Transforming Growth Factor β Receptor (TGFBR) genes 1 and 2. These genes were considered as likely candidates since mutations in these genes cause similar craniofacial abnormalities in mice. Furthermore, a TGFBR2 mutation had been identified in a large French family presenting clinical features of MFS (109).

Loeys-Dietz syndrome certainly includes many features of MFS (arachnodactyly, pectus deformity, scoliosis, dural ectasia, ascending aortic aneurysm with dissection), but also many findings not seen in MFS patients (e.g. hypertelorism, cleft palate/bifid uvula, craniosynostosis, generalized arterial tortuosity, aneurysms and dissection throughout the arterial tree). Although there definitely is phenotypic overlap between Loeys-Dietz syndrome and MFS, the distinction between the two disorders is important in view of marked differences in natural history and prognosis. For example, the arterial involvement in Loeys-Dietz syndrome is more widespread and has a worse outcome than in MFS (110). Follow-up and treatment are accordingly different for both syndromes.

Interestingly, TGFBR2 mutations have also been identified in patients with isolated thoracic AAD (111), suggesting that there is a phenotypic spectrum with thoracic AAD at the mild end and Loeys-Dietz syndrome at the other end.

I.3.6.2 Ehlers-Danlos syndrome - vascular type

The vascular type of Ehlers-Danlos syndrome (EDS) is caused by mutations in the *COL3A1* gene encoding type III collagen, an important constituent of blood vessel walls and hollow organs. Vascular EDS is an autosomal dominant disorder clinically characterized by widespread tissue fragility with joint hyperlaxity, easy bruising, atrophic scars, intestinal and/or uterine rupture and vascular fragility. Although the aorta can be involved, typically smaller arteries rupture or dissect in patients with this syndrome. Dissections often occur in the absence of previous dilatation. The vascular complications of vascular EDS are difficult to manage and cause premature death, with the median age of death being 48 years (112).

I.3.6.3 Ehlers-Danlos syndrome – kyphoscoliotic type

The kyphoscoliotic type of EDS is caused by deficient activity of the enzyme lysyl hydroxylase. It is an autosomal recessive disorder caused by mutations of the PLOD1 gene (Procollagen-Lysine, 2-Oxoglutarate 5-Dioxygenase). Clinical characteristics include general joint laxity, progressive and severe kyphoscoliosis and scleral fragility(113). Arterial rupture occurs occasionally and aortic dissection has been reported in one patient (114).

I.3.6.4 Arterial tortuosity syndrome

Arterial Tortuosity Syndrome is a recently identified autosomal recessive disorder characterized by widespread arterial tortuosity, along with large artery stenosis. Aneurysm formation of the aorta and large arteries may also be present. Arterial Tortuosity Syndrome is caused by mutations in the GLUT10 gene, encoding for a facilitative glucose transporter (115).

I.3.6.5 Familial Aortic Aneurysms and Dissections

Familial AAD comprise a genetically heterogeneous group of conditions. A few mutations in different genes have been identified, but the frequency of known mutations in this group is low, suggesting that many other unidentified genes exist.

Some patients with familial AAD present minor manifestations of MFS such as mild skeletal symptoms or myopia. In such cases, the differential diagnosis with MFS may be very difficult and additional molecular studies may be helpful to establish a correct diagnosis.

FBN1 mutations have been detected in one family and two sporadic patients with ascending aortic disease which did otherwise not meet the clinical diagnostic criteria for MFS (116, 117).

As already mentioned, mutations in the *TGFBR2* gene have also been identified in patients with isolated thoracic AAD (111)

Mutations in the Myosin Heavy Chain Gene 11 (*MYH11)* gene have recently been identified in a large family with autosomal dominant transmission of thoracic AAD associated with patent ductus arteriosus (118).

In addition, two other loci have been identified in families with autosomal dominant transmission of AAD: the *TAAD1* locus on chromosome 5 (5q13-14) (9) and the *FAA1* locus on chromosome 11 (11q23-24) (119).

I.3.6.6 Thoracic aortic aneurysms and dissections associated with bicuspid aortic valve

In patients with bicuspid aortic valves there is a nine fold increase in the risk of developing acute dissections when compared to patients with normal aortic valves. Recent studies suggest that there are associated congenital anomalies of the aortic wall in these patients. Aortic aneurysms and dissections occur irrespective of altered hemodynamics or age (120).

The minimal frequency of familial occurrence of bicuspid aortic valve ranges between 9.1 and 17.1% (121, 122). Analysis of these families indicated that the condition is inherited in an autosomal dominant manner with reduced penetrance (121).

II. Outline and aims

This thesis aims to provide a detailed study of cardiovascular characteristics of Marfan syndrome and to study their relationship to the genotype.

Although dilatation of the ascending aorta is the main cardiovascular complication in MFS, the diagnostic value of other cardiovascular features in the evaluation and follow-up for MFS should not be underestimated.

Defining cardiovascular involvement in the absence of proximal aortic dilatation may be crucial for a correct diagnosis of MFS. To this end, adequate cardiovascular diagnostic criteria are needed. Although these criteria exist for the diagnosis of MVP, they are largely lacking for the other minor cardiovascular manifestations, such as dilatation of the MPA, dilatation and dissection of the descending aorta and calcification of the mitral valve annulus. In **chapter 1** of this thesis, we provide a critical evaluation of the current "minor" cardiovascular diagnostic criteria for MFS and formulate practical guidelines for correct evaluation and diagnosis of these "minor" manifestations.

As a result of increased life expectancy in MFS it is expected that other cardiovascular problems such as LV dysfunction may arise in MFS patients. A few reports have mentioned the presence of diastolic and systolic cardiac dysfunction, however detailed studies are lacking. Therefore, we performed an in depth study combining classic echocardiography, Tissue Doppler Imaging (TDI) and MRI, for the evaluation of left ventricular systolic and diastolic function in MFS patients that are free of significant valvular heart disease. The results of this study are presented in **chapter 2**.

Aortic stiffness is increased in MFS patients, but the pathophysiological sequence underlying this process is largely unknown at present. It is unclear whether altered (proximal) aortic wall properties in MFS give rise to local wave reflections

and how these contribute to the global picture of wave reflection and the mechanical load imposed on the aortic wall. In **chapter 3**, we compared indices of local and global wave reflection in the aorta between MFS patients and control subjects using ultrasound and MRI.

An intriguing aspect of MFS is the high degree of clinical variability both within and between families. It has been postulated that the *FBN1* genotype plays a role in the determination of this variability. In **chapter 4**, we investigated this correlation. First, we studied the correlation between the *FBN1* genotype and parameters of aortic stiffness in a large group of MFS patients. Second we present detailed clinical descriptions in three large families harbouring a *FBN1* mutation and demonstrate extensive clinical variability among family members. We also illustrate the importance of repeated and thorough clinical follow-up, detailed family history taking and the value of additional molecular testing.

III. Materials and Methods

III.1 Patients and control subjects

To study the minor cardiovascular criteria, a total of 77 subjects, recruited both at the Ghent University Hospital (53 subjects) and at the Johns Hopkins University School of Medicine in Baltimore (24 subjects) with a mean age of 25.0 yrs (range 4months – 55years) and fulfilling the Gent clinical diagnostic criteria for MFS were studied with echocardiography. Patients were compared with 77 age- and sex-matched control subjects, recruited among colleagues and family members of the researchers. A subgroup of 29 adult MFS and 31 control subjects also underwent MRI of the aorta. From this subgroup, 26 patients (mean age 32±10.9, 12 men) without significant valvular heart disease and 26 age- and sex matched controls were selected for the detailed evaluation of the LV function, using a combination of MRI and echo-doppler parameters and for the evaluation of elastic parameters of the aorta, also using a combination of ultrasound and MRI.

For the assessment of LV dimensions and function and for the study of the correlation between aortic stiffness and the *FBN1* genotype, we collaborated with the group of Prof. B. Mulder at the Amsterdam Medical Centre (Amsterdam, NL).

For the study of LV dimensions, the 26 patients from our study were included in a multi-centre study, pooling data from 4 university hospitals. In total 234 MFS patients were studied with conventional echocardiography.

For the assessment of aortic stiffness parameters, a cohort of 67 MFS patients (31 men, mean age 32 ±10 years) fulfilling the Ghent criteria representing 51 families with an identified *FBN1* mutation underwent cardiac MRI in the Academic Medical Centre (Amsterdam, the Netherlands) (41 patients) and the Ghent University Hospital (Belgium) (26 patients).

III.2 Imaging techniques

III.2.1 Echocardiography

III.2.1.1 Conventional echocardiography

All echocardiographic measurements were performed on a Vivid 7 ultrasound machine (GE Vingmed Ultrasound, Horten, Norway). Conventional echodoppler techniques were used for the evaluation of dimensions of the heart chambers and vessels (aorta and MPA) and for the evaluation of valvular function. Aortic diameters were measured in the parasternal long axis view at the level of the annulus, the sinuses of Valsalva, the sinotubular junction and the ascending aorta. Measurements were performed at end diastole with the leading edge to leading edge principle. Diameters were corrected for the body surface area (BSA) and compared with age dependent nomograms as proposed by Roman et al (41).

Z-scores for the proximal aorta were calculated according to the formula:

$$\text{Z-score} = \frac{X - M}{SD}$$

X=obtained value; M=mean calculated value; SD=Standard Deviation
The mean calculated value is calculated with the regression equation provided in the graph in fig 4.
The standard deviation is calculated according to the following formula:
$SD = SEE / (\sqrt{(1-r^2)} \times \sqrt{(n/n-2)})$.
With r= correlation coefficient; n= study population

BSA and SD= Standard deviation. M and SD were obtained from the regression equations provided by Roman et al (41). Z-scores >2 indicates that the obtained value exceeds the mean value with more than two standard deviations and is considered as a significant increase.

Mitral valve prolapse was evaluated from the parasternal long axis view and from the apical four chamber view. Classic MVP was defined as leaflet displacement exceeding 2mm and leaflet thickness of at least 5mm; nonclassic MVP was defined as leaflet displacement exceeding 2mm and leaflet thickness less than 5mm, according to Freed et al (123).

The MPA diameter was measured a few millimetres distally from the valve insertion at the broadest portion of the pulmonary artery, visualized from the parasternal short axis window.

The LV dimensions were assessed in the parasternal long-axis view. Left ventricular end-diastolic and end-systolic diameters as well as septal and posterior end-diastolic wall thickness were measured in 2D mode. For the multi-centre trial in chapter 2, the predicted normal values for LV end diastolic diameter and LV end systolic diameter were calculated for each patient according to their age and body

surface area using Henry's regression equations (122). The LV dimension was expressed as a percentage of the predicted value according to the formula (observed dimension/predicted normal value X 100%). Fractional shortening was calculated using the formula: Fractional shortening = (LV end diastolic diameter- LV end systolic diameter)/LV end diastolic diameter X100%. Criteria for dilated cardiomyopathy were defined as a combination of (1) relative LV end diastolic diameter >117% (mean+2SD+5%); and (2) fractional shortening <25%. (123).

For the assessment of transmitral Doppler signals, the sample volume (size 2 mm) of the pulsed wave Doppler was placed between the tips of the mitral leaflets in the apical four chamber view. Early (E) and late (A) transmitral flow velocities, the ratio of early to late peak velocities (E/A) and deceleration time of early velocity (DT_E) were obtained. Isovolumic Relaxation Time was measured using continuous wave Doppler with the transducer beam angulated towards the left ventricular outflow tract, so that aortic and mitral flow was simultaneously recorded (124).

III.2.1.2 Tissue Doppler Imaging

For the detailed evaluation of left ventricular function, we applied a newly developed technique, namely tissue Doppler imaging (TDI).

Tissue Doppler Imaging is applicable for the quantification of myocardial velocities (125, 126). It can be used to assess global and regional systolic LV function and to identify abnormal LV relaxation in a variety of conditions.

Tissue Doppler Imaging has been applied in the early identification of cardiac involvement in asymptomatic mutation carriers in several genetic conditions such as hypertrophic cardiomyopathy, Becker Muscular Dystrophy and Fabry disease(127-130). We have summarized the results from different studies in a review paper (131). These findings indicate that TDI is a more sensitive technique for the detection of subtle (subclinical) impairment of LV function. TDI has not been previously applied in MFS patients.

In the preparation of the protocol for echocardiographic measurements for this thesis, the following papers were published:

1. De Sutter J, De Backer J, Velghe A, Van de Veire N, De Buyzere M, Gillebert T Determinants of septal mitral annulus velocity (E') and the ratio of transmitral early peak velocity to E' (E/E'): effects of age, gender and left ventricular mass. American Journal of Cardiology . 2005 Apr 18; 513 (1-2): 35-45
2. J. De Backer, D. Matthys, T.C. Gillebert, A. De Paepe , J. De Sutter. The use of Tissue Doppler Imaging for the assessment of changes in myocardial structure and function in inherited cardiomyopathies. European Journal of Echocardiography. 2005 Aug;6(4):243-50

III.2.2 Vascular studies

For the vascular studies a combination of different techniques was used.

Applanation tonometry (SPT 301, Millar Instruments, Houston, Texas) data were obtained at the level of the brachial artery using a dedicated data-acquisition platform. Waveforms were scaled to brachial diastolic and systolic blood pressure was used for the measurement of the brachial artery pressure waveform. Mean arterial pressure was obtained as the numeric integral of the scaled brachial tonometric curve, and brachial pulse pressure as the difference between systolic and diastolic brachial blood pressure. Diastolic blood pressure and mean arterial pressure obtained in this way were subsequently used to convert carotid diameter distension waveforms into carotid (central) pressure waveforms.

The carotid artery diameter distension waveform was measured with a 12 MHz vascular probe (12L) using a previously validated vessel wall tracking system (132). The central pressure waveform was derived from the carotid diameter distension waveform end from brachial diastolic and mean arterial pressure. The carotid distension waveform was converted into a carotid pressure waveform assuming that (i) the relation between pressure and diameter is linear and, (ii) diastolic and mean arterial pressure are similar at the brachial and carotid artery(133, 134). This carotid pressure waveform is further considered as a surrogate of the central pressure waveform. The maximum of the pressure waveform yielded carotid systolic blood pressure and carotid pulse pressure was calculated as systolic – diastolic pressure.

The validation of the technique used for measurement of carotid pressure waves was published in the following manuscript:

Patrick Segers, Stein Inge Rabben, Julie De Backer, Johan De Sutter, Thierry C Gillebert, Luc Van Bortel and Pascal Verdonck "Functional Analysis of the Common Carotid Artery : Relative Distension Differences over the Vessel Wall Measured In Vivo". J Hypertens 2004 May;22(5):973-81.

Local reflection, arising from impedance mismatch between levels xx and yy, was quantified using local wave reflection coefficients (Γxx-yy) calculated as

$$\Gamma_{xx-yy} = \frac{Z_{0-yy} - Z_{0-xx}}{Z_{0-yy} + Z_{0-xx}}$$

where Z_0-xx is the characteristic impedance at level xx, approximated as

$$Z_{0-xx} = \sqrt{\frac{\rho}{A_{xx}}\left(\frac{SBP - DBP}{A_{xxs} - A_{xxd}}\right)}$$

with ρ the density of blood (assumed 1030 kg/m3) and Axx the average value of Axxs and Axxd (s systolic and d diastolic respectively). SBP and DBP are central systolic and diastolic blood pressure, respectively.

The augmentation index (AIx) is calculated as

$$AIx = 100\frac{P_2 - DBP}{P_1 - DBP}$$

where P1 and P2 are either SBP, either the pressure associated with an inflection point visually identified on Pao. The pressure occurring first is labelled as P1. AIx<100% indicates arrival of the pressure wave in late systole; AIx > 100% is indicative for arrival in early systole. We also measured the time delay between the foot of Pao, and the moment of occurrence of the inflection point, ΔTf-b. This time interval is associated with the time needed for a wave to travel forth and back from the ascending aorta to its apparent reflection site (the effective length of the arterial system, Δx), calculated as

$$\Delta x = \frac{PWV.\Delta T_{f\text{-}b}}{2}$$

with PWV the aortic pulse wave velocity, assessed with magnetic resonance imaging. We refer to Figure 2 for illustration of the inflection point and timing intervals.

III.2.3 Magnetic Resonance Imaging

Subjects were scanned on a 1.5T MR system (Magnetom Symphony, Siemens, Erlangen, Germany) with ECG gating. Left ventricular volumes were acquired with an ECG triggered trueFISP (Fast Imaging with Steady-state Precession) sequence. Stroke volume was calculated as the difference between end-diastolic and end-systolic volume.

The aorta was with an angiographic examination, followed by through-plane phase-contrast images at the level of the ascending, descending thoracic, thoracic-abdominal (near the diaphragm) and low abdominal aorta. The distance between the different measuring sites was determined. The flow curves at these 4 levels were calculated with the Siemens Mean Curve software and interpolated to a temporal resolution of 1 ms. At each level, the time delay between the ECG R-top and the moment when flow reaches half of its peak value was calculated. With time and distance travelled by the propagating flow front known at 4 locations, pulse wave velocity (cm/sec) was calculated as the slope of the regression line through these data points (Mathlab, The Mathworks, Natick, Massachusetts)(51).

Aortic distensibility at the 4 levels was calculated according to the following formula:

$D = (Amax-Amin)/(Amin \times pulse\ pressure)$.

D = distensibility (millimeters of mercury^{-1}, mmHg^{-1}), Amax = maximal (systolic) aortic area (square millimeters, mm²), Amin = minimal (diastolic) aortic area (mm²), pulse pressure = systolic blood pressure - diastolic blood pressure (millimeters of mercury, mmHg).

III.3 Genetic studies

The techniques used for the molecular analysis of the *FBN1* gene have evolved during the course of this work from SSCP and CSGE to DHPLC and direct sequencing of the gene (27).

SSCP (Single Stranded Conformation Polymorphism) is based on detecting conformational differences between mutant and control wild-type single strand DNA sequences after electrophoresis on a non-denaturing polyacrylamide gel. This method is suitable for the analysis of fragments ranging from 150 – 600 basepairs (135).

CSGE (Conformation Sensitive Gel Electrophoresis) is a variant of heteroduplex analysis. The method is applicable for the analysis of DNA fragments up to 450 bp (136).

dHPLC (denaturing High Performance Liquid Chromatography) compares a mixture of denatured and re-annealed Polymerase Chain Reaction (PCR) amplicons, revealing the presence of a mutation by the differential retention of homo- and heteroduplexes. Fragments as large as 1.5 Kb can be analysed with this method.

Fragments showing an abnormal migration pattern with one of these three mutation-scanning methods were sequenced to determine the specific base change.

Recently, the development of high-throughput and automatized sequencing techniques allows us to perform direct sequencing of the entire gene. This technique is indicated in those cases with a high clinical suspicion for MFS in whom previous screening techniques were negative.

The causality of the different mutations was proven, based on conservation, functional importance, segregation of the mutation with the disease in one family and, if necessary on the absence of the mutation in 100 normal controls.

The development and evolution of *FBN1* mutation analysis has been described in detail in two papers from our group:

Loeys B, Nuytinck L, Delvaux I, De Bie S, De Paepe A. Genotype and phenotype analysis of 171 patients referred for molecular study of the fibrillin-1 gene FBN1 because of suspected Marfan syndrome. Arch Intern Med. 2001 Nov 12;161(20):2447-54.

Loeys B, De Backer J, Van Acker P, Wettinck K, Pals G, Nuytinck L, Coucke P, De Paepe A. Comprehensive molecular screening of the FBN1 gene favors locus homogeneity of classical Marfan syndrome. Hum Mutat. 2004 Aug;24(2):140-6.

The Variable Number Tandem Repeat (VNTR) polymorphism (TAAAA in intron 28 of *FBN1*) was amplified by means of PCR. The following primers were used: forward: 5' FAM ATC TCA GAG TAC ATA GAG TGT TTT AG 3' and reverse 5'AGT TGT TTG AAT GAC ATC ATT G 3'. Genotypes for VNTR were determined with the use of an ABI 3100 Genetic Analyzer (Applied Biosystems).

IV. Results

IV. 1 Chapter 1: Analysis of minor cardiovascular criteria in Marfan syndrome

A critical analysis of minor cardiovascular criteria in the diagnostic evaluation of patients with Marfan syndrome

Julie De Backer, Bart Loeys, Dan Devos, Hal Dietz, Johan De Sutter, Anne De Paepe
Genetics in Medicine. 2006 Jul;8(7):401-8

In this paper, the prevalence of each of the four current minor cardiovascular manifestation as defined in the Ghent nosology was assessed in 77 MFS patients (24 children<14years and 53 adults) and 77 age- and sex matched control subjects. All patients and control subjects underwent echocardiography. A subset of 29 patients and 29 control subjects also underwent MRI scanning of the entire aorta.

We found that the prevalence of MVP in MFS patients was 66.2%, with nearly equal proportions of classic and non-classic MVP.

Because criteria for the diagnosis of MPA dilatation are lacking, we compared the obtained values in MFS patients to those in normal control subjects. This enabled us to define a cut-off value of 23mm for MPA dilatation in subjects of ≥14 years of age. With this cut-off value, 85% of the adult MFS patients had MPA dilatation. Since age and body surface area (BSA) are important determinants of MPA diameter in children, we preferred the use of Z-scores in the age group <14 years, based on age and BSA. We used normal values provided by Snider et al (124). Fifty percent of MFS children had MPA dilatation, defined as a Z-score >2.

Calcification of the mitral valve annulus was not encountered in the MFS patients.

Diameters of the descending thoracic and abdominal aorta were larger in MFS patients than in control subjects. However, substantial overlap was observed between both populations, so that is was difficult to delineate a cut off-value to define dilatation of the descending thoracic and abdominal aorta.

The observed findings were used to formulate practical guidelines for the cardiovascular evaluation of patients referred for MFS.

A critical analysis of minor cardiovascular criteria in the diagnostic evaluation of patients with Marfan syndrome

Julie De Backer, MD[1,2], Bart Loeys, MD, PhD[1], Dan Devos, MD[3], Harry Dietz, MD, PhD[4], Johan De Sutter, MD, PhD[2], and Anne De Paepe, MD, PhD[1]

Purpose: The prevalence of most minor cardiovascular manifestations in Marfan syndrome (MFS) is unknown. We assessed the prevalence of minor cardiovascular manifestations in MFS to evaluate their usefulness in a diagnostic setting. **Methods:** Seventy-seven patients with MFS (aged 4 months to 55 years) underwent echocardiography to assess the presence of mitral valve prolapse and the diameter of the main pulmonary artery. A subset of 29 adult patients with MFS also underwent magnetic resonance imaging evaluation of the diameters of the thoracoabdominal aorta. **Results:** Mitral valve prolapse was encountered in 66% of patients with MFS, with an equal distribution of classic and nonclassic mitral valve prolapse. The main pulmonary artery diameter was significantly larger in patients with MFS at all ages when compared with controls. In the adult group (≥14 years), we were able to provide a cutoff value of 23 mm to define pulmonary artery dilatation. The descending aorta was enlarged, but with substantial overlap with controls, thus precluding the use of a cutoff value. **Conclusions:** Mitral valve prolapse and main pulmonary artery dilatation are common findings in MFS patients at all ages and are easy to assess with echocardiography. Cutoff values to define dilatation of the descending aorta are hard to define, making them of limited value in the diagnostic evaluation. We recommend echocardiographic evaluation of mitral valve proplase and main pulmonary artery diameter in patients referred for cardiovascular diagnostic assessment for MFS. ***Genet Med* 2006:8(7):401–408.**

Key Words: *Marfan syndrome, diagnostic criteria, cardiovascular*

Marfan syndrome (MFS) is an autosomal dominant connective tissue disorder characterized by a combination of clinical manifestations in different organ systems, including the skeletal, cardiovascular, ocular, and central nervous system. The diagnosis is mainly based on clinical characteristics and requires the identification of major and minor criteria, as summarized in the Gent nosology.[1] Major criteria are specific for MFS; minor criteria are much less specific and occur frequently in the general population. The diagnosis in an index patient is confirmed by the presence of major criteria in at least two different organ systems in association with involvement of a third organ system. In patients with a first-degree relative who independently meets these diagnostic criteria, the presence of a major criterion in one organ system together with the involvement of a second organ system confirms the diagnosis of MFS.

From the [1]Centre for Medical Genetics, [2]Department of Cardiology, and [3]Department of Medical Imaging, University Hospital Ghent, Ghent, Belgium; and [4]McKusick-Nathans Institute for Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland.

Julie De Backer, MD, Centre for Medical Genetics University Hospital Ghent, Belgium De Pintelaan 185 9000 Ghent, Belgium.

Submitted for publication January 18, 2006.

Accepted for publication March 31, 2006.

DOI: 10.1097/01.gim.0000223550.41849.e3

The importance of a correct interpretation of minor manifestations is twofold.

First, adequate interpretation of minor manifestations may be important in excluding the diagnosis of MFS. In cases with a marfanoid habitus with myopia, for example, the absence of any cardiovascular manifestation may be reassuring.

Second, it may be essential for the confirmation of the diagnosis in patients in whom involvement of a third organ system is required, for example, in children with lens luxation and major skeletal manifestations, but without striae.

With regard to the cardiovascular system, mitral valve prolapse (MVP) is the only manifestation for which the investigational method and definition are well delineated. Whereas MVP was initially diagnosed with M-mode techniques, the current guidelines recommend two-dimensional echocardiography.[2,3] MVP is further subdivided into classic and nonclassic MVP, according to whether valve thickening is present or not. Nonclassic MVP is often referred to as "mitral valve bulging" in cardiovascular literature. To the best of our knowledge, the prevalence of these two different forms of MVP has not been assessed in patients with MFS.

The true prevalence of the other minor cardiovascular criteria in MFS is largely unknown. Normal values for main pulmonary artery (MPA) diameters are only available for children,[4] and the prevalence of MPA dilatation has only been assessed with magnetic resonance imaging (MRI) in patients with MFS.[5] A few

reports on normal values for the diameters of the descending thoracic or abdominal aorta are available,[6,7] but these have not yet been evaluated systematically in patients with MFS.

The objectives of this study are as follows:

1. Evaluate the prevalence of minor cardiovascular manifestations in a large group of patients with MFS: MVP, dilatation of the MPA in a patient aged less than 40 years, dilatation or dissection of the descending thoracic or abdominal aorta in a patient aged less than 50 years, and calcification of the mitral annulus in a patient aged less than 40 years.
2. Propose recommendations for the assessment of minor cardiovascular criteria in MFS, based on the findings in this study.

MATERIALS AND METHODS

A total of 77 subjects with a mean age of 25.0 years (range 4 months to 55 years) who fulfilled the Gent criteria for MFS were studied. We defined two groups: children (aged <14 years; N = 24) and adults (aged ≥14 years; N = 53). Five patients (four men, one woman; all aged ≥14 years) had previously undergone aortic root surgery; one patient (28-year-old female) had previously undergone mitral valve replacement for severe mitral valve regurgitation based on prolapse.

Patients were compared with 77 age- and sex-matched control subjects, recruited among colleagues and family members of the researchers. None of the control subjects had known cardiovascular disease.

A subgroup of 29 adult patients with MFS and 31 control subjects also underwent MRI imaging of the aorta.

Echocardiography

Transthoracic echocardiography was performed on a VIVID 7 Vingmed-General Electric scanner (GE Vingmed Ultrasound, Horten, Norway). MVP was evaluated from the parasternal long-axis view and the apical four-chamber view. Classic MVP was defined as leaflet displacement exceeding 2 mm and leaflet thickness of at least 5 mm; nonclassic MVP was defined as leaflet displacement exceeding 2 mm and leaflet thickness less than 5 mm, according to Freed et al.[2]

The MPA diameter was measured a few millimeters distally from the valve insertion at the broadest portion of the pulmonary artery, visualized from the parasternal short-axis window (Fig. 1). Images were stored in digital format on a remote hard disk. Data were reviewed offline by a sonographer blinded to the diagnosis of the subject with a commercially available image-processing program (Echopac 6.3, GE Vingmed Ultrasound).

To test interobserver variability of the measurement, the images of a randomly selected subset of 10 patients with MFS and 10 controls were evaluated by an independent sonographer. For the evaluation of intraobserver variability, the acquired images of 31 patients with MFS were analyzed twice by the same sonographer. Intra- and interobserver variability were determined by the coefficients of variance by comparing the standard deviation (SD) of the test differences as a percentage of the average in both series.

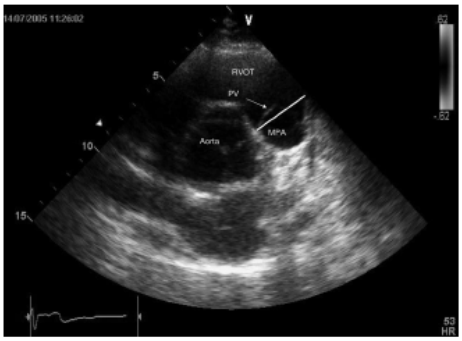


Fig. 1. Echocardiographic image of the MPA measurement. RVOT, right ventricular outflow tract; PV, pulmonary valve; MPA, main pulmonary artery.

Magnetic resonance imaging

MRI was performed on a 1.5T MR system (Magnetom Symphony, Siemens, Erlangen, Germany). Aortic diameter was assessed at the level of the ascending aorta (at the level of the pulmonary artery), descending thoracic aorta (at the level of the pulmonary artery), diaphragm, and abdomen (distally from the major abdominal branch vessels, just proximal to the bifurcation). A high temporal resolution (25 msec) balanced fast gradient (TrueFISP) cine sequence was used, positioned in an optimal transverse plane of the aorta. On these images the largest (systolic) diameter was measured. Aortic aneurysms and dissections were evaluated with magnetic resonance angiography.

Body surface area (BSA), was calculated according to the DuBois and DuBois formula:[8]

$$BSA = 0.20247 \times (H)^{0.725} \times (W)^{0.425}$$

with W = weight in kilograms; H = height in meters.

Statistical analysis

Baseline characteristics were compared with nonpaired *t* test for continuous variables and with chi-square tests for categoric data. Multivariate analysis was performed to assess the influence of covariates.

For patients and controls, mean diameters are given with their respective SD. The distribution of the diameters was normal. Student *t* test was used to compare the two groups. A *P* value of .05 or less was used to define statistical significance. SPSS version 11.0.1 (SPSS Inc., Chicago, IL) was used for the statistical analysis.

This study was approved by the local ethics committees (Ghent University Hospital; Johns Hopkins Institute).

RESULTS

Baseline characteristics

Baseline characteristics in adults are presented in Table 1.

Age, sex, BSA, and BMI were comparable in both groups. As expected, patients with MFS were taller than controls ($P < .0001$), and the diameter of the proximal aorta measured at the level of the sinus Valsalva was larger ($P < .0001$). The subgroup of patients with MFS who also underwent MRI was not significantly different from the other patients with MFS with respect to age, sex, BSA, and BMI.

Children were aged 4 months to 14 years; 50% were boys. The mean z score of the aortic sinus was 4.56 (SD 3.1).

Mitral valve prolapse and calcification of the mitral valve annulus

In total, 66% of patients with MFS had some form of MVP. Classic MVP was present in 35.1% of patients with MFS; nonclassic MVP was present in 31.2%. Mitral valve regurgitation was absent in all but two patients: One had moderate mitral valve regurgitation, and one (28-year-old female) had previously undergone mitral valve replacement (mechanical valve) for severe regurgitation because of underlying MVP. One control subject had nonclassic MVP without mitral valve regurgitation. Within the group of patients with MFS, MVP was not related to sex, length, or weight. Patients with nonclassic MVP were older than those without or with classic MVP (33.2 ± 16.6 years vs. 25.0 ± 11.5 years and 17.6 ± 13.7 years, respectively, $P = .001$).

MVP was more common in children with MFS than in adults (79% vs. 60%; $P = .003$).

In this patient population, nobody demonstrated significant calcification of the mitral valve annulus on echocardiography.

Dilatation of the main pulmonary artery

Measurement of the MPA diameter was possible in all but three adult patients with MFS and in all but two control patients.

The MPA diameter was significantly larger in patients with MFS compared with controls: 25.1 ± 4.7 mm versus 18.9 ± 3.7 mm ($P < .001$). In univariate analysis including age, BSA, sex, and the presence of MFS, BSA and the presence of MFS appeared as independent predictors of the MPA diameter. After multivariate analysis, the presence of MFS and BSA remained as the only independent predictors. The relationship between age and BSA and the MPA diameter for the different age categories (>14 years and ≤14 years) is illustrated in Figure 1A to D. It can be appreciated from this that the MPA is dilated from early in life. The strong relationship between age and BSA and the MPA diameter in the younger age group is illustrated in Figure 1A and B, whereas this relationship becomes less pronounced in adulthood.

Subdividing the patients in the two age categories (>40 years or <40 years), as proposed in the current diagnostic criteria, does not alter the global finding of increased diameter in the adult patients with MFS (Fig. 2).

We found a significant correlation ($r^2 = 0.54$) between the diameter of the proximal aorta and the pulmonary artery annulus diameter (Fig. 3), although it is noteworthy that some patients with MFS with a normal diameter of the proximal aorta had significant dilatation of the MPA.

A receiver operating characteristic curve to evaluate the value of measurement of the MPA root in the prediction of the disease was constructed. In a receiver operating characteristic curve, true positives are plotted against the false-positive rate for the different cutpoints of a diagnostic test. The area under the curve is a measure of test accuracy (with an area of 1 representing a perfect test and an area of 0.5 representing a worthless test). When applied on the entire study population, the area under the curve was 0.85.

Limiting this analysis to the age group 14 years and older increased this value to 0.94 (Fig. 4), indicating that measurement of the MPA diameter can adequately discriminate patients with MFS from controls in this age group. On the basis of this curve, cutoff values with a certain sensitivity and specificity are calculated. We propose a cutoff value of 23 mm for MPA diameter in subjects 14 years and older, corresponding to a sensitivity of 85% and a specificity of 94.2%.

In children, a uniform cutoff value is not applicable because of the strong relationship with BSA in this age group. We rec-

Table 1
Baseline characteristics for adult patients with Marfan syndrome and controls

	Marfan (N = 53)	Marfan MRI subgroup (N = 29)	Controls (N = 53)	P value[a]
Age (y)	33.0 ± 10.9	31.9 ± 10.4	37.3 ± 11.9	.06
Male sex (%)	47	48	56	.38
Length (cm)	184.3 ± 9.0	184.3 ± 9.7	172.4 ± 9.7	<.0001
Weight (kg)	73.7 ± 15.0	77.7 ± 13.7	70.7 ± 13.8	.27
BSA (m^2)	1.92 ± 0.3	2.0 ± 0.2	1.83 ± 0.2	.191
BMI (kg/m^2)	22.2 ± 4.1	23.0 ± 4.2	23.5 ± 3.8	.018
Aortic diameter at the level of the sinus Valsalva (mm)[b]	41.6 ± 1.0	39.8 ± 0.8	29.7 ± 0.3	<.0001

Continuous values given as mean ± standard deviation.
[a]Between patients with Marfan syndrome (MFS) and controls.
[b]Only assessed in patients without previous aortic root surgery.
MRI, magnetic resonance imaging; BSA, body surface area; BMI, body mass index.

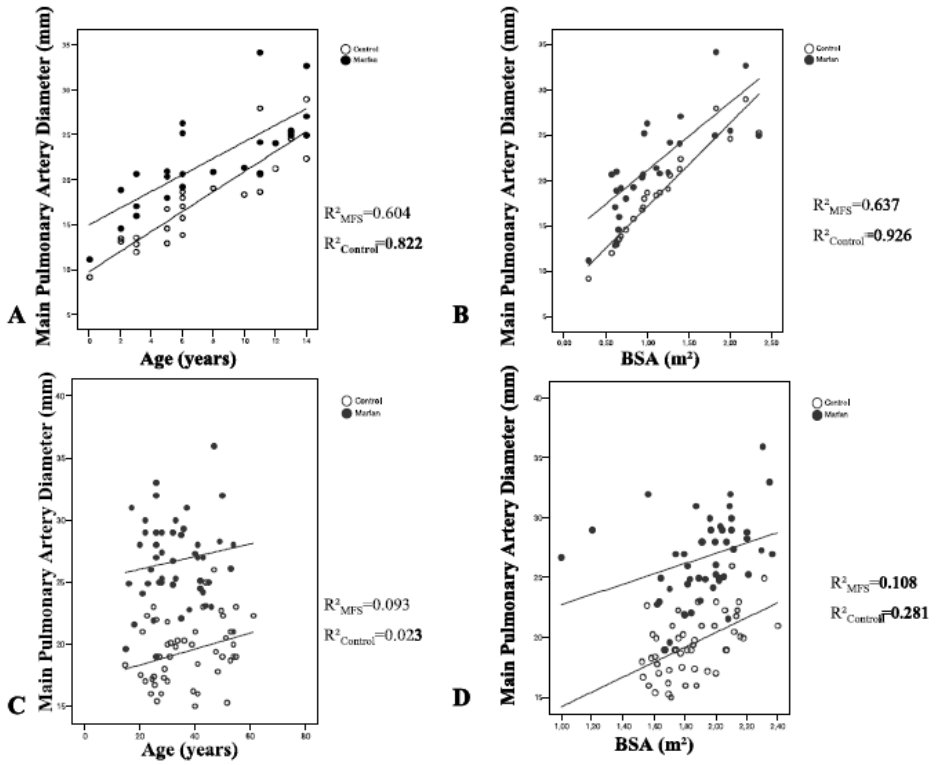


Fig. 2. A–D: Main pulmonary artery diameter in function of age (A and C) and BSA (B and D) in patients with Marfan syndrome and controls (A and B: age <14 years; C and D: age ≥14 years).

ommend the use of *z* scores in this age group; normal values were published by Snider et al.[9] They provide a regression equation for calculating the MPA diameter based on the BSA:

MPA diameter (in centimeters) = 0.0946 + 1.544 BSA½, with an SD of 0.32. From this, the *z* score for an individual patient can be calculated as

$$Z = X - M/SD$$

with X = the obtained value for a specific subject, M = the mean expected for that subject, and SD the standard deviation of the expected mean value, being 0.32 in this case.

The major benefit of the proposed cutoff value is its excellent negative predictive value. Nevertheless, it may occasionally be helpful for confirmation of the diagnosis. We identified cardiovascular involvement in five adult patients with MFS without dilatation of the proximal aorta (*z* score <2) and without MVP. The clinical characteristics of these patients are given in Table 2. All of these subjects were family members of patients who fulfilled the diagnostic criteria; they were all identified on the basis of the presence of a *FBN1* mutation. In many centers, however, mutation screening is not performed on a routine basis, and in these instances, correct assessment of clinical manifestations may be crucial.

Interobserver and intraobserver variability of the echocardiographic measurements were good with coefficients of variance of 3.5% for intraobserver variability and 6.5% for interobserver variability.

Dilatation and dissection of the descending thoracic or abdominal aorta

When the mean values for the aortic diameters at different levels (excluding one outlier with an aneurysm described further in the text) were compared, significant differences were found between patients with MFS and control subjects (Table 3). However, absolute differences were small, and there was

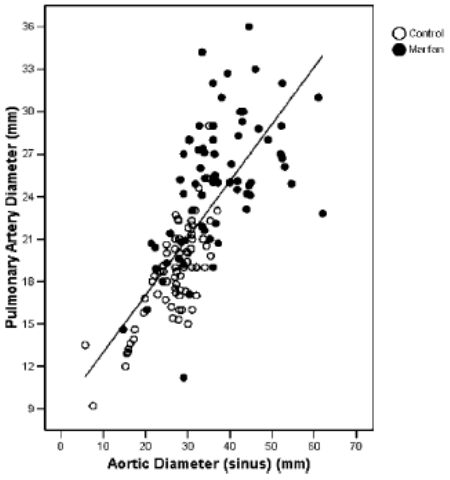


Fig. 3. Correlation between the diameter of the proximal aorta and the main pulmonary artery diameter.

substantial overlap between patients and controls, which compromised our attempt to set up reference values (Fig. 5).

We calculated *z* scores based on our findings in control subjects (*z* score = [obtained value − mean value]/SD). At the level of the descending thoracic aorta, six patients (21%) had a *z* score of 2 or greater, four of whom had previous aortic root surgery; at the level of the diaphragm, three patients (10%) had a *z* score of 2 or greater, two of whom underwent previous aortic root surgery; at the level of the abdominal aorta, two patients (7%) had a *z* score of 2 or greater, one of whom underwent previous aortic root surgery. Except for the patient with an aneurysm of the descending aorta, the *z* scores were all less than 3.

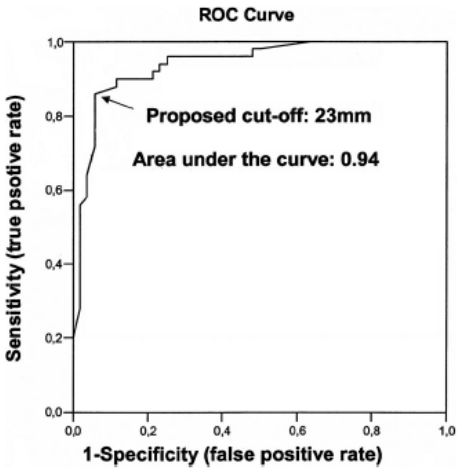


Fig. 4. Receiver operating characteristic curve assessing the accuracy of pulmonary artery root diameter measurement in the diagnosis of adult patients with Marfan syndrome.

In patients with MFS and in controls, the diameters at the different levels of the descending aorta correlated, but in patients with MFS, there was no significant correlation with the diameter at the aortic sinus or the pulmonary artery.

We detected an asymptomatic type B dissection in one patient (29-year-old woman) with a significant dilatation of the descending aorta (diameter of 5.7, 5.6, and 3.5 cm at the level of the descending thoracic aorta, diaphragm, and abdominal aorta, respectively). Of interest is that this particular patient had a normal diameter of the proximal aorta (3.6 cm at the level of the sinuses of Valsalva, *z* score 1.4). One control subject (52-year-old man) also had slightly elevated diameters of the descending thoracic aorta (diameter of 2.6, 3.4, and 2.6 cm at the level of the descending thoracic aorta, diaphragm, and abdominal aorta, respectively). This patient had no cardiovascular risk profile, and family history for abdominal aortic aneurysm was negative.

DISCUSSION

Cardiovascular manifestations in MFS have been defined in the Gent nosology in 1996.[1] At that time, however, clear guidelines for the assessment of these manifestations were limited to measurement of the diameter of the proximal aorta, as defined by Roman et al.,[10] and to the standardized imaging techniques for the assessment of ascending aortic dissection. Since then, standardized two-dimensional echocardiographic methods have been published, and large-scale trials have reported on the prevalence of classic and nonclassic MVP in the general population,[2,11] but the prevalence of these two forms of MVP in MFS according to these guidelines has not yet been assessed. The absence of validated techniques and reference values for the other minor criteria, namely, dilatation or dissection of the descending thoracic aorta, dilatation of the MPA, and calcification of the mitral valve annulus, have hampered the use of these criteria in the diagnosis of MFS.

In this study we evaluated the prevalence of these minor cardiovascular criteria in patients with MFS and attempted to formulate practical guidelines for cardiovascular assessment in patients referred for diagnostic evaluation of MFS.

The prevalence of MVP in patients with MFS was 66.2%, with nearly equal proportions of classic and nonclassic MVP. These figures clearly confirm a higher prevalence of MVP in patients with MFS when compared with the general population (1.3% for the classic form and 1.1% for the nonclassic form),[2] as already stated by other authors.[12,13] Nonclassic MVP, in cardiovascular literature often referred to as "mitral valve bulging," should equally be considered in the diagnostic evaluation.

Table 2
Clinical characteristics of the five patients with pulmonary artery dilatation as the only cardiovascular manifestation

Patient (age/sex)	Skeletal system	Eyes	Skin	Dural ectasia	Family/genetic
1 (27/F)	Pectus carinatum, severe scoliosis, arachnodactyly, increased arm span, flat feet, joint hypermobility, typical facial appearance	Myopia	Striae	Present	Brother affected FBN1: c. 7828G > A
2 (26/F)	Pectus excavatum, scoliosis, increased arm span, flat feet, facial appearance, hypermobility	Ectopia lentis	No striae	Not assessed	Brother affected FBN1: c. 1463 G > T
3 (28/F)	Arachnodactyly, increased arm span, reduced elbow extension, flat feet, facial appearance	Retinal detachment	No striae	Absent	Mother affected FBN1: IVS8-1G > C
4 (20/F)	Arachnodactyly, increased arm span, decreased elbow extension, flat feet, facial appearance, joint hypermobility	Myopia	No striae	Not assessed	Father affected FBN1: c. 408C > A
5 (21/F)	Arachnodactyly, flat feet, increased arm span, reduced elbow extension, joint hypermobility	Not affected	No striae	Not assessed	Father and brother affected FBN1: IVS8-1G > C

In accordance with the figures from previous publications, we found a relatively higher prevalence of MVP in children with MFS when compared with adults. This may be because MVP is often the presenting cardiovascular sign of MFS in children, whereas in adults other manifestations might have led to the diagnosis.

Calcification of the mitral valve annulus may be a specific manifestation in patients with MFS, but this parameter is difficult to quantify with echocardiography. Although it is not possible to exclude a higher prevalence of mitral annular calcification if more sensitive screening practices such as computed tomography were used, it seems unlikely that the yield would justify the added inconvenience and expense on patients.

Guidelines regarding measurement and normal values for MPA dilatation were lacking at the time of the publication of the diagnostic criteria.

Measurement of the MPA diameter may be useful in ruling out cardiovascular involvement suggestive of MFS.

The prevalence of MPA dilatation in patients with MFS was recently studied by Nollen et al.[5] with the use of MRI.

Complications arising from pulmonary artery dilatation are much milder, if any, compared with those resulting from progressive aortic dilatation. This is mainly attributable to the lower pressure in the pulmonary circulation. Pulmonary artery dissection is rare.

The excellent negative predictive value of the proposed cutoff value of 23 mm indicates that cardiovascular involvement can be ruled out with high certainty when measuring a diameter less than 23 mm in patients aged 14 years or more, given that no aortic root dilatation or MVP is present. On the other hand, we identified five patients with definite MFS, in whom no other cardiovascular sign was present except for an MPA diameter exceeding 23 mm.

Table 3
Thoracoabdominal aortic diameters at different levels (values in centimeters)

	Marfan (N = 27)	Control (N = 25)	*P* Value
Descending thoracic	24.1 ± 2.7	20.8 ± 2.7	<.001
Diaphragm	21.7 ± 2.7	19.3 ± 3.7	<.001
Abdomen	16.9 ± 2.6	14.9 ± 3.1	.005

Defining dilatation of the MPA in children is more problematic. Although the MPA is clearly dilated in the younger age group, one has to take both age and BSA into account. A uniform cutoff value in children cannot be defined. Instead, the use of *z* scores should be recommended.

MPA diameter correlated to the diameter of the proximal aorta, indicating that they are the result of the same underlying pathophysiologic process. We encountered patients without significant aortic root dilatation who already had a dilated pulmonary artery, which enabled us to define involvement of the cardiovascular system in these patients.

Because dilatation of the MPA was observed in the entire age range under study here, it is preferable to abolish the age limit of 40 years, as proposed in the Ghent criteria.

Nollen and colleagues[5] measured the diameter of the MPA with MRI in patients with MFS. By using a cutoff value of 28 mm at the level of the MPA root, Nollen et al. report a prevalence of MPA dilatation of 74%. There are several possible explanations for the difference in proposed cutoff values of 28 mm and our proposed cutoff value of 23 mm. First, the site of measurement was different; whereas Nollen et al. measured the anterior/right diameter, we were limited by the echocardiographic window to measure the anterior/left window. Second, a significantly higher proportion of the patients studied by Nollen et al. had a history of aortic root surgery, indicating that this group of patients had more advanced involvement of the cardiovascular system with higher diameters of the pulmonary artery. Last, but not least, the applied imaging techniques in both studies are different and thus are not comparable.

One of the major advantages of MRI is that image acquisition is not limited by patient factors such as thorax deformities. This may be important in the case of patients with MFS, although we encountered only two patients in whom imaging of the MPA was inadequate with echocardiography. Another in-

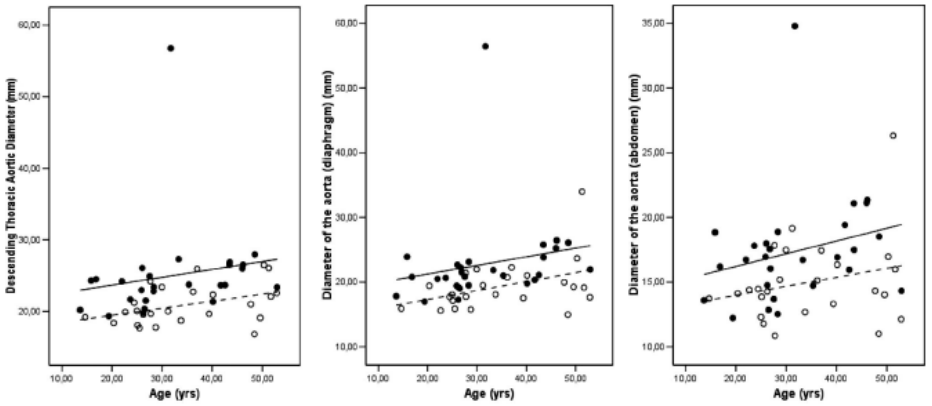


Fig. 5. Diameter of the descending aorta at three levels: descending thoracic, diaphragm, and abdominal.

teresting point is that both the aortic root and the MPA root are asymmetric, which has been nicely demonstrated using MRI.[14,15] This asymmetry may also be part of the explanation for the difference in obtained values between our study and the MRI study by Nollen et al.[15]

In a diagnostic setting, however, echocardiography is preferable compared with MRI, in view of the higher costs and lower accessibility of MRI.

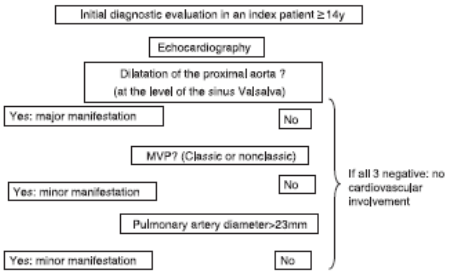
Data on dilatation of the descending aorta in patients with MFS are scarce. Patients with MFS presenting with thoracoabdominal aortic aneurysm/dissection are reported in a few case reports.[16,17] Other reports on the descending aorta in patients with MFS are mainly limited to surgical data describing the occurrence of primary or secondary complications in the descending aorta necessitating surgical intervention. Finkbohner and colleagues[18] report that 15% of their patients underwent initial surgery that involved portions of the descending aorta. Nollen and colleagues[19] report an increased growth (defined as >1 mm/year) in a small subset of patients (6% in the descending thoracic aorta and 7% in the abdominal aorta). Kawamoto and colleagues[20] studied the progression of thoracoabdominal aortic diameters in patients with MFS after surgical repair and defined a subgroup of patients showing progressive dilatation of the distal aorta (>3 mm/year).

Our data demonstrate that as a group, patients with MFS have increased diameters at different levels of the thoracoabdominal aorta when compared with controls. There is, however, substantial overlap between the upper values of controls and the lower values of patients with MFS, precluding the use of a specific cutoff value. For this reason, we believe that measurement of descending aortic diameters is of little value in a diagnostic screening setting.

Our findings obtained from the calculation of the *z* scores of the thoracoabdominal aorta show that increased *z* scores (≥2) occur only in a minority of patients and are more common in patients having previously undergone aortic root surgery. For this reason and in view of the results obtained in the studies mentioned earlier in postoperative patients with MFS,[20,21] imaging of the distal aorta should be performed on a regular basis in this subset of patients.

Guidelines for cardiovascular assessment in patients referred for diagnostic cardiovascular evaluation for Marfan syndrome

We propose the following flowchart for cardiovascular examination in adult patients referred for initial screening:



In children, the *z* score for the MPA diameter should be calculated as described above. In the flowchart, "pulmonary artery diameter >23 mm" should be replaced by "a *z* score >2."

Study limitations

Matching with controls was done for age and sex, not for weight and height, but this was overcome by including weight and height as covariants in the statistical analysis.

This study design was retrospective. Application of these guidelines in a prospective manner is necessary to confirm their validity.

ACKNOWLEDGMENTS

This study was supported by research grants from the Ghent University (BOF 011D4701) (J. De Backer) and the Fund for Scientific Research Belgium (FWO G029002) (A. De Paepe). Johan De Sutter and Bart Loeys are senior clinical investigators of the Fund for Scientific Research, Flanders (Belgium) (FWO-Vlaanderen).

References

1. De Paepe A, Devereux RB, Dietz HC, Hennekam RC, et al. Revised diagnostic criteria for the Marfan syndrome. *Am J Med Genet* 1996;62:417–426.
2. Freed LA, Levy D, Levine RA, Larson MG, et al. Prevalence and clinical outcome of mitral-valve prolapse. *N Engl J Med* 1999;341:1–7.
3. Levine R, Handschumacher M, Sanfilippo A, Hagege A, et al. Three-dimensional echocardiographic reconstruction of the mitral valve, with implications for the diagnosis of mitral valve prolapse. *Circulation* 1989;80:589–598.
4. Lorenz CH. The range of normal values of cardiovascular structures in infants, children, and adolescents measured by magnetic resonance imaging. *Pediatr Cardiol* 2000;21:37–46.
5. Nollen GJ, van Schijndel KE, Timmermans J, Groenink M, et al. Pulmonary artery root dilatation in Marfan syndrome: quantitative assessment of an unknown criterion. *Heart* 2002;87:470–471.
6. Garcier JM, Petitcolin V, Filaire M, Mofid R, et al. Normal diameter of the thoracic aorta in adults: a magnetic resonance imaging study. *Surg Radiol Anat* 2003;25:322–329.
7. Hager A, Kaemmerer H, Rapp-Bernhardt U, Blucher S, et al. Diameters of the thoracic aorta throughout life as measured with helical computed tomography. *J Thorac Cardiovasc Surg* 2002;123:1060–1066.
8. Dubois DA. A formula to estimate the approximate surface area if height and weight be known. *Arch Int Med* 1916;17:863.
9. Snider AR, Enderlein MA, Teitel DF, Juster RP Two-dimensional echocardiographic determination of aortic and pulmonary artery sizes from infancy to adulthood in normal subjects. *Am J Cardiol* 1984;53:218–224.
10. Roman MJ, Devereux RB, Kramer-Fox R, O'Loughlin J Two-dimensional echocardiographic aortic root dimensions in normal children and adults. *Am J Cardiol* 1989;64:507–512.
11. Freed LA, Benjamin EJ, Levy D, Larson MG, et al. Mitral valve prolapse in the general population: the benign nature of echocardiographic features in the Framingham Heart Study. *J Am Coll Cardiol* 2002;40:1298–1304.
12. van Karnebeek CDM, Naeff MSJ, Mulder BJM, Hennekam RCM, et al. Natural history of cardiovascular manifestations in Marfan syndrome. *Arch Dis Child* 2001; 84:129–137.
13. Yetman AT, Bornemeier RA, McCrindle BW. Long-term outcome in patients with Marfan syndrome: is aortic dissection the only cause of sudden death? *J Am Coll Cardiol* 2003;41:329–332.
14. Meijboom LJ, Groenink M, van der Wall EE, Romkes H, et al. Aortic root asymmetry in Marfan patients: evaluation by magnetic resonance imaging and comparison with standard echocardiography. *Int J Card Imaging* 2000;16:161–168.
15. Nollen GJ, van Schijndel KE, Timmermans J, Groenink M, et al. Magnetic resonance imaging of the main pulmonary artery: reliable assessment of dimensions in Marfan patients on a simple axial spin echo image. *Int J Cardiovasc Imaging* 2003;19:141–147; discussion 9 –50.
16. van Ooijen B. Marfan's syndrome and isolated aneurysm of the abdominal aorta. *Br Heart J* 1988;59:81–84.
17. Pruzinsky MS, Katz NM, Green CE, Satler LF. Isolated descending thoracic aortic aneurysm in Marfan's syndrome. *Am J Cardiol* 1988;61:1159–1160.
18. Finkbohner R, Johnston D, Crawford ES, Coselli J, et al. Marfan syndrome. Long-term survival and complications after aortic aneurysm repair. *Circulation* 1995;91: 728–733.
19. Nollen GJ, Groenink M, Tijssen JG, Van Der Wall EE, et al. Aortic stiffness and diameter predict progressive aortic dilatation in patients with Marfan syndrome. *Eur Heart J* 2004;25:1146–1152.
20. Kawamoto S, Bluemke DA, Traill TA, Zerhouni EA Thoracoabdominal aorta in Marfan syndrome: MR imaging findings of progression of vasculopathy after surgical repair. *Radiology* 1997;203:727–732.
21. Finkbohner R, Johnston D, Crawford ES, Coselli J, et al. Marfan syndrome: long-term survival and complications after aortic aneurysm repair. *Circulation* 1995;91: 728–733.

IV.2 Chapter 2: Study of the Left Ventricular function in Marfan syndrome

2.1 Evaluation of Left Ventricular Dimensions and Function in the Marfan Syndrome without Significant Valvular Regurgitation.

Lilian J. Meijboom, Janneke Timmermans, Johan P. van Tintelen, Gijs J. Nollen, Julie De Backer, Maarten P. van den Berg, Gerard H.Boers, Barbara J.M. Mulder.
American Journal of Cardiology 2005;95:795-797

In this study 234 MFS patients, free of significant valvular heart disease were studied with conventional echocardiography. Left ventricular dimensions (end systolic- and end diastolic diameter) and systolic function (fractional shortening) were assessed and compared to predicted values from the literature.

We demonstrated slightly elevated LV dimensions in these patients (relative LV end diastolic diameter 102±10%). Significantly increased LV end diastolic diameter defined as a relative dimension>117% occurred in 17 patients (7%). LV systolic function assessed with fractional shortening was decreased in 21 patients (8%).

Evaluation of Left Ventricular Dimensions and Function in Marfan's Syndrome Without Significant Valvular Regurgitation

Lilian J. Meijboom, MD, Janneke Timmermans, MD, Johan P. van Tintelen, MD, Gijs J. Nollen, MD, PhD, Julie De Backer, MD, Maarten P. van den Berg, MD, PhD, Gerard H. Boers, MD, PhD, and Barbara J.M. Mulder, MD, PhD

Left ventricular dimensions and systolic function were studied using echocardiography in 234 patients with Marfan's syndrome without significant valvular regurgitation. Left ventricular dimensions and systolic function were found to be normal in most patients with Marfan's syndrome. Some involvement of the left ventricle may have been present in a small group of these patients. No patients, however, fulfilled the criteria for dilated cardiomyopathy. ©2005 by Excerpta Medica Inc.

(Am J Cardiol 2005;95:795–797)

Marfan's syndrome is an autosomal, dominantly inherited disorder of connective tissue caused by a mutation in the fibrillin-1 gene.[1] As fibrillin-1 is a component of the myocardium, it has been speculated that the fibrillin defect may predispose patients with Marfan's syndrome to left ventricular (LV) dilation and reduced LV function.[2] LV dimensions are often increased in patients with Marfan's syndrome because of aortic or mitral regurgitation. This study investigated LV dimensions and systolic function in a large group of patients with Marfan's syndrome who were free of significant heart valve disease.

• • •

A total of 529 consecutive patients diagnosed with Marfan's syndrome (according to the revised Ghent criteria) were identified in 4 university hospitals. Only the 234 patients without previous aortic root surgery and without significant aortic and/or mitral valve regurgitation noted on echocardiograms were included in our study. In these 234 patients, LV end-diastolic diameter (EDD) and LV end-systolic diameter (ESD) were derived from M-mode or 2-dimensional echocardiograms.[3] The predicted normal values for LVEDD and LVESD were calculated for each patient according to their age and body surface area using Henry's regression equations.[4] The LV dimension was expressed as a percentage of the predicted value according to the formula (observed dimension/predicted normal value × 100). We defined this as the relative LVEDD (percent) or relative LVESD (percent). Fractional shortening was calculated using the formula ([LVEDD− LVESD]/LVEDD × 100).

We analyzed the incidence of dilated cardiomyopathy. Two criteria had to be fulfilled for a diagnosis of dilated cardiomyopathy: (1) relative LVEDD >117% (mean +2 SD +5%); and (2) fractional shortening <25%.[5] The first and most recent echocardiograms were compared in 196 patients to determine any change in LV size or systolic function over time.

Data are described as frequencies or as means with SDs. Follow-up times are expressed as mean and range. Differences between patient subgroups with respect to proportions were tested with the chi-square test and differences on continuous variables with the Student's *t* test. A 2-sided significance level of 0.05 was used for each statistical test.

The clinical characteristics of 234 adult patients with Marfan's syndrome without significant valvular

TABLE 1 Clinical Characteristics of 234 Patients With Marfan's Syndrome Without Significant Valvular Regurgitation

Variables	n = 234
Men	115 (49%)
Age at first echocardiogram (yrs)	29 ± 11
Range	17–70
Mean body surface area (m^2)	2.0 ± 0.2
Mean aortic root diameter (mm)	39 ± 6
β-blocking therapy	70 (30%)
Mean follow-up (yrs)	6
Range	3 mo–15 yrs

Values are expressed as means ± SD or as number (percentages).

From the Department of Cardiology, Academic Medical Center, Amsterdam; the Departments of Cardiology and General Internal Medicine, University Hospital, Nijmegen; and the Departments of Clinical Genetics and Cardiology, University Hospital, Groningen, The Netherlands; and Department of Medical Genetics, Ghent University Hospital, Ghent, Belgium. Dr. Mulder's address is: Department of Cardiology, Room B2-240, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands. E-mail: b.j.mulder@amc.uva.nl. Manuscript received September 23, 2004; revised manuscript received and accepted November 10, 2004.

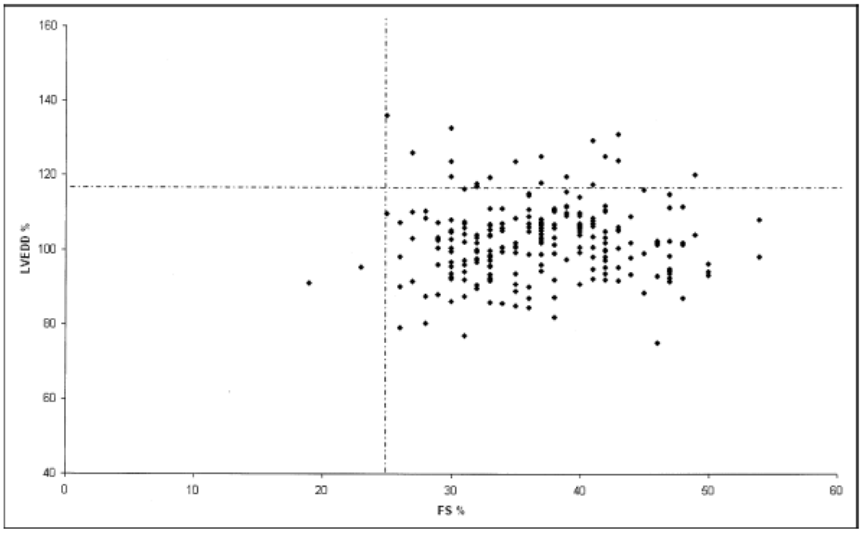


FIGURE 1. Relative LVEDD and fractional shortening *(FS)* in 234 patients with Marfan's syndrome without significant valvular regurgitation. *Dashed lines,* borders of dilated cardiomyopathy (see text). In none of the patients were the criteria for dilated cardiomyopathy fulfilled for LVEDD and FS *(left upper quadrant)*.

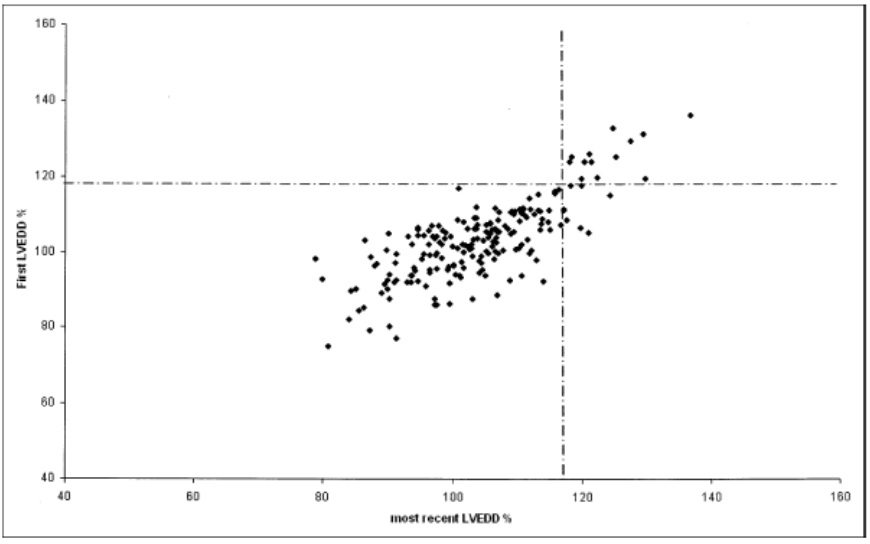


FIGURE 2. Comparison of the relative LVEDD on the first and most recent echocardiograms. During the 6-year follow-up, another 6 patients surpassed the cut-off value of 117% of the predicted LVEDD, again without decreased fractional shortening.

regurgitation are listed in Table 1. On the first echocardiogram, the mean LVEDD was 50 ± 6 mm (relative LVEDD, 102 ± 10%; range 75% to 136%) and mean fractional shortening was 37 ± 6% (range, 19% to 54%). Mean LVESD was 32 ± 5 mm (relative LVESD, 101 ± 14%; range 63% to 160%).

The LVEDD in 17 patients (7%) was >117% (2 SD +5%) of the predicted value (relative LVEDD 124 ±

6%; range 118% to 136%). However, in none of these patients was the fractional shortening <25% (Figure 1) according to the second criterion for dilated cardiomyopathy. The records of these 17 patients with LVEDD >117% were reviewed for systemic arterial hypertension, signs of coronary artery disease, alcohol consumption, sustained supraventricular arrhythmias, systemic disease (e.g., amyloidosis and hemochromatosis), pericardial disease, diabetes mellitus, and drug-induced cardiomyopathy. None of these factors could explain the increased LVEDD in these patients.

In these 17 patients, the relative LVESD and aortic root diameter were significantly increased compared with patients with a relative LVEDD ≤117% (relative LVESD, 124 ± 15% vs 99 ± 12%, p <0.01, respectively; aortic root diameter, 44 ± 6 vs 39 ± 5 mm, p <0.01, respectively).

In 45 patients, LV dimensions were not entirely normal: 22 patients had a LVEDD >112% (2 × SD), 21 patients had fractional shortening <30%, and 2 patients had a LVEDD of >112% and fractional shortening of <30%.[6,7]

No significant difference in LVEDD or in heart frequency was observed in patients with and without β-blocking therapy (p = 0.17, p = 0.08, respectively).

The first and most recent echocardiograms were compared in 196 patients with Marfan's syndrome to determine any change in LV size or function over time. During a mean follow-up of 6 years (range 3 months to 15 years) no abnormal change in the mean relative LVEDD percentage (first 103 ± 10% vs last 104 ± 11%, p = 0.08) was observed (Figure 2). Also, no change was observed in the mean relative LVESD (first 101 ± 14% vs last 101 ± 15%, p = 0.6) or mean fractional shortening (first 37 ± 6% vs last 38 ± 7%, p = 0.06). In the patients with a relative LVEDD >117%, no significant change between the first and last measurement was seen during the 6-year follow-up (mean relative LVEDD, 125 ± 6% vs 124 ± 5%, p = 0.4; mean fractional shortening, 35 ± 6% vs 35 ± 6%, p = 0.4). Again, none of these patients fulfilled the criteria for cardiomyopathy. Another 6 patients surpassed the cutoff value of 117% of the predictive LVEDD, also without a fractional shortening of <25%.

• • •

Our study provides LV dimensions and systolic function in a very large population of patients with Marfan's syndrome without significant valvular regurgitation. LV dimensions and systolic function were normal in most patients with Marfan's syndrome. Although the left ventricle was dilated in 7% of these patients, none of the patients fulfilled the criteria for idiopathic dilated cardiomyopathy. No abnormal change in LV dimensions or systolic function was observed during the 6-year follow-up.

Speculations have been made that LV dilation and reduced LV function is a common finding in patients with Marfan's syndrome because of a fibrillin defect in the myocardium or increased aortic wall stiffness that may lead to increased LV afterload and associated LV dilation.[2,6,8] Yetman et al[6] observed LV dilation in 68% of 70 young patients with Marfan's syndrome (median age,17 years), including, however, patients with valvular regurgitation. Heart transplantation for heart failure has been reported in patients with Marfan's syndrome, but the prevalence of valvular regurgitation in these studies was unclear.[9–11]

Two studies investigated the incidence of LV dilation and function in a small patient group with Marfan's syndrome without valvular regurgitation. Savolainen et al[12] found no statistically significant difference in LV size and systolic function in 22 children with Marfan's syndrome compared with 22 age-matched healthy children. In the study by Chatrath et al,[13] 7 of the 36 adult patients (19%) with Marfan's syndrome without significant valvular regurgitation showed increased LVEDD with normal LV systolic function.

In our study, the left ventricle was dilated in 17 of 234 patients (7%) with Marfan's syndrome; however, the diagnosis for dilated cardiomyopathy could not be made in any of our patients. The incidence of a dilated left ventricle was lower than that reported by Chatrath et al,[13] even if we used a milder criterion for dilated LVEDD (relative LVEDD +2 × SD). This is probably because our population was larger and no selection bias occurred.

During 6 years of follow-up, no abnormal change in LV dimensions and systolic function was observed; nonetheless, an enlarged LVEDD developed in 6 patients. Again, none of these 6 patients fulfilled the criteria for idiopathic dilated cardiomyopathy.

1. McKusick VA. The cardiovascular aspects of Marfan's syndrome: a heritable disorder of connective tissue. *Circulation* 1955;11:321–342.
2. Pyeritz RE. The Marfan syndrome. *Annu Rev Med* 2000;51:481–510.
3. Cheitlin MD, Alpert JS, Armstrong WF, Aurigemma GP, Beller GA, Bierman FZ, Davidson TW, Davis JL, Douglas PS, Gillam LD. ACC/AHA guidelines for the clinical application of echocardiography. A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on Clinical Application of Echocardiography). Developed in collaboration with the American Society of Echocardiography. *Circulation* 1997;95:1686–1744.
4. Henry WL, Gardin JM, Ware JH. Echocardiographic measurements in normal subjects from infancy to old age. *Circulation* 1980;62:1054–1061.
5. Mestroni L, Maisch B, McKenna WJ, Schwartz K, Charron P, Rocco C, Tesson F, Richter A, Wilke A, Komajda M. Guidelines for the study of familial dilated cardiomyopathies. Collaborative Research Group of the European Human and Capital Mobility Project on Familial Dilated Cardiomyopathy. *Eur Heart J* 1999;20:93–102.
6. Yetman AT, Bornemeier RA, McCrindle BW. Long-term outcome in patients with Marfan syndrome: is aortic dissection the only cause of sudden death? *J Am Coll Cardiol* 2003;41:329–332.
7. Rumington A, Chambers J. Echocardiography; A Practical Guide for Reporting. New York: Parthenon Publishing Group, 1998:3.
8. Nollen GJ, Meijboom LJ, Groenink M, Timmermans J, Barentsz JO, Merchant N, Webb GD, Lamb HJ, Tijssen JG, Van der Wall EE, Mulder BJM. Comparison of aortic elasticity in patients with the Marfan syndrome with and without aortic root replacement. *Am J Cardiol* 2003;91:637–640.
9. Kesler KA, Hanosh JJ, O'Donnell J, Faust S, Turrentine MW, Mahomed Y, Brown JW. Heart transplantation in patients with Marfan's syndrome: a survey of attitudes and results. *J Heart Lung Transplant* 1994;13:899–904.
10. Kocher A, Ehrlich M, Khazen C, Ankersmit J, Nourani F, Itescu S, Edwards NM, Wolner E, Laufer G. Repair of an aortic aneurysm at the time of heart transplantation: report of two cases and review of the literature. *Transplant Proc* 1999;31:3184–3186.
11. Mullen JC, Lemermeyer G, Bentley MJ. Recurrent aortic dissection after orthotopic heart transplantation. *Ann Thorac Surg* 1996;62:1830–1831.
12. Savolainen A, Nisula L, Keto P, Heikali P, Viitasalo M, Kaitila I, Kupari M. Left ventricular function in children with the Marfan syndrome. *Eur Heart J* 1994;15:625–630.
13. Chatrath R, Beauchesne LM, Connolly HM, Michels VV, Driscoll DJ. Left ventricular function in the Marfan syndrome without significant valvular regurgitation. *Am J Cardiol* 2003;91:914–916.

IV.2.2 Primary Impairment of left ventricular function in Marfan syndrome.

Julie De Backer, Dan Devos, Patrick Segers, Dirk Matthys, Katrien François, Thierry Gillebert, Anne De Paepe, Johan De Sutter.
Int J Cardiol. 2006. 112:353-358

This paper describes the evaluation of LV systolic and diastolic function in 26 MFS patients without significant valvular heart disease. The findings are compared to those of 26 age- and sex-matched control subjects.

For this purpose, we used a combination of conventional echocardio-graphy, TDI and MRI.

We clearly demonstrated abnormalities in both systolic and diastolic function, as summarized in the table1.

	Marfan	Control (N=26)	P value
LVESV/BSA (ml/m²)	36.0±9.5	29.5±6.7	0.007
EF (%)	53.5±9.0	59.6±6.7	0.009
S_m basal septum (cm/sec)	5.2±1.4	6.4±1.3	0.001
DT_E (msec)	171.8±40.5	141.1±36.2	0.001
E_a (cm/sec)	9.6±2.5	11.9±3.3	0.003
A_a (cm/sec)	6.9±2.3	8.2±2.2	0.023
Ea/Sm basal septum	1.9±0.6	1.9±0.5	0.8

Table 1: summary of the systolic and diastolic function parameters in MFS patients and controls. LVESV: Left ventricular end systolic volume; BSA: Body Surface Area; EF: Ejection Fraction; S_m: peak Systolic velocity at the mitral valve annulus; DT_E: Deceleration time of the E wave; E_a: early mitral annulus velocity; A_a: late mitral annulus velocity

When compared to normal control subjects, we found increased end systolic volume, decreased ejection fraction and decreased myocardial velocities in MFS patients, indicating impaired systolic function. In addition, MFS patients presented an increased deceleration time, lower early mitral valve inflow velocities and decreased late mitral annular velocities, indicating impaired diastolic function.

The ratio of Ea to peak Sm at the basal septum was comparable between both groups, indicating a concordant reduction in contraction and relaxation velocities, with a preserved contraction/relaxation coupling in patients with MFS. As a

consequence the observed diastolic dysfunction should be interpreted as secondary to primary contractile dysfunction.

ELSEVIER

International Journal of Cardiology 112 (2006) 353 – 358

International Journal of Cardiology

www.elsevier.com/locate/ijcard

Primary impairment of left ventricular function in Marfan syndrome☆

Julie F. De Backer [a,b,*], Daniel Devos [c], Patrick Segers [d], Dirk Matthys [e], Katrien François [f], Thierry C. Gillebert [b], Anne M. De Paepe [a], Johan De Sutter [b]

[a] *Department of Medical Genetics, Ghent University Hospital, Gent Belgium*
[b] *Department of Cardiovascular Medicine, Ghent University Hospital, Gent Belgium*
[c] *Department of Medical Imaging, Ghent University Hospital, Gent Belgium*
[d] *Hydraulics Laboratory, Institute of Biomedical technology, Ghent University, Gent Belgium*
[e] *Department of Pediatrics, Ghent University Hospital, Gent Belgium*
[f] *Department of Cardiovascular Surgery, Ghent University Hospital, Gent Belgium*

Received 30 June 2005; received in revised form 26 September 2005; accepted 2 October 2005
Available online 28 November 2005

Abstract

Background: Cardiovascular involvement in Marfan syndrome is mainly characterized by progressive dilatation of the proximal aorta. Whether left ventricular dysfunction is present in these patients is not clear at present.
Objectives: Assess left ventricular function in patients with Marfan syndrome, free of significant valvular heart disease, using a combination of MRI and Tissue Doppler imaging (TDI).
Methods and results: A total of 26 Marfan patients (mean age = 32.0 ± 10.9, 12 men) without significant valvular heart disease, and 26 age- and sex-matched controls were studied. Left ventricular volumes and ejection fraction were measured with magnetic resonance imaging. Systolic and diastolic function parameters were assessed using conventional echocardiography and TDI. When compared to controls, Marfan patients showed impairment of left ventricular contractile function as expressed by a reduced ejection fraction (53.5 ± 9.0% vs. 59.6 ± 6.7%, $p = 0.009$), an increased end-systolic volume (36.0 ± 9.5 vs. 29.5 ± 6.7 ml/m^2, $p = 0.007$), and reduced peak systolic velocities at the basal septal and lateral myocardial wall (5.2 ± 1.4 vs. 6.4 ± 1.3 cm/s, $p = 0.003$ and 6.0 ± 2.2 vs. 7.5 ± 2.3 cm/s, $p = 0.03$, respectively). Diastolic function was impaired with an increased deceleration time of the *E* wave (171 ± 41 ms vs. 141 ± 36 ms, $p = 0.006$). Peak early diastolic velocity at the mitral valve annulus was significantly lower (9.6 ± 2.4 cm/s vs. 11.9 ± 3.3 cm/s, $p = 0.006$).
Conclusion: These data provide evidence for mild, but significant impairment of left ventricular systolic and diastolic function in Marfan patients, not related to valvular heart disease.

Keywords: Marfan syndrome; Systolic function; Diastolic function; Left ventricular function

1. Introduction

Marfan syndrome (MFS, MIM#154700) is an inherited connective tissue disorder characterized by manifestations in different organ systems, including the ocular, skeletal and cardiovascular systems [1]. The clinical diagnosis is based on the presence of major and minor criteria in these different organ systems, as formulated in the "Ghent Nosology" [2]. Cardiovascular involvement in MFS is characterized by progressive dilatation of the proximal aorta, which may lead to aortic dissection, acute aortic

☆ This study was supported by a research grant from the Ghent University (BOF 011D4701) (J. De Backer) and by a research grant from the Fund for Scientific Research Belgium (FWO G029002) (A. De Paepe). Johan De Sutter is a senior clinical investigator of the Fund for Scientific Research – Flanders (Belgium) (FWO – Vlaanderen).

* Corresponding author. Department of Medical Genetics and Department of Cardiovascular Medicine, Ghent University Hospital, Belgium, De Pintelaan 185, 9000 Gent, Belgium. Tel.: +32 9 240 36 03; fax: +32 9 240 49 70.
E-mail address: Julie.debacker@UGent.be (J.F. De Backer).

0167-5273/$ - see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.ijcard.2005.10.010

regurgitation or rupture and sudden death [3]. Life expectancy in patients with MFS is mainly determined by these cardiovascular complications [4]. Other cardiovascular findings in MFS include mitral valve prolapse, pulmonary artery dilatation and dilatation or dissection of the descending aorta [5]. Impairment of left ventricular (LV) function in MFS may occur as a consequence of significant valvular disease, but there is also recent evidence for impaired LV function, in the absence of valvular heart disease, as expressed by increased LV diameters in a small subset of patients [6,7]. Similarly, there are indications that left ventricular diastolic function is impaired in MFS, but the available data are scarce [8,9].

Up to date, a combined study of left ventricular systolic and diastolic function in Marfan syndrome using recently developed sensitive techniques, i.e. magnetic resonance imaging (MRI) and Tissue Doppler imaging (TDI) has not yet been performed.

We conducted a case-control study, combining echocardiography and MRI in order to evaluate LV systolic and diastolic function in patients with Marfan syndrome, without significant valvular heart disease.

2. Methods

Thirty-one patients with established Marfan syndrome were screened with echocardiography. Twenty-six of them were considered suitable for further analysis. Five cases were excluded because of significant valvular heart disease (2 cases) or previous aortic root surgery (3 cases). The 26 remaining patients were free of significant valvular heart disease. For these 26 remaining patients, matched control subjects were recruited among healthy volunteers. Care was taken as to match for sex and age (range ±5 years). All patients were in New York Heart Association (NYHA) class 1. Twenty-one patients (72%) were on beta-blocking therapy but patients taking beta-blockers were asked to stop the medication for 3 days before the study.

Control subjects were healthy volunteers recruited among colleagues and family members of the researchers. None of them was known with cardiovascular disease. None had hypertension or valvular heart disease was excluded in all of them.

All patients and controls underwent echocardiography and MRI on the same day.

The study was approved by the local ethics committee. All patients and controls gave oral and written informed consent.

2.1. Echocardiographic examination

All patients and all normal subjects underwent a standard echocardiographic examination, using a VIVID 7 Vingmed-General Electric ultrasound scanner (GE Vingmed Ultrasound, Horten, Norway). Subjects were examined in the left lateral recumbent position using standard parasternal short- and long-axis and apical views. The left ventricular dimensions were assessed in the parasternal long-axis view. Left ventricular end-diastolic and end-systolic diameters (LVEDD and LVESD) as well as septal and posterior end-diastolic wall thickness (IVS and PW) were measured in 2D mode. Sample volume (size = 2 mm) of the pulsed wave Doppler was placed between the tips of the mitral leaflets in the apical four chamber view. Early (E) and late (A) transmitral flow velocities, the ratio of early to late peak velocities (E/A) and deceleration time of E velocity (DT_E) were obtained. Isovolumic Relaxation time (IVRT) was measured using continuous wave Doppler with the transducer beam angulated towards the left ventricular outflow tract, so that aortic and mitral flows were simultaneously recorded [10].

Pulsed wave TDI was performed with the sample volume positioned at the septal side of the mitral annulus. Early (E_a) and late (A_a) mitral annulus velocities were obtained. The mean value of at least two different cycles was obtained.

Color Doppler TDI images obtained in 2 apical views (4-chamber and 2-chamber) were stored in digital format on a remote hard-disk. Data were reviewed offline with a commercially available image processing program (Echopac 6.3, GE Vingmed Ultrasound, Horten, Norway). Regional myocardial velocities were measured in basal segments of the lateral, septal, inferior and anterior left ventricular walls. Peak systolic velocity (S_m) was measured ignoring the initial peak that is observed during isometric ventricular contraction. Adequate apical 2-chamber view was difficult to obtain in 7 Marfan patients, due to underlying thoracic deformities. Therefore, assessment of peak S_m at the inferior and anterior wall was omitted in the analysis.

Left ventricular mass was calculated according to Devereux et al. [11]: $1.04((LVEDD+PWT+IVST)^3)*0.8+0.4$; with LVEDD = internal diameter, PWT = posterior wall thickness, IVST = interventricular septal thickness.

Echocardiographic assessment of left ventricular volumes was not possible, again due to the inability to obtain adequate apical 2-chamber views.

Meridional wall stress (σ_m) was calculated according to the formula: $\sigma_m = LVESP * LVESD^2/(4h(LVESD+h))$ [12]; with LVESD = end-systolic pressure in the left ventricle, estimated as peak systolic pressure; LVESD = end-systolic left ventricular diameter, h = mean wall thickness in systole (PWT + ISWT)/2.

2.2. MRI study

Left ventricular volumes were measured on a 1.5 T MR system (Magnetom Symphony, Siemens, Erlangen, Germany). Ten to twelve 6-mm slices with 1.2-mm interslice gap were scanned in the short axis direction, from base to apex. A 15-segment, ECG-triggered trueFISP sequence was used with a repetition time (T_R) of 50 ms, and an echo time

(T_E) of 1.82 ms. Volumes were calculated by semi-automated delineation of left ventricular lumen on all slices in end-diastolic and end-systolic phase, and consecutively adding the luminal volume (surface × (slice thickness + interslice gap)) of all the slices.

2.3. Statistical analysis

Results are presented as mean ± S.D. Independent sample *t*-test was used to compare continuous variables with a normal distribution; non-normal distributed values were compared using Mann–Whitney *U* test; χ^2 test was used to compare categorical variables. A *p*-value of <0.05 was used to define statistical significance. Univariate and when appropriate, multivariate analysis, were applied to assess the interaction between different parameters. SPSS version 11.0.1 was used for the statistical analysis (SPSS Inc, Chicago, IL, USA).

Although patients were matched for age, age was included as a covariant in the statistical analysis. The reason for this approach is that the covered range of age in the population is rather wide (13–60), introducing considerable age-induced variability for parameters varying with age, which may overpower the variation of parameters due to the pathology.

3. Results

3.1. Baseline characteristics

Baseline characteristics in patients and controls are presented in Table 1.

As expected, Marfan patients were significantly taller when compared to normal subjects and they were also slightly heavier, resulting in an increased BSA. Heart rate and blood pressure were comparable between both groups.

The aortic sinus was significantly larger in Marfan patients, when compared to controls (3.9 ± 0.6 cm vs. 2.9 ± 0.3 cm, $p<0.0001$). This difference remained significant after correction for BSA ($p<0.0001$).

A total of 5 patients and 1 control subject had mild, yet insignificant aortic insufficiency (pressure half-time > 600 ms). Four patients had mild mitral valve insufficiency, which was caused by mitral valve prolapse in 2 of them. None of the control subjects had mitral valve insufficiency.

Table 1
Baseline characteristics

	Marfan ($N=26$)	Control ($N=26$)	*P* value
Age (years)	32.0 ± 10.9	35.3 ± 12	0.3
Men/Women	12/14	12/14	1
Length (m)	1.83 ± 0.10	1.74 ± 0.11	0.001
Weight (kg)	75.4 ± 14.3	67.8 ± 13.5	0.06
BMI (kg/m^2)	22.5 ± 4.3	22.4 ± 3.0	0.9
BSA (m^2)	2.0 ± 0.2	1.8 ± 0.2	0.01
Heart rate (bpm)	62.6 ± 9.0	67.0 ± 10.1	0.11
Systolic blood pressure (mmHg)	113.8 ± 8.2	111.9 ± 10.4	0.5
Diastolic blood pressure (mmHg)	64.2 ± 10.1	63.4 ± 7.2	0.7
Mean arterial blood pressure (mmHg)	80.7 ± 8.2	79.6 ± 7.6	0.6
Aortic sinus diameter (cm)	3.9 ± 0.6	2.9 ± 0.3	<0.0001

Table 2
Systolic function parameters

	Marfan	Control	*P* value
MRI			
LVEDV (ml)	154.7 ± 41.3	133.4 ± 30.6	0.04
LVEDV/BSA (ml/m^2)	78.4 ± 18.8	73.1 ± 12.0	0.24
LVESV (ml)	71.1 ± 21.7	53.8 ± 15.1	0.001
LVESV/BSA (ml/m^2)	36.0 ± 9.5	29.5 ± 6.7	0.007
EF (%)	53.5 ± 9.0	59.6 ± 6.7	0.009
Echocardiography			
LVEDD (mm)	51.4 ± 6.8	47.1 ± 4.8	0.01
LVEDD/BSA (mm/m^2)	26.2 ± 3.1	26.3 ± 2.0	0.8
LVESD (mm)	33.4 ± 6.8	30.1 ± 5.9	0.17
LVESD/BSA (mm/m^2)	17.0 ± 3.4	17.2 ± 2.8	0.8
LV mass (g)	152.3 ± 55.5	112.0 ± 36.7	0.2[a]
S_m basal septum (cm/s)	5.2 ± 1.4	6.4 ± 1.3	0.001[b]
S_m basal lateral (cm/s)	6.0 ± 2.2	7.5 ± 2.3	0.04[b]
Average S_m (cm/s)	5.7 ± 1.4	7.0 ± 1.5	0.006[b]
Meridional wall stress (mmHg)	57.1 ± 21.5	57.6 ± 20.7	0.5[b]
LV mass/volume ratio (g/ml)	2.2 ± 0.8	2.2 ± 0.6	0.9

LVEDV and EDD: left ventricular end-diastolic volume and diameter; BSA: body surface area; LVESV and LVEDD: left ventricular end-systolic volume and diameter; EF: ejection fraction; S_m: peak systolic myocardial velocity.
[a] After correction for BSA.
[b] After correction for age, BSA and heart rate.

3.2. Left ventricular systolic function parameters

Left ventricular systolic function parameters assessed with conventional echo, color Doppler TDI and MRI are presented in Table 2.

Left ventricular diameters assessed with echocardiography from the parasternal long axis view were not significantly different between both groups. In contrast, MRI assessment of left ventricular volumes and EF showed

Table 3
Diastolic function parameters

	Marfan	Control	*P* value
Peak *E* (cm/s)	74.0 ± 14.7	82.2 ± 15.0	0.18
Peak *A* (cm/s)	49.9 ± 11.3	57.9 ± 14.9	0.02
E/*A* ratio	1.5 ± 0.4	1.6 ± 0.4	0.7
DT of the *E* wave (ms)	171.8 ± 40.5	141.1 ± 36.2	0.001
E_a (cm/s)	9.6 ± 2.5	11.9 ± 3.3	0.003
A_a (cm/s)	6.9 ± 2.3	8.2 ± 2.2	0.023
E/E_a	8.2 ± 2.3	7.1 ± 1.4	0.06
E_a/S_m basal septal	1.9 ± 0.6	1.9 ± 0.5	0.8

E: early filling wave velocity; *A*: atrial contraction wave velocity; DT: deceleration time; E_a: TDI peak early diastolic velocity at the mitral valve annulus; A_a: TDI peak late diastolic velocity at the mitral valve annulus.

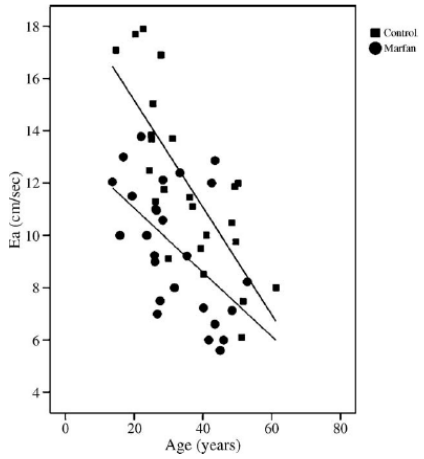


Fig. 1. Relationship between E_a and age in both groups.

a significant increase in end-systolic volume corrected for BSA ($p=0.007$) and a decrease in EF ($p=0.009$) in Marfan patients.

Peak S_m assessed with color Doppler TDI at the lateral and septal border of the left ventricular wall was significantly lower in Marfan patients. In a multivariate analysis including age, BSA, mitral valve insufficiency and aortic insufficiency as covariates, the presence of Marfan syndrome appeared as the only factor independently associated with EF, left ventricular end-systolic volume and peak S_m.

3.3. Left ventricular diastolic function parameters

Diastolic parameters measured with conventional echocardiography and with pulsed tissue Doppler are described in Table 3.

Marfan patients had a significantly increased DT_E, suggesting impaired relaxation. This finding was not influenced by heart rate. Both peak early (E_a) and late (A_a) diastolic velocities at the mitral valve annulus were decreased when compared to normal subjects ($p=0.003$ and 0.023, respectively), indicating reduced longitudinal movement of the left ventricle. Multivariate analysis including age, aortic insufficiency, mitral valve insufficiency and ejection fraction showed that age and the presence of Marfan syndrome were the only independent determinants of E_a.

There was a linear relationship between age and E_a in both study groups. E_a was lower in Marfan patients at all age groups, but this difference was most pronounced in the younger age group (Fig. 1).

A summary of the main results is displayed in Fig. 2.

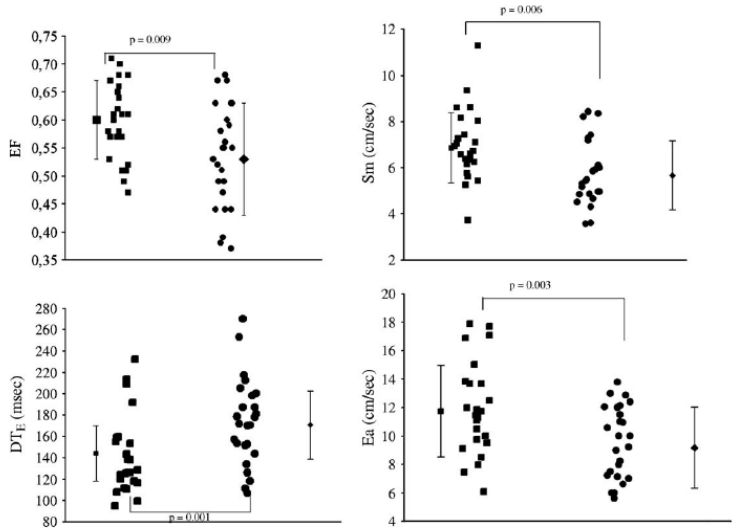


Fig. 2. Overview of the main findings in this study. EF: ejection fraction (%); S_m: average peak systolic velocity (septal and lateral corner); DT_E: deceleration time of the E wave; E_a: early mitral annulus velocity (septal); solid squares: controls; solid circles; Marfan patients.

4. Discussion

This study demonstrated mild, though significant primary impairment of LV function in patients with MFS, including both systolic and diastolic dysfunction. Left ventricular dysfunction in the patient group under study here could not be attributed to underlying valvular heart disease.

Left ventricular *diastolic function* in MFS has been evaluated in a few studies. A first study conducted with MRI and echocardiography in children demonstrated impaired LV diastolic function with an increased deceleration time and isovolumic relaxation time ascribed to weakened elastic recoil [8]. A subsequent echocardiographic study showed an unusual pattern of transmitral diastolic flow in which a decreased ventricular compliance (decreased deceleration time) and reduced myocardial relaxation (increased isovolumic relaxation time) coexist [9]. Our study demonstrated a prolongation of the early filling phase of the left ventricle. The reduced tissue Doppler velocities of the early filling wave at the mitral annulus (E_a) indicate that myocardial tissue relaxation is impaired.

Studies evaluating *LV systolic* function in Marfan syndrome are even rarer. In a recent study [6], we were able to demonstrate increased LVEDD and LVEDS in a subset of Marfan patients, results which were in line with a study by Chatrath et al. [7]. In contrast to these previous trials in which the obtained values in Marfan patients were compared with normal values from literature, we compared our patient group with a matched control group and we used MRI and TDI for the evaluation of LV function. Our study equally could not demonstrate clear differences in left ventricular diameters assessed with conventional echocardiography. However, the strength of this study lies in the fact that additional techniques to assess left ventricular function were applied. Both MRI and TDI did show left ventricular abnormalities in the MFS group, indicating that conventional echocardiography may not be sensitive enough to detect subtle abnormalities. Our results indicate that both MRI and TDI are more appropriate techniques for the correct evaluation of LV function in Marfan patients. In view of the lower costs and wider availability of TDI, which has become an established part of routine echocardiography in clinical practice, TDI should be recommended. The findings from the TDI study are in line with the observations in several forms of inherited cardiomyopathies, in which TDI appeared to be a more sensitive technique for the evaluation of myocardial dysfunction [21].

The ratio of E_a to peak S_m at the basal septum was comparable between both groups, indicating a concordant reduction in contraction and relaxation velocities, with a preserved contraction/relaxation coupling in patients with Marfan syndrome. As a consequence, the observed diastolic dysfunction is most likely secondary to primary contractile dysfunction. Further evidence for primary contractile dysfunction comes from the observation that meridional wall stress was comparable between both groups, meaning that afterload is not altered in Marfan patients and indicating that the observed differences in ejection fraction are likely due to impaired contractility.

We observed that the differences in both systolic and diastolic parameters were most pronounced in the younger age group. We assume that this is to be attributed to the design of our study in which we excluded those subjects with significant valvular disease. As these valvular abnormalities tend to increase with age, it is probable that we have excluded more severely affected older subjects from our study.

The pathogenesis of left ventricular dysfunction in Marfan patients is not yet completely understood. Structural and/or functional abnormalities of the fibrillin-1 protein, as a result of the underlying fibrillin-1 mutation in Marfan patients are likely to be responsible for the observed myocardial dysfunction.

Fibrillin-1 is one of the major constituents of the 10–12 nm microfibrils composing the extracellular matrix. These are located primarily around the periphery of the amorphous elastin component of the elastic fibers. Elastin plays an important role in mediating elastic recoil [13]. Microfibrils appear to subserve several global functions including scaffolding for tropoelastin deposition and elastic fiber formation and anchoring endothelial and epithelial cells to elastic fibers. Microfibrils are extensible themselves and may contribute to the mechanical properties of mature elastic tissues by means of load redistribution between individual elastic fibres [14].

Immunohistochemical studies of the myocardium with antibodies directed to fibrillin-1 demonstrated that microfibrils form myofiber–collagen fiber linkages at sites where the power of myocardial contraction is being transmitted to the extracellular connective tissue framework in the myocardium [15]. Mutations in the FBN1 gene might cause structural and/or functional abnormalities in the microfibrils leading to impairment of myocardial contraction. Another pathway through which fibrillin-1 likely interferes with myocardial function is through the complex transforming growth factor-β (TGF-β) signaling process. Recent studies have shown that the amount of fibrillin-1 in the matrix may be one determinant of the reservoir for TGF-β [16]. Elevated TGF-β1 gene expression has been measured in ventricular biopsies from hypertrophic and dilated cardiomyopathy patients [17,18]; the Leu^{10}→Pro polymorphism in the TGF-β1 gene is associated with end stage heart failure in dilated cardiomyopathy patients [19]. Further studies are needed to elucidate this complex interaction of fibrillin-1 with cytokines in patients with Marfan syndrome. It would also be interesting to study the possible role of fibrillin 1 in the pathogenesis of cardiomyopathies.

It is to be acknowledged that there are some limitations related to this study. Firstly, the study groups are relatively small. Secondly, it is always difficult to assess "normality" of normal control subjects. However, our control values for

left ventricular volumes and TDI measurements were found to be comparable with available data from literature [20]. An important aspect is the wash-out period for beta-blockers, which was 3 days in our study. One could argue that some residual effect might have been present and/or that the observed differences might be attributable to chronic beta blockade. We cannot rule this out for some of the observed differences, but seems unlikely that a reduced EF would be a result of chronic beta blocking therapy. On the other hand, in many other studies, beta blocker therapy is not interrupted at all [21] or stopped for a shorter period [22]. For ethical reasons, we considered withdrawal of beta-blockers for a longer period in this patient population unwise.

In conclusion, we demonstrated abnormalities in systolic and diastolic left ventricular function in patients with MFS using a combination of MRI and tissue Doppler imaging. Follow-up studies are needed to evaluate the evolution of these left ventricular abnormalities over time. With respect to cardiovascular treatment in MFS patients, these results could suggest a possible role for drugs supporting myocardial function, such as ACE inhibitors.

Acknowledgements

We are indebted to Dirk De Bacquer for his advice on the statistical analysis.

References

[1] Pyeritz RE, McKusick VA. The Marfan syndrome: diagnosis and management. N Engl J Med 1979;300(14):772–7.
[2] De Paepe A, Devereux RB, Dietz HC, Hennekam RC, Pyeritz RE. Revised diagnostic criteria for the Marfan syndrome. Am J Med Genet 1996;62(4):417–26.
[3] Gray JR, Davies SJ. Marfan syndrome. J Med Genet 1996;33(5): 403–8.
[4] Murdoch JL, Walker BA, Halpern BL, Kuzma JW, McKusick VA. Life expectancy and causes of death in the Marfan syndrome. N Engl J Med 1972;286(15):804–8.
[5] Pyeritz RE, Dietz HC. Marfan syndrome and other microfibrillar disorders. In: Royce PM, Steinmann B, editors. Connective tissue and its heritable disorders: molecular, genetic and medical aspects. New York: Wiley-Liss; 2002. pp. 585–626.
[6] Meijboom LJ, Timmermans J, van Tintelen JP, et al. Evaluation of left ventricular dimensions and function in Marfan's syndrome without significant valvular regurgitation. Am J Cardiol 2005;95(6): 795–7.
[7] Chatrath R, Beauchesne LM, Connolly HM, Michels VV, Driscoll DJ. Left ventricular function in the Marfan syndrome without significant valvular regurgitation. Am J Cardiol 2003;91(7):914–6.
[8] Savolainen A, Nisula L, Keto P, et al. Left ventricular function in children with the Marfan syndrome. Eur Heart J 1994;15(5): 625–30.
[9] Porciani MC, Giurlani L, Chelucci A, et al. Diastolic subclinical primary alterations in Marfan syndrome and Marfan-related disorders. Clin Cardiol 2002;25(9):416–20.
[10] Oh JK, Seward JB, Tajik AJ. The echo manual. second ed . Mayo Foundation.
[11] Devereux RB, Alonso DR, Lutas EM, et al. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. Am J Cardiol 1986;57(6):450–8.
[12] Falsetti HL, Mates RE, Grant C, Greene DG, Bunnell IL. Left ventricular wall stress calculated from one-plane cineangiography. Circ Res 1970;26(1):71–83.
[13] Rosenbloom J, Abrams WR, Mecham R. Extracellular matrix 4: the elastic fiber. Faseb J 1993;7(13):1208–18.
[14] Robinson PN, Godfrey M. The molecular genetics of Marfan syndrome and related microfibrillopathies. J Med Genet 2000;37(1):9–25.
[15] Vracko R, Thorning D, Frederickson RG. Spatial arrangements of microfibrils in myocardial scars: application of antibody to fibrillin. J Mol Cell Cardiol 1990;22(7):749–57.
[16] Byers PH. Determination of the molecular basis of Marfan syndrome: a growth industry. J Clin Invest 2004;114(2):161–3.
[17] Li RK, Li G, Mickle DA, et al. Overexpression of transforming growth factor-beta1 and insulin-like growth factor-I in patients with idiopathic hypertrophic cardiomyopathy. Circulation 1997;96(3): 874–81.
[18] Pauschinger M, Knopf D, Petschauer S, et al. Dilated cardiomyopathy is associated with significant changes in collagen type I/III ratio. Circulation 1999;99(21):2750–6.
[19] Holweg CT, Baan CC, Niesters HG, et al. TGF-beta1 gene polymorphisms in patients with end-stage heart failure. J Heart Lung Transplant 2001;20(9):979–84.
[20] Nikitin NP, Witte KK, Thackray SD, de Silva R, Clark AL, Cleland JG. Longitudinal ventricular function: normal values of atrioventricular annular and myocardial velocities measured with quantitative two-dimensional color Doppler tissue imaging. J Am Soc Echocardiogr 2003;16(9):906–21.
[21] Wilson DG, Bellamy MF, Ramsey MW, et al. Endothelial function in Marfan syndrome: selective impairment of flow-mediated vasodilation. Circulation 1999;99(7):909–15.
[22] Jondeau G, Boutouyrie P, Lacolley P, et al. Central pulse pressure is a major determinant of ascending aorta dilation in Marfan syndrome. Circulation 1999;99(20):2677–81.

IV.3 Chapter 3: Investigation of elastic properties of the aorta in patients with Marfan Syndrome

Aortic reflection coefficients and their association with global indices of wave reflection in healthy controls and patients with Marfan disease.

Patrick Segers*, Julie De Backer*, Dan Devos, Stein-Inge Rabben, Thieryy Gillebert, Luc Van Bortel, Johan De Sutter, Anne De Paepe, Pascal Verdonck.
Am J Physiol Heart Cir Physiol 2006 Jun;290(6):H2385-92
*both contributed equally to this work

In this paper, we used a fully non-invasive protocol for the evaluation of aortic stiffness and local and global indices of wave reflection. We compared 26 MFS patients with 26 age- and sex matched control subjects. We used a combination of ultrasound and MRI techniques.

First, we assessed aortic stiffness through calculation of the pulse wave velocity (PWV) with MRI. Pulse wave velocity was significantly higher in MFS patients when compared to normal control subjects (4.86±1.10 and 5.19±1.00 m/s in normal controls versus MFS patients respectively, $p<0.05$).

Next, we studied aspects of local and global wave reflection in the aorta of MFS patients. Early return of reflected waves boosts systolic pressure and presents an extra load for the heart and the central vessels. As such, these wave reflections are regarded as one of the important determinants of central blood pressure and can contribute to the development of aortic dilatation in MFS. Local wave reflection, assessed at four different levels in the aorta through calculation of local characteristic impedance, was not different between patients and controls, except at the level of the diaphragm where it was lower in MFS patients. Global wave reflection as quantified by the augmentation index (AIx) was not higher in MFS patients than in a control population. Nevertheless, wave reflection appeared to be enhanced in young MFS patients. Our data demonstrated that the major determinants of AIx were PWV and the effective length of the arterial system.

Am J Physiol Heart Circ Physiol 290: H2385–H2392, 2006.
First published January 6, 2006; doi:10.1152/ajpheart.01207.2005.

Aortic reflection coefficients and their association with global indexes of wave reflection in healthy controls and patients with Marfan's syndrome

P. Segers,[1,*] J. De Backer,[2,3,*] D. Devos,[4] S. I. Rabben,[5] T. C. Gillebert,[3] L. M. Van Bortel,[6] J. De Sutter,[3] A. De Paepe,[2] and P. R. Verdonck[1]

[1]*Cardiovascular Mechanics and Biofluid Dynamics, Institute of Biomedical Technology, Ghent University; Departments of* [2]*Medical Genetics,* [3]*Cardiovascular Medicine, and* [4]*Medical Imaging, and* [6]*Heymans Institute of Pharmacology, Ghent University Hospital, Ghent, Belgium; and* [5]*Institute for Surgical Research, Rikshospitalet University Hospital, Oslo, Norway*

Submitted 15 November 2005; accepted in final form 2 January 2006

Segers, P., J. De Backer, D. Devos, S. I. Rabben, T. C. Gillebert, L. M. Van Bortel, J. De Sutter, A. De Paepe, and P. R. Verdonck. Aortic reflection coefficients and their association with global indexes of wave reflection in healthy controls and patients with Marfan's syndrome. *Am J Physiol Heart Circ Physiol* 290: H2385–H2392, 2006. First published January 6, 2006; doi:10.1152/ajpheart.01207.2005.—Early return of reflected pressure waves increases the load on central arteries and may increase the risk of aortic rupture in patients with Marfan's syndrome (MFS). To assess whether wave reflection is elevated in MFS, we used ultrasound and MRI to measure central pressure and flow waveforms in 26 patients (13–54 yr of age) and 26 age- and gender-matched controls. Aortic systolic and diastolic cross-sectional areas were measured at the ascending and descending aorta (AA and DA), diaphragm (DIA), and lower abdominal aorta (AB). From these measurements, local characteristic impedance ($Z_{o\text{-}xx}$) and local reflection coefficients ($\Gamma_{xx\text{-}yy}$) were calculated. Calculated global wave reflection indexes were the augmentation index (AIx) and the ratio of backward to forward pressure wave (P_b/P_f). The aorta was wider in MFS patients at AA ($P < 0.01$) and DA ($P < 0.01$). Aortic pulse wave velocity was 42 cm/s higher in MFS patients ($P < 0.05$). $Z_{o\text{-}xx}$ was not different between groups, except at DA, where it was lower in MFS patients. In controls, $\Gamma_{AA\text{-}DA}$ was 0.31 ± 0.08, $\Gamma_{DA\text{-}DIA}$ was 0.00 ± 0.11, and $\Gamma_{DIA\text{-}AB}$ was 0.31 ± 0.16. Mean values of $\Gamma_{xx\text{-}yy}$ were not different between MFS patients and controls. In controls, aging diminished $\Gamma_{AA\text{-}DA}$ but increased $\Gamma_{DIA\text{-}AB}$. Clear age-related patterns were absent in MFS patients. AIx or P_b/P_f was not higher in MFS patients than in controls. There were indications for enhanced wave reflection in young MFS patients. Our data demonstrated that the major determinants of AIx were pulse wave velocity and the effective length of the arterial system and, to a lesser degree, HR and P_b/P_f.

magnetic resonance imaging; augmentation index

THE PROPAGATION AND REFLECTION of pressure and flow waves along the arterial tree have been the subject of early fundamental biofluid mechanical research, but it was only in the early 1980s that the pathophysiological effect of pressure wave reflection was most clearly demonstrated by Murgo et al. (13). Early return of reflected waves boosts systolic pressure and presents an extra load for the heart and central vessels (15, 22, 24).

In the past few years, the study of arterial wave reflection has also reached the medical/clinical community, mainly because of the effort of Kelly and colleagues (9), who developed the "augmentation index" (AIx). This easy-to-use index can be derived from central pressure (or diameter) waveforms and formally quantifies the wave contour classification scheme of Murgo et al. (13) and O'Rourke and co-workers (16). Because AIx is a composite index, its interpretation is not always straightforward. It is dependent not only on the magnitude of wave reflection (the reflection coefficient), but also on the time delay between the forward and the reflected wave. As such, AIx is also determined by body stature, stiffness of the aorta [aortic pulse wave velocity (PWV)], and even heart rate (HR). Wave reflection, however, is still not fully understood, especially with respect to the origin of the reflected waves. In this in vivo study, we assessed local and global reflection (coefficients) in normal subjects (controls) as well as in patients with Marfan's syndrome (MFS), a genetically determined connective tissue disorder primarily affecting the (proximal) aorta. In addition to the effects of age, which spanned four decades in both groups, several aspects of the disease potentially alter the contributions of arterial wave reflection: *1*) Elevated aortic PWV due to global aortic stiffening, which would favor the early return of pressure waves from the periphery, has been reported in MFS patients (4, 5, 8). *2*) MFS primarily affects the proximal part of the aorta (6) and may change the gradual proximal-to-distal evolution of the mechanical properties of the aorta and give rise to reflections originating from an "impedance mismatch" along the aorta. *3*) MFS patients are, in general, taller than the normal population (one of the visual landmarks of the disease), and their height affects the distance to reflection sites. *4*) It has been suggested that the global wave reflection coefficient may be elevated in MFS patients (25).

Local reflection coefficients along the aorta will be assessed through calculation of changes in characteristic impedance (Z_o) along the vessel, with Z_o estimated from MRI recordings of the systolic and diastolic cross-sectional area at different levels along the aorta. Global reflection will be estimated via AIx and linear wave separation analysis. The ratio of the amplitude of the backward (P_b) to the forward (P_f) wave (P_b/P_f) will be used as an estimate of the global reflection coefficient (24). The in vivo data should thus provide local aortic reflection coefficients and have the potential to reveal a possible relation between local reflection along the aorta and the global wave reflection indexes, such as AIx and P_b/P_f.

* P. Segers and J. De Backer contributed equally to this work.

Address for reprint requests and other correspondence: P. Segers, Cardiovascular Mechanics and Biofluid Dynamics, Hydraulics Laboratory, Institute Biomedical Technology, Ghent Univ., Sint-Pietersnieuwstraat 41, 9000 Ghent, Belgium (e-mail: patrick.segers@ugent.be).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Table 1. *Population characteristics and general hemodynamic data*

	Control	MFS	*P*
M/F	12/14	12/14	1.00
Age, yr	35.5±11.8 (14–60)	32.7±11.5 (13–54)	0.37
Height, m	1.73±0.11 (1.52–1.93)	1.83±0.10 (1.72–2.10)	0.001
Weight, kg	67.0±13.0 (50–95)	75.4±14.3 (47–105)	0.03
BSA, m^2	1.80±0.21 (1.53–2.16)	1.98±0.20 (1.56–2.36)	0.003
DBP, mmHg	62.6±8.2 (48–80)	61.6±8.6 (47–80)	0.68
MAP, mmHg	84.4±9.4 (65.6–103.6)	84.8±9.35 (68.8–107.5)	0.87
SBP_{ca}, mmHg	104.8±11.8 (83.3–127.5)	106.4±11.7 (87.8–142.8)	0.64
HR, beats/min	67.0±10.0 (52.5–90.4)	61.8±10.0 (41.2–78.9)	0.07
SV, ml	78.9±22.4 (48.8–128.0)	82.0±27.4 (33.3–149.9)	0.67
CO, l/min	5.2±1.2 (3.0–7.4)	4.9±1.4 (2.4–7.8)	0.51

Values are means ± SD, with range in parentheses; $n = 26$ in each group. MFS, Marfan's syndrome; M, male; F, female; BSA, body surface area; DBP, diastolic blood pressure; SBP_{ca}, carotid artery, systolic blood pressure; MAP, mean arterial pressure; HR, heart rate; SV, stroke volume; CO, cardiac output. *P* values are results from *t*-test analysis of control vs. MFS patients.

MATERIALS AND METHODS

The study population consisted of 26 patients with confirmed MFS (13–54 yr of age) and 26 age- and gender-matched controls (Table 1). All MFS patients and controls underwent a 1-day measurement protocol, including MRI and echocardiography for the assessment of systolic and diastolic dimensions of the aorta at different levels, as well as central pressure and flow waveforms. This study was approved by the ethical committee of the Ghent University Hospital, and all subjects gave informed consent to participate in the study.

MRI: PWV, aortic dimensions, and local reflection coefficients. All MFS patients and controls were scanned on a 1.5-T magnetic resonance system (Magnetom Symphony, Siemens, Erlangen, Germany) with ECG gating. Aortic systolic and end-diastolic cross-sectional areas [$A_{xx(s)}$ and $A_{xx(d)}$] were measured using trueFISP images (fast imaging with steady-state precession, temporal resolution of 25 ms, and spatial resolution of 1.33 mm/pixel in *x* and y direction) obtained at four levels (indicated by *xx*) along the aorta: ascending and descending thoracic aortas (AA and DA), thoracic-abdominal aorta near the diaphragm (DIA), and lower abdominal aorta (AB; Fig. 1). The distance between the different aortic levels was assessed.

Through-plane phase-contrast images were obtained at these levels to assess the flow curves, which were calculated with Siemens Mean Curve software. The curves were interpolated to obtain a temporal resolution of 1 ms, and the time to half-peak (i.e., the time between the R-top of the ECG and the moment when flow reaches half of its peak value) was calculated. With time and distance traveled by the propagating flow front known at four locations, PWV (cm/s) was calculated as the slope of the regression line through these data points (Matlab, Mathworks, Natick, MA; Fig. 1).

Local reflection, arising from impedance mismatch between levels *xx* and *yy*, was quantified using local wave reflection coefficients ($\Gamma_{xx\text{-}yy}$) calculated as

$$\Gamma_{xx-yy} = \frac{Z_{0-yy} - Z_{0-xx}}{Z_{0-yy} + Z_{0-xx}} \quad (1)$$

where $Z_{0\text{-}xx}$ is the characteristic impedance at level *xx*, approximated as

$$Z_{0-xx} = \sqrt{\frac{\rho}{A_{xx}\left(\frac{SBP - DBP}{A_{xx(s)} - A_{xx(d)}}\right)}} \quad (2)$$

where ρ is the density of blood (assumed 1,030 kg/m^3) and A_{xx} is the average value of $A_{xx(s)}$ and $A_{xx(d)}$. SBP and DBP represent central systolic and diastolic blood pressure, respectively (see below).

Assessing central blood pressure waveforms. Central blood pressure waveforms were obtained via calibration of the common carotid artery diameter distension waveforms (Fig. 2) (17). With the subject in the supine position, a sequence of common carotid artery diameter distension traces typically containing three to five complexes was measured with a commercially available ultrasonographic system (Vivid 7, GE Vingmed Ultrasound, Horten, Norway) and a 12-MHz vascular probe (model 12L). The trace was averaged to obtain one representative waveform, which was subsequently transformed into a carotid artery pressure waveform (20), which was further used as a

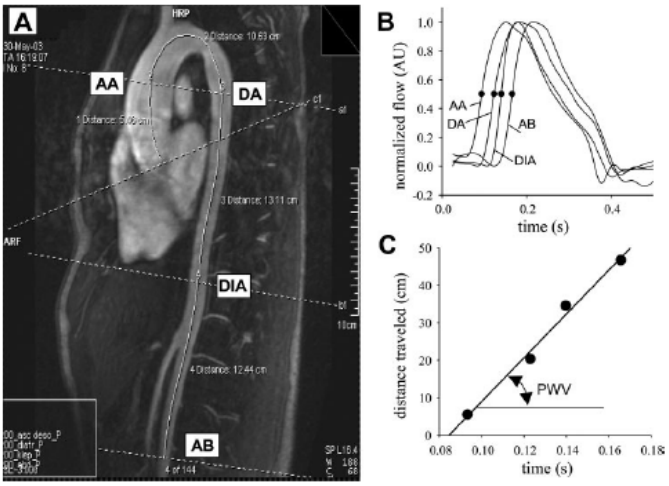


Fig. 1. *A*: MRI of the aorta used to assess distance between the 4 aortic measuring locations: ascending aorta (AA), descending aorta (DA), diaphragm (DIA), and lower abdominal aorta (AB). *B*: measured normalized flows at AA, DA, DIA, and AB, with indication of the moment when half-peak flow is reached. AU, arbitrary units. *C*: plot of distance traveled by the propagating flow front as a function of this time; slope of regression line yields aortic pulse wave velocity (PWV, cm/s).

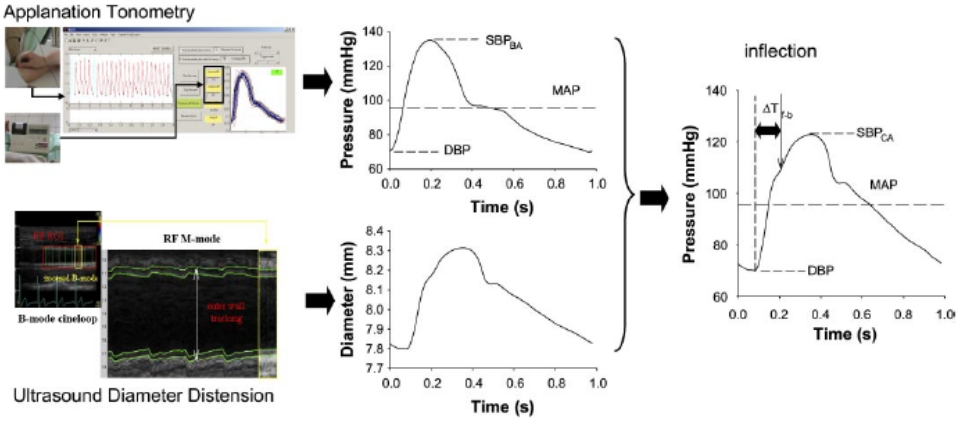


Fig. 2. Assessment of central waveforms. Applanation tonometry yields brachial arterial pressure waveform, which is calibrated using sphygmomanometer brachial systolic (SBP_{BA}) and diastolic (DBP) blood pressure. Averaging this curve gives mean arterial pressure (MAP). Diameter distension curves, measured using ultrasound, yield carotid diameter distension waveform, which is used as a surrogate for carotid pressure waveform. Waveform is calibrated using DBP and MAP. Peak value of the calibrated waveform yields carotid systolic pressure (SBP_{CA}). Calibrated curve clearly shows an inflection point, which is used to assess time delay (ΔT_{f-b}) between the onset of pressure rise and inflection point. RF, radio frequency.

surrogate of the central pressure waveform (P_{ao}). To do so, it was assumed that the relation between pressure and diameter was linear and that DBP and mean arterial pressure (MAP) were similar at the brachial and carotid arteries. Central SBP was taken as the maximum value of P_{ao}. MAP was assessed following a procedure recently described by Verbeke et al. (21) that includes three steps: *1*) applanation tonometry at the brachial artery to obtain the brachial artery waveform, *2*) calibration of this waveform using DBP and SBP as measured with a brachial cuff sphygmomanometer, and *3*) averaging of the scaled brachial artery pressure wave to yield MAP (21).

Assessing central flow waveforms. Blood flow velocities were acquired in the left ventricular outflow tract using a pulsed-wave Doppler 3.5-MHz probe in the apical five-chamber view. Images were stored as DICOM files for offline analysis, where contours were semiautomatically traced with a dedicated software interface written in Matlab. An ensemble average was constructed of at least three cycles, and the average curve was scaled, so that the area under the curve matched stroke volume (SV) as determined from MRI: left ventricular volumes were acquired with an ECG-triggered trueFISP sequence, and SV was calculated as the difference between end-diastolic and end-systolic volume. We judged this approach to be the most accurate, because left ventricular outflow tract diameters were difficult to assess accurately in MFS patients with dilated aortic roots. The aortic flow waveform is further indicated as $\dot{Q}_{ao}$. Cardiac output was obtained as the product of SV and HR. It was verified that HR was similar (±5 beats/min) during MRI and ultrasound measurements, which was the case in all subjects.

AIx and distance to the apparent reflection site. AIx is calculated as

$$AIx = 100\frac{P_2 - DBP}{P_1 - DBP} \quad (3)$$

where P_1 and P_2 represent SBP, i.e., the pressure associated with an inflection point visually identified on P_{ao} (Fig. 2, *right*). The pressure occurring first is labeled P_1. AIx <100% indicates arrival of the pressure wave in late systole, and AIx >100% indicates arrival in early systole. We also measured the time delay between the foot of P_{ao} and the moment of the inflection point (ΔT_{f-b}), which is associated with the time needed for a wave to travel from the ascending aorta to its apparent reflection site [effective length of the arterial system (Δx)], calculated as

$$\Delta x = \frac{PWV(\Delta T_{f-b})}{2} \quad (4)$$

with PWV assessed by MRI (see below; Fig. 2).

Linear wave separation analysis: P_b/P_f. As demonstrated by Westerhof et al. (23), the pressure wave is composed of a forward (P_f) and a reflected or backward (P_b) traveling component, which can be separated from each other provided that P_{ao} and $\dot{Q}_{ao}$, as well as Z_o, are known

$$P_f = \frac{(P_{ao} + Z_o\dot{Q}_{ao})}{2};\ P_b = \frac{(P_{ao} - Z_o\dot{Q}_{ao})}{2} \quad (5)$$

One can then define the global wave reflection coefficient as P_b/P_f. Z_o was estimated as the average value of the modulus of the high-frequency components of input impedance (12, 14).

Statistical analysis. Values are means ± SD. Population means were compared using Student's *t*-test. To study the evolution of parameters with age, data were organized in tertiles of age (≤27, >27 to ≤40, and >40 yr). The relation between parameters was studied using Pearson's correlation and linear regression analysis. When appropriate, the differences between MFS patients and controls were studied using ANOVA with age tertile and disease as fixed factors. All analyses were performed in SPSS (version 11.5, SPSS, Chicago, IL).

RESULTS

The MFS patients were taller, with greater body weight and body surface area than controls (Table 1). There was no difference between MFS patients and controls for age, body mass index, brachial and central blood pressure, HR, SV, and cardiac output. General hemodynamic data and patient characteristics are summarized in Table 1. Mean ages were 22.7 ±

Table 2. *Aortic cross-sectional area in systole and diastole, local characteristic impedance along the aorta, and local reflection coefficients*

	$A_{xx(s)}$, cm²	$A_{xx(d)}$, cm²	$Z_{0\text{-}xx}$, mmHg·ml⁻¹·s	$\Gamma_{xx\text{-}yy}$
AA				
Control	6.34±1.53	4.89±1.32	0.065±0.019	
MFS	7.79±2.24†	6.30±2.13†	0.059±0.016	
DA				
Control	3.44±0.95	2.77±0.85	0.127±0.030	
MFS	4.42±0.90†	3.57±0.85*	0.099±0.023*	
DIA				
Control	3.11±1.44	2.35±1.34	0.127±0.030	
MFS	3.62±0.83	2.78±0.76	0.111±0.029	
AB				
Control	1.84±0.91	1.43±0.86	0.264±0.111	
MFS	2.19±0.65	1.77±0.53	0.228±0.098	
AA-DA				
Control				0.31±0.08
MFS				0.27±0.11
DA-DIA				
Control				0.00±0.11
MFS				0.05±0.08
DIA-AB				
Control				0.31±0.16
MFS				0.30±0.16

Values are means ± SD. $A_{xx(s)}$ and $A_{xx(d)}$, cross-sectional area in systole and diastole; $Z_{0\text{-}xx}$, local characteristic impedance; $\Gamma_{xx\text{-}yy}$, local reflection coefficient; AA and DA, ascending and descending aorta; DIA, thoracic abdominal aorta near the diaphragm; AB, lower abdominal aorta. *$P < 0.05$; †$P < 0.01$ vs. control.

4.6, 34.6 ± 4.7, and 49.5 ± 4.8 yr in *tertiles 1, 2,* and *3,* respectively.

Aortic dimensions, Z_0, and local reflection coefficients. Population-averaged cross-sectional area measured at four levels along the aorta are given in Table 2. On average, the aorta was significantly wider in MFS patients than in controls at the two most proximal measuring locations: AA ($P < 0.01$) and DA ($P < 0.01$). To better appreciate the evolution of aortic size with age, data are also plotted as a function of age in Fig. 3. Aortic cross-sectional area progressively increased with age in MFS patients and controls ($P < 0.01$) at all levels. For the ascending aorta, the progression of dilatation with age was higher in MFS patients than in controls ($P < 0.05$), leading to a significantly higher aortic cross-sectional area in *tertile 3* ($P < 0.05$).

Z_0 was similar at the ascending aorta and the two most distal locations and significantly lower in MFS patients for the descending aorta ($P < 0.05$; Table 2). For the lower abdominal aorta, the evolution of $Z_{0\text{-}AB}$ with age is significantly different between the two groups ($P < 0.01$), increasing with age in controls and decreasing in MFS patients (Fig. 3). The difference was statistically significant in *tertile 3* ($P < 0.05$).

Local reflection coefficients are displayed as a function of age in Fig. 3. In the control population, a positive reflection coefficient was found: $\Gamma_{AA\text{-}DA}$ was, on average, 0.31 ± 0.08. In the midaortic region, $\Gamma_{DA\text{-}DIA}$ was close to zero, whereas the most distal reflection coefficient, $\Gamma_{DIA\text{-}AB}$, was again positive (0.31 ± 0.16). When analyzed as a function of age, $\Gamma_{AA\text{-}DA}$ decreased with age ($P < 0.05$), whereas $\Gamma_{DIA\text{-}AB}$ increased with age ($P < 0.05$). For the MFS patients, a similar global pattern was observed, with a positive proximal and distal reflection coefficient and no reflection in the midaortic region. In contrast with the control population, no correlation with age was found in any segment. By simple *t*-test analysis, it was determined that there were no differences in $\Gamma_{xx\text{-}yy}$ between controls and MFS patients. With use of ANOVA, a marginal difference between controls and MFS patients was found for $\Gamma_{AA\text{-}DA}$ ($P = 0.049$), with a lower reflection coefficient in the MFS patients.

Global reflection: AIx and distance to reflection site. AIx was, on average, virtually identical in controls and MFS patients: 102.1 ± 15.0 vs. 103.3 ± 10.6% ($P = 0.75$). When the data were displayed as a function of age (Fig. 4*A*), AIx tended to be higher in MFS patients than in controls in *tertile 1* and lower in MFS patients than in controls in *tertile 3*, but the differences were not statistically significant. PWV was 486 ± 110 and 519 ± 100 cm/s in controls and MFS patients, respectively [$P = 0.27$ (not significant) by *t*-test]. ANOVA, however, indicated a significant offset, estimated to be 42 cm/s, between both groups ($P = 0.03$; Fig. 4*C*). ΔT_{f-b} was not different between controls and MFS patients (0.165 ± 0.033 vs. 0.170 ± 0.039 s, $P = 0.70$). Δx, on the other hand, was shorter in controls than in MFS patients: 38.7 ± 6.0 vs. 43.4 ± 9.3 cm ($P < 0.05$). Data split per tertile of age is shown in Fig. 4*D*.

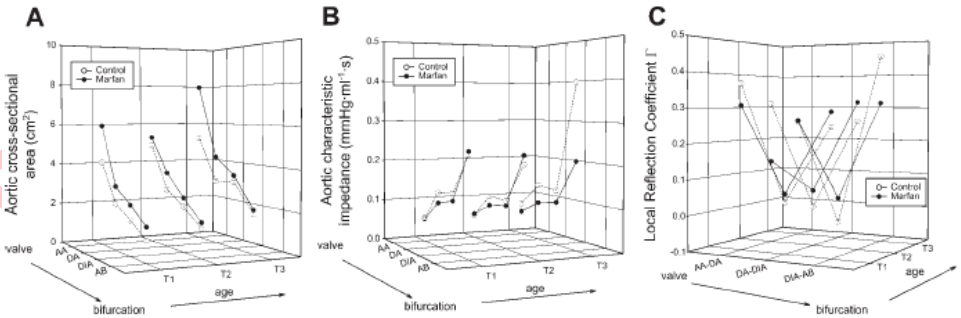


Fig. 3. Evolution in time [*tertiles 1–3* (T_1–T_3)] and along the aorta (AA, DA, DIA, and AB) of aortic cross section (*A*), characteristic impedance (*B*), and local reflection coefficients (*C*) in controls and patients with Marfan's syndrome.

Table 3. *Contribution of PWV, Δx, HR, and P_b/P_f to augmentation index as assessed by linear regression analysis*

	P	Standardized β-coeff	r^2
PWV, cm/s	0.000	0.791	0.373
Δx, cm	0.000	−0.635	0.694
HR, beats/min	0.001	−0.269	0.772
P_b/P_f	0.041	0.160	0.794

P, statistical significance of the parameter in the model; β, standardized coefficient, indicating relative importance of the parameter; r^2, predictive value of the model after additional inclusion of the parameter. PWV, pulse wave velocity; Δx, effective length of arterial tree; P_b/P_f, global reflection coefficient (i.e., ratio of backward to forward pressure wave).

whereas it increases with age in the distal aorta. Clear age-related patterns are absent in MFS patients. *3*) On average, global wave reflection, as quantified by AIx or P_b/P_f, is not higher in MFS patients than in controls. Nevertheless, there are indications for elevated wave reflection in young MFS patients. *4*) The major determinants of AIx are PWV and Δx and, to a lesser degree, HR and P_b/P_f.

Consistent with common knowledge (4, 6, 8), we found that the aorta was widened in MFS patients at the ascending and descending levels only. In controls and MFS patients, aortic enlargement with age was observed at all levels (Fig. 4). Enlargement appeared to progress at the same rate, except at the ascending aorta, where aortic dilatation occurred at a higher pace in MFS patients. In this study focusing on wave reflection, it is Z_0 and, more importantly, the changes in Z_0 that deserve attention, inasmuch as these may locally provoke wave reflection. Vessel caliber and stiffness affect Z_0 (see *Eq. 2*), and our data suggest that both effects counterbalance each other, with no net effect on Z_0 for the most proximal part of the aorta. This observation also supports the findings of Yin et al. (25), who derived aortic Z_0 from central pressure and flow and found Z_0 within the normal range in MFS patients. In this study, Z_0 was assessed from central pressure and flow as well as from changes in cross-sectional area along the aorta measured with MRI, which allowed us to study the aorta at different levels. It is generally accepted that, in the normal population, there is a gradual increase in impedance along the aorta [due to geometric and elastic taper (12, 14, 18)] but that impedance mismatch is most important in the periphery, where small arteries make the transition to arterioles and capillaries. For the descending aorta, the dilatation in the MFS patients seems to "overcompensate" for an increase in stiffness, with lower Z_0 in the MFS patients.

Despite the absence of significant differences in mean values of many calculated parameters, there are trends in the data when they are studied as a function of age (Fig. 3). In the ascending-descending section, there is a more pronounced gradient in Z_0 (increasing distally) in young controls than in older controls and MFS patients. The result is a positive reflection coefficient in the proximal aorta in young controls that decreases with age when the proximal aorta stiffens and the difference in Z_0 with adjacent sections becomes less. In the lower abdominal aorta, opposite changes were observed. In controls, the age-related increase in $Z_{0\text{-AB}}$ results in an increase in local positive reflection coefficients in the distal aorta. Again, we did not observe any age-related changes in the MFS patients. We speculate that interpatient differences in severity of the disease complicate the detection of eventual age-related patterns in MFS patients.

The literature on the subject of local reflection coefficients in the aorta in the general population, and in MFS patients in particular, is scarce. Ting et al. (19), analyzing apparent phase velocity, reported that local wave reflection can differ markedly along different regions in the aorta, with pronounced reflections in the ascending aorta and from just proximal to the renal arteries to the aortoiliac bifurcation, but not in the midthoracic region. This report (19) is consistent with data presented in Fig. 3, where $\Gamma_{\text{DA-DIA}}$ is indeed much lower than reflection coefficients in the other sections. Also, more rapid mechanical aging near the aortic bifurcation has been reported by Gillessen et al (2). Greenwald et al. (3) studied the effect of aging on the local reflection coefficient of the aortic bifurcation. They concluded that the progressive increase of lower aortic Z_0 (as also found in our study) decreases the impedance mismatch with the iliac arteries, decreasing the reflection coefficient from the bifurcation.

The following question remains: To what extent do local aortic properties and local reflection coefficients impact the global picture of arterial wave reflection, as quantified with indexes such as AIx? Our data seem to suggest that the effect, if any, is marginal. We found a (relatively weak) correlation between local and global indexes of wave reflection only between AIx and $\Gamma_{\text{DIA-AB}}$ ($r = 0.29$, $P < 0.05$). However, none of the local reflection coefficients entered the model for AIx in multiple linear regression analysis. The impact of local reflection properties along the aorta thus seems to be negligible compared with the other coexisting sources of wave reflection.

There is growing clinical interest in arterial wave reflection and AIx. Meijboom et al. (10) found elevated AIx in MFS patients, but in their MFS patients ($n = 4$) the aorta had been surgically repaired with a Dacron prosthesis, which may cause a drastic increase in PWV and compliance mismatch at the site of the anastomosis. Although it is recognized that AIx is a composite measure (14), no study has truly focused on dissecting the index into its determining factors. We could demonstrate that the main determinants of AIx were PWV and Δx and, to a lesser extent, reflection coefficient and HR (Table 3).

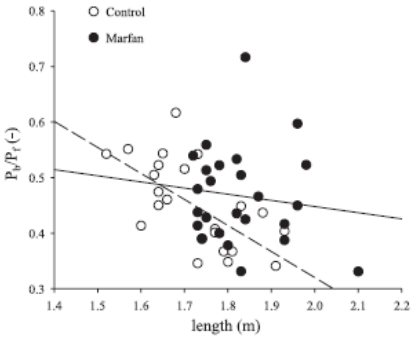


Fig. 6. Association between body length and global wave reflection coefficient (P_b/P_f) in control subjects and patients with Marfan's syndrome. Association was significant in control subjects only (dashed line; $r = -0.64$, $P = 0.001$).

This analysis also reveals why we could not demonstrate a (anticipated) difference in AIx between controls and MFS patients. The elevated PWV and the lower HR in the MFS patients are counterbalanced by the larger Δx in MFS patients. This is not necessarily a pathophysiological consequence of the disease but, rather, probably a reflection of the difference in body length between both groups. The taller stature of the MFS patients thus seems to have a protective effect in terms of wave reflection, delaying the return of the reflected wave. These mechanistic determinants of AIx also apply to other diseases affecting the functional properties of the aorta, such as atherosclerosis, hypertension, and diabetes (1), where we speculate that the hemodynamic burden caused by early wave reflection may be higher, particularly in smaller subjects. Also, different classes of blood pressure-lowering drugs may induce alterations in HR (β-blockers) and in the location of reflection sites (vasoactive drugs) and, hence, differentially affect the impact of wave reflection independent of the level of blood pressure decrease (7).

In this study, input impedance was calculated as an intermediate step in the linear wave separation analysis. The data confirm that, when studied in a global manner, the arterial system in MFS patients is not drastically different from that in controls, an observation that confirms the findings of Yin et al. (25) that were based on invasive data. We also want to draw attention to a finding concerning P_b/P_f. When P_b/P_f is plotted as a function of body length (Fig. 6), it immediately becomes clear that this factor is not independent of body size, as one might expect of a true reflection coefficient (in the control population, there was a significant inverse association between P_b/P_f and body length). We believe that the influence of length on P_b/P_f is explained by the fact that, in taller subjects, P_f and P_b travel longer distances. So, on arrival at the reflection site, the amplitude of P_b is smaller because of damping. In addition, the reflected wave needs to travel a longer distance back up the aorta and is more damped as well. As a result, the taller the subject is, the smaller the amplitude of the reflected component P_b and, thus, P_b/P_f.

In our opinion, one of the strong aspects of this study is its fully noninvasive character, which allows transfer of fundamental hemodynamic research from the experimental laboratory to the clinical setting. At the same time, it is acknowledged that this brings along methodological considerations and limitations that deserve some attention and comments. *1*) We scaled carotid diameter distension waveforms to assess carotid systolic pressure. Although the methodology of scaling diameter distension waveforms was found adequate (20), the relation between diameter and pressure is nonlinear (11), and we may have underestimated carotid systolic pressure, especially in the older subjects or in those with high blood pressure. Nevertheless, carotid arteries appear less affected by MFS, and MFS patients and controls were matched for age and blood pressure, so the eventual impact on the data should be the same for both groups. *2*) The local aortic reflection coefficients are derived from data measured at four distant, discrete locations, so that the aorta is implicitly approximated as a tube consisting of four segments, with discrete changes in mechanical properties. The computation of $Z_{0\text{-}xx}$ along the aorta is also based on carotid blood pressure, which was assumed to adequately represent blood pressure along the entire aorta. Pressure amplification is present along the aorta (14), and we may have underestimated pulse pressure (and $Z_{0\text{-}xx}$), especially at the most distal locations (AB). The extent to which this assumption has affected our findings is an open question. For the controls, our findings on the variation of local reflection coefficients along the aorta are consistent with the data from Ting et al. (19) and Gillessen et al. (2) (see above), suggesting that the effect is not important enough to affect these general findings. Our data do not allow us to directly make a similar statement for the MFS patients. Nevertheless, using estimated regional PWV in the abdominal aorta segment and the relation between $Z_{0\text{-}xx}$, cross-sectional area (A), and blood density (ρ; $Z_{0\text{-}xx} = \rho PWV/A$), the different evolution in $Z_{0\text{-}AB}$ between MFS patients and controls in *tertile 3* was confirmed (data not shown) using a method independent of blood pressure. *3*) As evident from our data and the discussion above, body size is an important confounding factor in the analysis of wave reflection. Although the different stature allowed us to enlarge the range of physiological parameters affecting wave reflection, ill-matched populations, in terms of body stature, may pose an important limitation in clinical studies. *4*) By inclusion of older MFS patients, it cannot be excluded that the population is biased, in the sense that older patients would have a "milder" manifestation of the disease, inasmuch as they have reached a higher age without surgery, despite the presence of the disease. *5*) For the wave separation analysis, we combined central flow with a surrogate for central pressure (and not the true central pressure). Although this methodology is quite commonly applied, it is acknowledged that this assumption may affect the accuracy of the wave separation and, hence, the value of P_b/P_f.

In conclusion, we have demonstrated that stiffening and dilatation of the proximal aorta in MFS patients do not lead to an increase in local aortic Z_0. In controls and MFS patients, local reflection coefficients are positive in the proximal and distal aorta and virtually zero in the midaortic region. In healthy subjects, the proximal reflection coefficient diminishes with age but increases in the distal region. We could not demonstrate an association between local reflection coefficients along the aorta and AIx, which is primarily determined by PWV and Δx.

GRANTS

This study was supported by Ghent University Grant BOF 011D4701 (to J. De Backer) and Fund for Scientific Research Belgium Grant FWO G029002 (to A. De Paepe). J. De Sutter is a senior clinical investigator of the Fund for Scientific Research-Flanders (FWO-Vlaanderen).

REFERENCES

1. **Boudoulas H, Toutouzas P, and Wooley C.** *Functional Abnormalities of the Aorta.* Armonk, NY: Futura, 1996.
2. **Gillessen T, Gillessen F, Sieberth H, Hanrath P, and Heintz B.** Age-related changes in the elastic properties of the aortic tree in normotensive patients: investigation by intravascular ultrasound. *Eur J Med Res* 1: 144–148, 1995.
3. **Greenwald SE, Carter AC, and Berry CL.** Effect of age on the in vitro reflection coefficient of the aortoiliac bifurcation in humans. *Circulation* 82: 114–123, 1990.
4. **Groenink M, de Roos A, Mulder BJ, Spaan JA, and van der Wall EE.** Changes in aortic distensibility and pulse wave velocity assessed with magnetic resonance imaging following β-blocker therapy in the Marfan syndrome. *Am J Cardiol* 82: 203–208, 1998.
5. **Groenink M, de Roos A, Mulder BJ, Verbeeten B Jr, Timmermans J, Zwinderman AH, Spaan JA, and van der Wall EE.** Biophysical properties of the normal-sized aorta in patients with Marfan syndrome: evaluation with MR flow mapping. *Radiology* 219: 535–540, 2001.

6. **Hirata K, Triposkiadis F, Sparks E, Bowen J, Wooley CF, and Boudoulas H.** The Marfan syndrome: abnormal aortic elastic properties. *J Am Coll Cardiol* 18: 57–63, 1991.
7. **Hirata K, Vlachopoulos C, Adji A, and O'Rourke MF.** Benefits from angiotensin-converting enzyme inhibitor "beyond blood pressure lowering": beyond blood pressure or beyond the brachial artery? *J Hypertens* 23: 551–556, 2005.
8. **Jeremy RW, Huang H, Hwa J, McCarron H, Hughes CF, and Richards JG.** Relation between age, arterial distensibility, and aortic dilatation in the Marfan syndrome. *Am J Cardiol* 74: 369–373, 1994.
9. **Kelly R, Hayward C, Avolio A, and O'Rourke M.** Noninvasive determination of age-related changes in the human arterial pulse. *Circulation* 80: 1652–1659, 1989.
10. **Meijboom LJ, Westerhof BE, Nollen GJ, Spaan JA, de Mol BA, Jacobs MJ, and Mulder BJ.** β-Blocking therapy in patients with the Marfan syndrome and entire aortic replacement. *Eur J Cardiothorac Surg* 26: 901–906, 2004.
11. **Meinders JM and Hoeks AP.** Simultaneous assessment of diameter and pressure waveforms in the carotid artery. *Ultrasound Med Biol* 30: 147–154, 2004.
12. **Milnor WR.** *Hemodynamics*. Baltimore, MD: Williams & Wilkins, 1989.
13. **Murgo JP, Westerhof N, Giolma JP, and Altobelli SA.** Aortic input impedance in normal man: relationship to pressure wave forms. *Circulation* 62: 105–116, 1980.
14. **Nichols W and O'Rourke M.** *McDonald's Blood Flow in Arteries. Theoretical, Experimental and Clinical Principles*. London: Arnold, 2005.
15. **O'Rourke MF.** Mechanical principles. Arterial stiffness and wave reflection. *Pathol Biol (Paris)* 47: 623–633, 1999.
16. **O'Rourke MF, Avolio A, and Qasem A.** Clinical assessment of wave reflection. *Hypertension* 42: e15–e16; author reply e15–e16, 2003.
17. **Segers P, Rabben SI, De Backer J, De Sutter J, Gillebert TC, Van Bortel L, and Verdonck P.** Functional analysis of the common carotid artery: relative distension differences over the vessel wall measured in vivo. *J Hypertens* 22: 973–981, 2004.
18. **Segers P and Verdonck P.** Role of tapering in aortic wave reflection: hydraulic and mathematical model study. *J Biomech* 33: 299–306, 2000.
19. **Ting CT, Chang MS, Wang SP, Chiang BN, and Yin FC.** Regional pulse wave velocities in hypertensive and normotensive humans. *Cardiovasc Res* 24: 865–872, 1990.
20. **Van Bortel LM, Balkestein EJ, van der Heijden-Spek JJ, Vanmolkot FH, Staessen JA, Kragten JA, Vredeveld JW, Safar ME, Struijker Boudier HA, and Hoeks AP.** Non-invasive assessment of local arterial pulse pressure: comparison of applanation tonometry and echo-tracking. *J Hypertens* 19: 1037–1044, 2001.
21. **Verbeke F, Segers P, Heireman S, Vanholder R, Verdonck P, and Van Bortel LM.** Noninvasive assessment of local pulse pressure: importance of brachial-to-radial pressure amplification. *Hypertension* 46: 244–248, 2005.
22. **Westerhof N and O'Rourke MF.** Haemodynamic basis for the development of left ventricular failure in systolic hypertension and for its logical therapy. *J Hypertens* 13: 943–952, 1995.
23. **Westerhof N, Sipkema P, van den Bos CG, and Elzinga G.** Forward and backward waves in the arterial system. *Cardiovasc Res* 6: 648–656, 1972.
24. **Westerhof N, Stergiopulos N, and Noble M.** *Snapshots of Hemodynamics. An Aid for Clinical Research and Graduate Education*. New York: Springer, 2004.
25. **Yin FC, Brin KP, Ting CT, and Pyeritz RE.** Arterial hemodynamic indexes in Marfan's syndrome. *Circulation* 79: 854–862, 1989.

IV.4 Chapter 4: Study of the correlation between the cardiovascular phenotype and the genotype

4.1 Variability of aortic stiffness is not associated with the fibrillin1 genotype in patients with Marfan syndrome.

Julie De Backer, Gijs Nollen, Dan Devos, Gerard Pals, Paul Coucke, Koen Verstraete, Ernst E van der Wall, Anne De Paepe, Barbara JM Mulder.
Heart. 2006 Jul;92(7):977-8

In this paper we measured PWV and distensibility of the aorta with MRI as parameters of aortic stiffness. We compared aortic stiffness between on the one hand patients with missense and/or in frame-deletion mutations in *FBN1* (group 1, N=35) and on the other hand patients with nonsense and/or out-of frame *FBN1* mutations (group 2, N=29). In group 1, we performed a subanalysis in 18 patients with mutations involving a cystein residue (group 1_{sub}).

We also investigated the relation between aortic stiffness and a VNTR polymorphism in the *FBN1* gene, thought to be related to aortic stiffness in non-Marfan patients. The three most common haplotypes of this polymorphism were studied (2-2, 2-3 and 2-1).

No significant differences regarding age, body surface area or Mean Arterial Pressure were observed between the different study groups.

As demonstrated in table 2, no significant differences were observed between both groups of mutations for any of the aortic stiffness parameters.

In a subanalysis comparing cystein substitutions versus nonsense or out of frame deletion/insertion mutations, no significant differences were observed.

In addition, aortic stiffness within ten families with two affected subjects and one family with five affected subjects showed marked variability.

No differences in aortic stiffness parameters were observed between the different genotypes identified by the VNTR polymorphism.

	Mutation type			*VNTR polymorphism*		
Variable	Group1 (n=35)	Group1$_{sub}$ (n=18)	Group2 (n=29)	2-2 (n=32)	2-3 (n=16)	2-1 (n=9)
Pulse Wave Velocity	5.3±1.7	5.7±1.9	5.4±1.2	5.0±1.0	5.8±2.2	5.4±1.1
Distensibility						
Ascending aorta*	3.8±1.8	3.3±2.1	3.4±2.3	3.9±2.5	5.6±2.6	3.9±2.2
Descending Thoracic aorta	3.7±2.0	3.3±1.8	3.2±1.7	3.6±2.6	3.6±2.2	4.5±2.6
Abdominal aorta	4.0±2.2	3.6±2.4	3.0±1.6	3.3±1.9	3.9±2.2	5.5±5.8

Table 2 Aortic stiffness parameters in both mutation groups, in the subgroup of cystein mutations and in the different polymorphism groups. * = measured in patients without aortic root replacement. Distensibility in 10^{-3} $mmHg^{-1}$. Pulse Wave Velocity in m/s

Variability of aortic stiffness is not associated with the fibrillin 1 genotype in patients with Marfan's syndrome

J De Backer, G J Nollen, D Devos, G Pals, P Coucke, K Verstraete, E E van der Wall, A De Paepe, B J M Mulder

Heart 2006;**92**:977–978. doi: 10.1136/hrt.2005.071720

Marfan's syndrome (MFS) is an autosomal dominant connective tissue disorder, with clinical manifestations in the skeletal, ocular, and cardiovascular organ systems, caused by mutations in the fibrillin 1 gene (*FBN1*). MFS shows full penetrance but with considerable clinical variability both between and within families. More than 500 different mutations have been identified so far, scattered throughout the gene and usually unique to individual families.

Prognosis in MFS is mainly determined by progressive dilatation of the aorta, potentially leading to aortic dissection and death at young age. Recently, we have shown that increased aortic stiffness is an independent predictor of progressive aortic dilatation.[1]

It has been suggested that genetic variation in *FBN1* as assessed by analysis of an intragenic polymorphism (variable number tandem repeat (VNTR) polymorphism in intron 28) is an important factor contributing to risk associated with pulse pressure and aortic stiffness in healthy middle aged men and in patients with coronary artery disease.[2 3] In patients with MFS the association between aortic stiffness and the *FBN1* genotype or *FBN1* mutations has not been investigated previously.

Our purpose was to investigate the association between aortic stiffness parameters and the FBN1 genotype in patients with MFS. The genotype was characterised by the mutation on the one hand and by a specific intragenic FBN1 polymorphism on the other.

PATIENTS AND METHODS

A cohort of 67 patients with MFS (31 men, mean (SD) age 32 (10) years) representing 51 families with an identified *FBN1* mutation underwent cardiac magnetic resonance imaging in the Academic Medical Centre (Amsterdam, the Netherlands) (41 patients) and the Ghent University Hospital (Belgium) (26 patients). MFS had been diagnosed according to the Ghent criteria. Eighteen patients (27%) had previously undergone an elective aortic root replacement. Fifty eight patients (83.6%) were taking β blockers. Twelve families were represented with two patients and one family with five patients.

The *FBN1* gene was molecularly analysed by established techniques.[4] The VNTR polymorphism (TAAAA in intron 28 of *FBN1*) was analysed by previously described techniques.[2]

Magnetic resonance images were acquired with a 1.5 T system (Magnetom Vision; Siemens Medical Systems, Erlangen, Germany). Aortic distensibility and pulse wave velocity were assessed as previously described.[5]

Data are given as mean (SD). Comparisons were performed by χ^2 test for categorical variables and analysis of variance for continuous variables. Data were statistically analysed with the SPSS statistical package (SPSS Inc, Chicago, Illinois, USA). The level of significance was set at $p < 0.05$.

RESULTS

In this group of 67 patients, 51 different mutations were present. Thirty five patients (51%) had either a missense or in-frame deletion mutation (group 1). Twenty nine patients (45%) had a mutation leading to a premature termination codon (group 2). Eighteen of the 29 (64%) missense mutations were cysteine substitutions ($group1_{sub}$).

The VNTR polymorphism was identified in 59 patients. In total, three alleles were identified as 2, 3, and 4 according to the number of TAAAA repeats. These corresponded to four genotypes, with 2–2, 2–3, and 2–4 accounting for 97% of the population. The major genotypes were used for further analysis.

The 2–2 genotype was present in 54% of patients, 2–3 in 27%, and 2–4 in 15%. When compared with a control sample of 37 healthy subjects from the same locality, this distribution was very similar.

Baseline characteristics according to mutation types or to VNTR polymorphisms did not differ with regard to age, sex, previous Bentall procedure, or β blocker use. Patients with a nonsense mutation had a slightly higher body surface area.

We found no significant differences between the groups of mutations in any of the aortic stiffness parameters (table 1).

In a subanalysis comparing cysteine substitutions versus premature termination codon mutations, no significant differences were observed. Aortic stiffness within the 10 families with two affected and in one family with five affected varied greatly. No differences in aortic stiffness parameters were observed between the genotypes identified by the VNTR polymorphism.

This study found no association between the *FBN1* genotype and aortic stiffness parameters in patients with MFS.

The functional consequences of the different *FBN1* mutation types are difficult to assess, mainly because the precise function of fibrillin 1 is not completely understood. Fibrillin 1 is one of the major constituents of the 10–12 nm microfibrils composing the extracellular matrix. Microfibrils are extensible themselves and may contribute to the mechanical properties of mature elastic tissues by means of load redistribution between individual elastic fibres.

From recent observations, it is becoming clear that fibrillin 1 is not merely a structural protein. Fibrillin 1 subserves an important functional role in the complex transforming growth factor β signalling pathway. At least part of the clinical spectrum of the disease, such as mitral valve prolapse, is related to transforming growth factor β induced mechanisms. The precise link between the aortic manifestations and this signalling pathway has not been elucidated yet, but the effect of the *FBN1* mutations will at least partly be explained through these complex mechanisms.

Several attempts have been made to identify possible genotype–phenotype correlations in MFS, but none has been convincing so far.

Table 1 Aortic stiffness parameters in both mutation groups, in the subgroup of cysteine mutations (groups 1_{sub}), and in each polymorphism group

	Mutation group			VNTR polymorphism		
Variable	Group 1 (n = 35)	Group 1_{sub} (n = 18)	Group 2 (n = 29)	2–2 (n = 32)	2–3 (n = 16)	2–1 (n = 9)
Pulse wave velocity (m/s)	5.3 (1.7)	5.7 (1.9)	5.4 (1.2)	5.0 (1.0)	5.8 (2.2)	5.4 (1.1)
Distensibility (10^{-3}/mm Hg)						
Ascending aorta*	3.8 (1.8)	3.3 (2.1)	3.4 (2.3)	3.9 (2.5)	5.6 (2.6)	3.9 (2.2)
Descending thoracic aorta	3.7 (2.0)	3.3 (1.8)	3.2 (1.7)	3.6 (2.6)	3.6 (2.2)	4.5 (2.6)
Abdominal aorta	4.0 (2.2)	3.6 (2.4)	3.0 (1.6)	3.3 (1.9)	3.9 (2.2)	5.5 (5.8)

Data are mean (SD).
*Measured in patients without aortic root replacement.
VNTR, variable number tandem repeat.

Another observation reinforcing the lack of evidence for genotype–phenotype correlations is the high degree of intrafamilial variability with respect to onset of disease, organ system involvement, and severity. This intrafamilial variability is also seen for aortic stiffness in patients with MFS, as shown in the present study. It seems likely that aortic stiffness resembles the extreme clinical variability in patients with MFS.

In two recent studies an association between an *FBN1* polymorphism and different parameters of aortic stiffness in normal subjects and in patients with coronary artery disease has been suggested.[2 3]

We assessed the influence of this polymorphism in our patient group, thus also taking the effect of the normal allele into account. We found no association between any of the assessed aortic stiffness parameters and the different polymorphisms. In patients with an *FBN1* mutation further explanations for the variation in aortic stiffness should be sought outside the *FBN1* gene (such as genetic modifying gene loci or environmental factors).

We showed that, similar to other genotype–phenotype associations, correlations between *FBN1* genotype and aortic stiffness are very poor in patients with MFS. This reflects the high variability of disease severity in these patients. This variability cannot be accounted for by an effect of the normal allele, as shown by the lack of an association between aortic stiffness parameters and a VNTR polymorphism. Other modifiers of phenotypic expression must be implicated, which is the subject for further studies.

Authors' affiliations
J De Backer*, P Coucke, A De Paepe, Center for Medical Genetics of the Ghent University Hospital, Ghent, Belgium
G J Nollen*, B J M Mulder, Department of Cardiology of the Academic Medical Center, Amsterdam, the Netherlands
D Devos, K Verstraete, Department of Radiology and Medical Imaging of the Ghent University Hospital, Ghent, Belgium
G Pals, Department of Molecular Genetics, VU University Medical Center, Amsterdam, the Netherlands
E E van der Wall, Department of Cardiology of the Leiden University Medical Center, Leiden, the Netherlands

*Both authors contributed equally to this manuscript

This study was supported by a grant from the Netherlands Heart Foundation (2000.108) (G J Nollen), by a research grant from Ghent University (BOF 011D4701) (J De Backer), and by a research grant from the Fund For Scientific Research Belgium (FWO G029002) (A De Paepe).

The study was approved by the local ethics committees (University Hospital Ghent, Belgium and Academic Medical Centre Amsterdam, the Netherlands) and individual oral and written informed consent was obtained from each patient.

Correspondence to: Dr Barbara J M Mulder, Department of Cardiology, Room B2-240, Academic Medical Centre, Meibergdreef 9, 1105 AZ Amsterdam, Netherlands; b.j.mulder@amc.uva.nl

Accepted 29 September 2005

REFERENCES

1 **Nollen GJ**, Groenink M, Tijssen JG, *et al*. Aortic stiffness and diameter predict progressive aortic dilatation in patients with Marfan syndrome. *Eur Heart J* 2004;**25**:1146–52.
2 **Medley TL**, Cole TJ, Gatzka CD, *et al*. Fibrillin-1 genotype is associated with aortic stiffness and disease severity in patients with coronary artery disease. *Circulation* 2002;**105**:810–5.
3 **Powell JT**, Turner RJ, Henney AM, *et al*. An association between arterial pulse pressure and variation in the fibrillin-1 gene. *Heart* 1997;**78**:396–8.
4 **Loeys B**, De Backer J, Van Acker P, *et al*. Comprehensive molecular screening of the FBN1 gene favors locus homogeneity of classical Marfan syndrome. *Hum Mutat* 2004;**24**:140–6.
5 **Groenink M**, de Roos A, Mulder BJ, *et al*. Biophysical properties of the normal-sized aorta in patients with Marfan syndrome: evaluation with MR flow mapping. *Radiology* 2001;**219**:535–40.

IV.4.2 Challenges in the diagnostic evaluation for Marfan syndrome.

J. De Backer, B. Loeys, B. Leroy , P. Coucke, H. Dietz, A. De Paepe
Clinical Genetics, in press

In this paper, we present three families which illustrate the variable presentations of MFS, between as well as within families, and highlight the place of molecular testing in the diagnosis of MFS.

Detailed clinical investigations including physical examination by an experienced clinical geneticist, slit lamp examination of the eyes, echocardiography and magnetic resonance imaging for assessment of dural ectasia, were performed in 36 subjects from three unrelated families in whom an underlying *FBN1* mutation was identified.

In family 1, the proband presented manifestations of MFS on the one hand and Weill-Marchesani syndrome on the other hand. Additional molecular analysis of the *FBN1* gene and detailed clinical examination in first degree relatives were necessary to establish the correct diagnosis.

In family 2, the proband presented as a child displaying overlap between MFS and the kyphoscoliotic form of Ehlers-Danlos syndrome. Follow-up over time and additional molecular and biochemical testing allowed us to make the correct diagnosis. Furthermore, molecular testing enabled us to identify several family members with incomplete expression of MFS.

In family 3 an example is provided of extensive intrafamilial variability in MFS. Additional molecular testing was very helpful to identify subjects with very mild expression.

Utility of molecular analyses in the exploration of extreme intrafamilial variability in the Marfan syndrome

J. De Backer, B. Loeys, B. Leroy, P. Coucke, H. Dietz, A. De Paepe
Clinical Genetics 2007 – in press

Abstract

Background: The diagnosis of Marfan syndrome may be hampered by the existence of very mild and atypical cases as well as by marked intrafamilial variability. In these instances, molecular analysis of the fibrillin-1 gene (FBN1) can be helpful to identify individuals at risk. The underlying molecular mechanism for the clinical variability is presently unknown.
Methods: We performed clinical and molecular studies in 36 subjects from 3 unrelated families. Expression studies of both FBN1 alleles were performed and related to the clinical severity.
Results: In family 1 an overlapping phenotype between MFS and Weill-Marchesani syndrome is presented. The diagnosis necessitated molecular studies and clinical examination in first degree relatives.
In family 2, the young proband presented with a phenotype overlapping between MFS and the kyphoscoliotic type of Ehlers-Danlos syndrome. Follow-up over time and identification of a FBN1 mutation allowed confirmation of the diagnosis. Mutation analysis enabled us to identify family members with mild expression.
Family 3 illustrates the extensive intrafamilial variability in the clinical severity of MFS. Identification of a FBN1 mutation was helpful to identify subjects with mild expression and for the timely diagnosis in a neonate.
In families 2 and 3, the relative expression of both FBN1 alleles was not related to clinical severity.
Conclusion: Confirmation of the diagnosis of MFS may require detailed and repeated clinical evaluation and thorough family history taking. FBN1 mutation analysis is supportive for the diagnosis in mild and atypical presentations.

Key words

Marfan syndrome; fibrillin1 gene; intrafamilial variability; diagnostic criteria

Introduction

Marfan syndrome (MFS) is an autosomal dominant pleiotropic connective tissue disorder caused by mutations in the fibrilllin-1 gene (FBN1) (1). The diagnosis is based on a combination of major and minor clinical criteria, involving different organ systems defined in the Ghent nosology (2). In an index patient the diagnosis of MFS requires the presence of major manifestations in at least two organ systems with involvement of a third organ system. In a relative of an index patient that independently fulfils the diagnosis of MFS, the diagnosis is confirmed in the presence of a major criterion in one organ system and involvement of a second organ system.

Major criteria include the presence of 4 out of 8 specific skeletal manifestations, ectopia lentis, dilatation/dissection of the ascending aorta at the level of the sinuses of Valsalva, and dural ectasia. These have high diagnostic specificity, because they are uncommon in the general population, but all can be observed in other systemic connective tissue disorders. Minor criteria such as mitral valve prolapse, striae distensae, pneumothorax and joint hypermobility are less specific and occur frequently in the general population (3).

Early diagnosis of individuals at risk for MFS is extremely important as timely treatment of cardiovascular manifestations can greatly improve life expectancy in MFS (4).

The majority of adults with MFS meet the clinical criteria for MFS, allowing straightforward diagnosis on clinical grounds. For example in individuals with bilateral lens dislocation and dilatation of the proximal aorta, demonstrating the involvement of a third organ system will easily lead to the diagnosis of MFS. However, in some instances, confirmation of a clinical diagnosis is more challenging. This is especially true in children in which some of the manifestations of MFS are not yet present. Mild or atypical presentation of MFS is sometimes present, in adult patients as well. In particular the differentiation between MFS or a MFS-like condition can be very difficult in the absence of typical ocular or cardiovascular manifestations of the MFS.

In these challenging diagnostic cases, additional confirmation with molecular analysis of the FBN1 gene should be considered. The size of the gene and the presence of significant allelic heterogeneity have been major drawbacks for the routine application of molecular testing, but over the past decade, screening techniques have been refined and detection rates have significantly increased, thus justifying the use of molecular testing in clinical practice.

In sporadic cases, molecular analysis may be indicated in patients presenting major manifestations in one organ system such as “isolated ectopia lentis” or a thoracic aortic aneurysm in young normotensive patients. In relatives of a patient in whom an

underlying FBN1 mutation has been identified, molecular testing may allow the diagnosis of MFS to be established in individuals presenting a very mild or "emerging" MFS phenotype.
The underlying mechanisms for the intrafamilial variability are still poorly understood. It is suggested that both genetic and environmental factors are involved. An interesting mechanism, proposed by Hutchinson et al, is that the expression level of the non-mutated allele may play a role in the determination of intrafamilial variability (5).

In this paper, we want to highlight some challenges in the diagnosis of MFS by three illustrative case histories. Based on the clinical and molecular findings obtained in these families we want (1) to stress the importance of detailed history taking and repeated clinical examination in patients referred for possible MFS, (2) to illustrate the variable presentation and clinical severity of the MFS, (3) to demonstrate the role of molecular testing in the confirmation or exclusion of MFS in individuals in families with extreme variability and/or atypical presentations , (4) to explore the relevance of the expression level of either the mutant or wild-type allele as a determinant of intrafamilial phenotypic variability.

Methods

The three families described in this manuscript were selected based on their high didactic value, namely the availability of detailed clinical and molecular information in a large number of family members and the presence of striking intrafamilial clinical variability.

1. Clinical evaluation

Each patient underwent extensive physical examination by a clinical geneticist with experience in diagnosing heritable connective tissue disorders. Skeletal and cutaneous manifestations, as defined in the Ghent nosology (2), were looked for in detail.
Slit lamp examination of the eyes was performed in every patient to investigate whether lens dislocation or another ocular manifestation of MFS was present.
Each patient underwent a detailed echocardiographic study, for the assessment of the following cardiovascular manifestations: aortic root dilatation, mitral valve prolapse and main pulmonary artery dilatation. Z-scores for the aortic root at the level of the sinuses of Valsalva were calculated using the formula: Z-score= (obtained value – mean calculated value)/standard deviation. Mean calculated values and standard deviations related to age and body surface area were derived from the formulas provided by Roman et al (6).

Magnetic Resonance Imaging (MRI) was performed to look for dural ectasia in patients in whom the definitive diagnosis could not be established. T1 weighted spin-echo sequences were obtained in both axial and sagittal planes with a 1.5 T magnet. Dural ectasia was defined using previously published criteria (7).

2. Molecular studies

1. Mutation screening of the FBN1 gene

FBN1 mutational analysis was performed using a combination of different mutation screening techniques including SSCP (Single Strand Conformation Polymorphism analysis), CSGE (Conformation-Sensitive Gel Electrophoresis), and DHPLC analysis (Denaturing High Performance Liquid Chromatography). Aberrantly migrating fragments, detected by SSCP, CSGE or DHPLC were subsequently sequenced.
In the proband, both complementary DNA (cDNA), reverse transcribed from mRNA from cultured fibroblasts, and genomic DNA (gDNA), extracted from blood or cultured fibroblasts, was studied.
In samples which remained mutation-negative after SSCP, CSGE or DHPLC, direct sequencing analysis of the entire coding region of the FBN1 gene was carried out.
In relatives, the presence of the mutation was studied on gDNA using PCR, followed by direct sequencing of the involved exon.

2. Expression studies

2.1 Family 1

We did not obtain fibroblasts from the patients in family 1, thus precluding expression studies in this family. Using a splice site prediction program, we tried to assess whether altered splicing was likely
(http://www.fruitfly.org/seq_tools/splice.html/)

2.2 Family 2

We obtained fibroblasts for cDNA extraction from ten family members, representing two generations. Quantitative cDNA fragment analysis was performed on an ABI 3100 Prism using a forward cDNA primer. (3'CAAACACAGTCAGCAGTTAC5') and a FAM-labeled reverse cDNA primer (5'TGCAGCGTCCATTTTGACAG3')

flanking the mutation. The peak height obtained was used as a marker of expression level.
Since one normal (482bp) and two abnormal (325 and 470bp) fragments were obtained, we calculated 2 ratio's, comparing the peak height of the wild-type allele to each of the mutated fragments. These ratio's were then correlated to the Z-score of the aortic root as a parameter of clinical severity.

2.3 Family 3

To investigate the segregation of the wild-type allele in affected individuals, we analysed several FBN1 markers, as previously described (8). The amount of mutant transcript was determined by PCR-based methods that have previously been shown to be quantitative(9). RNA extraction, reverse-transcription PCR, and allele-specific oligonucleotide (ASO) hybridization analysis for mutation R1596X were performed by use of standard procedures, without modification (10). The primers used to amplify fibroblast or lymphoblast cDNA were F6-S (5'-ACCTGGATTCCAGCTGCC-3') and F6-AS (5'-TCAGGATCTAGTGCACATTC-3'). The probes used for ASO analysis were R1596-WT (5'-GGATTTGGTCAGAAACCTTCC-3') and X1596-MUT (5'-CCTAAACCAAGCTTTGGAAGG-3'). The final wash temperature for both probes was 62C. Signal strength was quantified by use of an INSTANTIMAGER system (Packard Instrument). A correction factor was calculated in order to equalize the wild-type and mutant signals observed after sequential hybridization of the probes to PCR-amplified patient genomic DNA to adjust for varying exposure times and differential affinity of the two probes for their target sequences.

Case presentations

1. Clinical and molecular findings

Family 1

This family was assessed through the 62-year-old male proband (F1-II: 1 in figure 1) in which a routine echocardiogram for palpitations revealed an aneurysm of the proximal aorta. The diameter of the aortic root at the level of the sinuses of Valsalva was 57mm (Z-score=6.2). Aortic root replacement was done using a composite graft: replacement of the proximal aorta by a Dacron graft and replacement of the aortic valve with a mechanical prosthesis (Bentall procedure). Subsequently, the patient was referred to us for diagnostic work-up for MFS.

His medical history revealed that he had ocular surgery at the age of 47 years for bilateral cataract and for glaucoma five years thereafter. Ocular examinations prior to these interventions had identified microspherophakia and very shallow anterior eye chambers, suggestive for Weill-Marchesani syndrome.

On physical examination, the proband presented a muscular build. His height was 1m72 and his weight 110 kg. His armspan (183cm) –to-height ratio was increased (ratio 1.06) but the upper/lower segment ratio was normal (0.92). He had a round face and brachycephaly of the skull. There was generalized stiffness of the finger joints. No skeletal nor skin manifestations reminiscent of MFS were present. Dural etasia was not assessed.

The proband's family history revealed that his elder brother and sister had a very similar phenotypic appearance. Reportedly, they both had ocular problems and died suddenly at the ages of 63 and 45 years respectively. The proband's mother and maternal uncle had also been diagnosed with glaucoma. However they were tall and had long, slender fingers. They both died suddenly at age 84 and 70 years respectively from an "unspecified cardiac cause". The proband had three children, but had limited contact with them and refused to refer them for further studies.

Based on the cardiovascular findings and the family history of sudden death, FBN1 mutation analysis was performed in the proband. A heterozygous 12nt deletion was detected in exon 20 of the FBN1 gene (c.2502-2513delTGAAAGTACTTT; p.Glu 835-Leu838del). Upon communication of the results of the molecular study to the proband, he gave permission to contact his children for further clinical evaluation.

Physical examination in his eldest son (F1-III: 1) showed the presence of typical skeletal features of MFS. In addition, he had severe myopia and bilateral upward luxation of the lenses and moderate dilatation of the proximal aorta at the level of the sinuses of Valsalva (43mm, Z-score 3.14). As such, the proband's son unequivocally met the diagnostic criteria for MFS with major cardiovascular, ocular and skeletal manifestations.

Examination of the proband's 7 year old grandson revealed that he presented typical skeletal manifestations of MFS, as well as bilateral subluxation of the lenses and myopia. Echocardiographic evaluation was completely normal. Molecular studies confirmed the presence of the FBN1 deletion in the proband's son and grandson.

The proband's daughter (F1-III:4) was clinically evaluated and did not present any manifestation of MFS. Molecular analysis confirmed the absence of the FBN1 mutation.

The proband's granddaughter (F1-IV:2) was not clinically evaluated, but did not carry the FBN1 mutation.

Family 2

The proband of family 2 (F2- IV:6 in figure 2) was initially referred to us at the age of 12 years for suspicion of MFS. He presented marked kyphoscoliosis, arachnodactyly, generalized joint hypermobility, flat feet, mild myopia and hyperextensible skin with one atrophic scar. Echocardiographic examination revealed mitral valve prolapse but a normal diameter of the proximal aorta. Slitlamp examination of the eyes excluded lens dislocation.
Family history at that time revealed that the proband's father (F2-III:3) had had surgical repair for an abdominal aortic aneurysm at the age of 44 years. He was tall, but presented no other skeletal or ocular features of MFS and the diameter of the proximal aorta was normal on echocardiography (37mm, Z-score 0.3), precluding clinical diagnosis of MFS. The paternal grandmother of the proband died suddenly at the age of 87 years.
Based on the clinical findings in the proband and the family history, both MFS and the kyphoscoliotic form of Ehlers Danlos Syndrome (EDS-VI) were considered. A biochemical analysis of radio-labelled collagens extracted from cultured fibroblasts of the proband showed a normal migration pattern of both the α1(I) and α2(I) chains of type I collagen with no arguments for underhydroxylation of the collagen type Iα chains, making the diagnosis of kyphoscoliotic EDS unlikely.
During the pubertal period, his kyphoscoliosis worsened and he developed pectus excavatum and stretch marks on the shoulders. By the age of 22 years, he had developed dilatation of the proximal aorta at the level of the sinuses of Valsalva. This evoked the possible diagnosis of MFS in him, since he presented major skeletal manifestations (8 out of 8 skeletal manifestations), aortic dilatation, stretch marks on the shoulders and mild myopia.
When the proband was 32 years old, his father died at the age of 60 years during surgery for a thoracic aortic aneurysm.

Molecular study of the FBN1 gene was performed in the proband and revealed a splice-site mutation (c. 989-1G>C) at the intron 9 – exon 9 junction.
Subsequently, clinical and molecular investigations were performed in 28 additional family members, the results of which are summarized in Table 1.
The FBN1 mutation was identified in 19 additional family members of the proband. None of these 19 relatives presented a classic MFS phenotype. Lens dislocation was absent in all of them; five individuals had mild myopia (F2-IV:4, IV:6, IV:9, IV:21 and V:3); one had a history of retinal detachment in association with mitral valve prolapse (F2-V:19). Major skeletal manifestations were present in only one female patient of 25 years (F2-V:10), who had neither cardiovascular nor ocular features of Marfan syndrome. Significant aortic root dilatation was present in two boys of 13

and 16 years respectively (F2-V:16 and F2-V:4) as well as in six adult relatives (F2-IV:1, IV:4, IV:10, IV:12, IV:16 and V:1). None of the individuals with aortic root dilatation had major skeletal or ocular manifestations but two had dural ectasia (F2-IV:1 and V:1), one of them (F2-IV:16) had a pectus carinatum and flat feet and one had mild myopia (F2-IV:4). Three family members had died from a thoracic aortic dissection between 57 and 60 years (F2-III:3, III:6 and III:9), prior to the identification of the mutation in this family. The father of the proband (F2-III:3) had been clinically examined but he presented no physical manifestations of MFS; family members F2-III:6 and III:9 were never clinically examined but reportedly had normal stature and no ocular problems.

Overall, the phenotypic features of individuals who tested positive for the presence of the FBN1 mutation and presented no aortic root dilatation were strikingly mild. One of them had mitral valve prolapse (F2-V:19), one had main pulmonary artery dilatation (F2-V:6), both without skeletal or ocular manifestations. One individual had pectus carinatum, arachnodactyly and decreased elbow extension (F2-V:8) without cardiovascular or ocular manifestations. Two female relatives in their mid fifties had no organ system involvement at all, except for mild myopia (F2-IV:9 and IV:21), although the diameter of the proximal aorta in F2-IV:9 had slightly increased over the last 3 years (last diameter 39mm, Z-score 1.7).

Among the nine relatives who did not carry the mutation, mild connective tissue abnormalities reminiscent of MFS were present in three. One boy (F2-V:15) presented mild scoliosis, a pectus carinatum and a high arched palate. The proband's 34 year old sister (F2-IV:7), presented with borderline enlargement of the proximal aorta (35mm, Z-score 2.1) and mild scoliosis; on a follow-up examination 3 years later, the aortic root diameter remained stable at 35 mm. One 46 year old male had a history of retinal detachment with not other manifestations of MFS (F2-IV:3).

Family 3

The 28 year old female proband initially sought medical advice because of suspicion of MFS in her 4 year old son (F3-III:2 in figure 3). He had surgery for a right sided inguinal hernia at the age of 2 years. Clinical evaluation showed tall stature (103cm, 97th percentile) and a prominent Marfanoid phenotype, with severe pectus excavatum, arachnodactyly and flat feet. Echocardiography showed dilatation of the proximal aorta (24mm at the level of the sinus Valsalva, Z-score=3.2) and slit lamp examination of the eyes revealed bilateral subluxation of the lenses.

The proband (F3-II:2) underwent bilateral lens extraction at the age of 7 years, but no further diagnostic work-up was done at that time. Clinical examination at the age of 28 years demonstrated facial and skeletal manifestations of MFS. Stretch marks were present on the abdomen and on the lower back. Echocardiography showed

mild aortic root dilatation (37 mm at the sinus of Valsalva, Z-score=2.36) and the presence of a dissection flap at the level of the aortic arch. Subsequent MRI of the aorta revealed marked dilatation of the descending thoracic aorta (53mm at the level of the diaphragm) and the presence of a type B aortic dissection (extending from the aortic arch to the abdominal aorta, just proximal of the renal arteries).
Based on these findings, the clinical diagnosis of MFS was made in both individuals (major skeletal and cardiovascular manifestations and stretch marks in the proband and major cardiovascular manifestation and involvement of the skeleton in her son). Molecular analysis demonstrated a nonsense mutation (p.R1596X) in exon 38 of the FBN1 gene in both of them.
Subsequently, the proband's three year old daughter (F3-III:3) and her sister (F3-II:3) were also evaluated. While mild skeletal features were observed in the proband's daughter, physical examination in her sister was completely normal except for the presence of contractures of her fifth digits (camptodactylia) and mild facial characteristics (dolichocephaly). Additional echocardiographic and ophtalmological examinations were normal in both. Dural ectasia was excluded by MRI in the proband's sister who was shown to carry pR1596X.
FBN1 mutation analysis demonstrated the presence of the mutation also in the proband's father (F3-I:2) and half-brother (F3-II:5). Her father was reportedly healthy but refused further physical examination. Her half-brother had lens subluxation, mild aortic dilatation and severe dural ectasia, thus fulfilling the diagnostic criteria for MFS.
At birth, a baby girl born to the proband's sister (F3-III:4)presented no clinical manifestations of MFS, but was shown to carry the same FBN1 mutation as her mother and the other affected relatives. At the age of 6 months, proximal aortic dilatation was detected for which treatment with beta-blockers was initiated.

2. Expression studies

1. Family 1

To determine if the 12 bp deletion created a new cryptic splice donor or acceptor site, we used a splice site prediction software package. However, this search revealed no new splice site indicating that this specific deletion probably did not result in altered mRNA splicing. As no fibroblasts were available from patients of this family, no experiments could be performed to investigate mRNA expression.

2. Family 2

We were able to demonstrate that the splice site mutation generates two different transcripts on the cDNA level: one in which exon 9 was skipped and another which was alternatively spliced and carried an in-frame deletion of 12 basepairs (c989-1000delATGTTCGCCCAG).

We found no correlation between the ratio's (wild-type/alternatively spliced fragment, wild-type/exon skipped fragment or the alternatively spliced/exon skipped fragment) and the Z-score of the aortic root, indicating that the expression level of the alleles does not determine the cardiovascular severity in this family (figure 4).

To exclude the influence of age on the obtained curves, we compared the mean age in the 5 patients with the lowest Z-score to the mean age in the 5 patients with the highest Z-score. No significant difference was observed (35.8yrs ± 17.7 in the group with lowest Z-scores versus 35.8yrs ± 11 in the group with the highest Z-scores, p=0.15 in an independent samples t-test).

In comparing skeletal features in relation to the obtained ratio's, we could not demonstrate significant differences. Three subjects (F2-V:9, IV:16 and IV:6) had skeletal features whereas seven others (F2-V:19, V:6, IV:21, IV:9, V:3, IV:1 and V:1) had no skeletal features. The mean value in both groups was not significantly different: see table 2.

3. Family 3

Segregation analysis revealed that individuals II:2 and II:3 share the same maternal wild-type allele and children III:2 and III:3, share the same paternal wild-type allele making it unlikely that the wild type allele would act as a modifier of the clinical severity.

No significant differences were found between both sisters regarding the expression level of wild-type and mutant fragments (figure 4), indicating that the relative expression of both alleles is not determining the clinical severity.

Discussion

These three families clearly illustrate that the clinical presentation of MFS can be very mild or atypical and display remarkable variability within families. The diagnosis of MFS was challenging in at least some of the relatives and required careful and repeated clinical evaluations over time as well as additional support from FBN1 testing.

In the first family, the proband presented phenotypic features reminiscent of both MFS and Weill-Marchesani syndrome (WMS). Although the aortic root aneurysm definitely is a major manifestation of MFS, no second major criterion could be demonstrated in him. The skeletal (brachydactyly, stocky stature and stiff joints) and the ocular (microspherophakia) manifestations were suggestive for WMS. The diagnosis of MFS in the proband could only be established after the demonstration of a FBN1 mutation and the subsequent identification of typical features of MFS in the proband's son and other family members, stressing the importance of careful clinical evaluation of first degree relatives.

The apparent co-occurrence of WMS and MFS in one family had already been reported in 1959 by Bowers (11). WMS is a rare disorder characterized by short stature, brachydactyly, joint stiffness, microspherophakia with severe myopia, glaucoma and ectopia lentis. In the family described by Bowers, sixteen individuals were diagnosed with MFS, whereas two family members (one 3 year old boy and one adult) who presented a large head, short extremities and short stature were diagnosed with WMS. Re-evaluation of the family four years later, showed that WMS features in the 3-year old child were no longer present and that his physical appearance had evolved to that of classic MFS (12). This clearly illustrates the importance of repeated follow-up in patients with a suspicion or family history of MFS.

In WMS, both autosomal dominant and recessive transmission has been described. While the autosomal recessive form is caused by mutations in the ADAMTS10 gene (13), the dominant form has been associated with FBN1 mutations. Faivre and co-workers identified a 24nt in-frame deletion in exon 41 of the FBN1 gene in four individuals of a three-generation WMS family (14). Since then three additional FBN1 mutations have been reported in WMS patients: G214S in exon 6 (15), R1596P in exon 38 (16) and an in-frame exonic deletion of exons 9-11 (16). It is unclear from the available literature data whether these WMS patients had aortic root dilatation, but our observations in family 1 warrant caution and regular cardiovascular evaluation in these patients.

It is intriguing that the FBN1 mutations encountered in our family and in the WMS family reported by Faivre and co-workers are small in-frame deletions, which are

unusual FBN1 mutations. Only seven other small in-frame deletions have been reported. in the FBN1 mutation database(17). Three of them in neonatal MFS (18-20), three in classical MFS (21-23), and one in a patient not fulfilling the Ghent criteria for MFS(24), as was the case in our proband in family 1. However, it appeared that the patient described by Liu and co-workers had typical skeletal manifestations of MFS but did not have major cardiovascular manifestations – in contrast to the proband in family 1.

In the second family the proband presented overlapping clinical features of both the MFS and kyphoscoliotic Ehlers-Danlos syndrome. Two subtypes of EDS should be considered in the differential diagnosis of MFS. Firstly, in cases presenting with aortic dissection in the absence of prior significant aneurysm formation, the vascular type of EDS should be excluded. Second, the kyphoscoliotic type should be considered when a combination of scoliosis and tissue fragility is present, as illustrated in the proband of family 2. Both types of EDS can be diagnosed by demonstration of abnormal electrophoretic patterns of radio-labelled collagens extracted from skin fibroblast cultures (25).

The most important issue illustrated in family 2, is that the phenotype in MFS patients may be misleadingly mild. In fact the identification of the FBN1 mutation was the only tool that allowed us to identify at risk individuals in the family. Identification of a mutation in these individuals facilitated correct counselling in these patients and gave us a powerful argument to convince them of the importance of follow-up.
Furthermore, we were able to identify affected children early in life with the help of FBN1 screening, which is important since it is recommended that medical treatment with beta-blockers be initiated at the earliest age possible(26).
Three of the individuals in the family that died from a thoracic aortic dissection prior to the availability of molecular testing reportedly had limited MFS features. Based on the segregation of the mutation in this family and the occurrence of a thoracic aortic aneurysm, we have convincing evidence to assume that they had MFS. This clearly illustrates the risk for aortic disease in this family and mandates life-long follow-up in affected individuals.
Molecular testing in family 2 also allowed us to exclude MFS in two young subjects presenting some “minor”, less specific features of MFS including scoliosis, high arched palate and pectus deformity in F2-V:15 and scoliosis and borderline diameter of the proximal aorta in F2-IV:7. Demonstration of the absence of the FBN1 mutation alleviated the need for repeated follow-up in these two individuals.
The splice-site mutation in family 2 has not been reported previously. In the FBN1 database(17), a total of 13 splice-site mutations are reported that are known to cause

exon-skipping in association with neonatal MFS, classical MFS as well as in "incomplete" MFS.

The third family again illustrates that clinical expression may vary significantly within the same family. If not for the additional molecular studies, the proband's sister would not have been identified as a carrier since she presented very mild clinical features of MFS. The diagnosis in her baby girl (F3-III:4) was established very early in life through the identification of the FBN1 mutation. This girl is now under regular ophthalmologic, orthopaedic and cardiovascular follow-up.

The three families presented here illustrate the striking variability in clinical severity that can be encountered in MFS. The mechanisms for this underlying intrafamilial variability are still poorly understood. It is suggested that both genetic and environmental factors are involved. An interesting mechanism, proposed by Hutchinson et al, is that the expression level of the normal allele may play a role in the determination of the intrafamilial variability (5). We studied the relative expression of the mutant and the normal FBN1 alleles in families 2 and 3 but could not demonstrate any correlation between the clinical severity and expression levels of either of the two FBN1 alleles, thus not supporting the data from Hutchinson. Several explanations are possible for this discrepancy: first of all, the molecular techniques used for the quantification of allele expression are different and thus not comparable. Secondly, with regards to the second family, the type of mutation was different (splice site mutation versus nonsense mutation in the manuscript by Hutchinson), which might result in differences in expression of the FBN1 alleles. Thirdly, it is possible that the expression level of the normal allele plays a role in the determination of clinical severity in certain families as in the one presented by Hutchinson and not in others, as in our families. Lastly, and importantly, it is very difficult, not to say impossible, to compare the clinical severity encountered in MFS patients. As MFS is a pleiotropic disorder with manifestations in different organ systems which are in addition each dependent on the age of the studied individual, it is very hard to define a global "severity score". To deal with this difficulty, we applied two different strategies in these families. As we had data on a large number of individuals in family 2, we compared our molecular results with the Z-score of the diameter of the proximal aorta on the one hand and presence or absence of skeletal features on the other hand. The advantage of the use of the Z-score is that this parameter is linear, as opposed to the other clinical features which are categorical (either "present" or "absent"); in addition, age is taken into account in the calculation of the Z-score and we could compare the age in the group with the highest Z-score to that in the group with the lowest Z-score. In family 3, we had very distinct phenotypes in both sisters and were able to compare both with respect

to their expression level of the FBN1 alleles. Obviously, these results need confirmation in a larger group of patients.

From the observations in these families, several practical recommendations can be formulated which can help to improve early and accurate diagnosis of MFS.
First, diagnostic assessment of MFS in patients and, importantly, also in relatives, should at least include a thorough clinical evaluation by a clinician with experience in connective tissue disorders, in addition to an echocardiographic examination and slit-lamp examination of the eyes. Whether assessment of dural ectasia is necessary for the diagnosis, is a matter of debate. In the families described here, dural ectasia was present in two patients in family 2 (F2-V:1 and IV:1) and could confirm the diagnosis of MFS on clinical grounds in them. However, dural ectasia was absent in both a 21y old and a 55 y old female in family 2 (F2-V:3 and IV:9) as well as in the sister of the proband in family 3. Thus, dural ectasia assessment did not allow us to identify them as affected subjects, despite they all harboured a FBN1 mutation.
Second, repeated evaluation, sometimes into adulthood is required in children suspected with MFS in view of the age related expression of the skeletal and cardiovascular manifestations. In addition, life-long follow-up may be necessary in adult patients who present major manifestations in only one organ system such as major skeletal involvement or lens dislocation and "minor" involvement of a second organ system. This has previously been demonstrated by Black and co-workers (27). They reported the development of aortic root dilatation in the 5th decade in patients presenting familial ectopia lentis. This confirms the need for life-long screening in adult patients carrying a FBN1 mutation in the absence of major manifestations, as was the case in several subjects reported in this paper (F2-IV:9 and IV:21; F3-II:3).
Third, we show that molecular testing can help to (1) diagnose children with MFS early and (2) establish or refute the diagnosis in patients with atypical or very mild presentation of MFS.

The large size of the gene, the extreme allelic heterogeneity and low mutation detection rates have been quoted as major drawbacks to advocate routine molecular testing. The identification TGFBR2 mutations in so-called "MFS2" have led to further confusion (28). However, evidence for locus homogeneity in MFS is high and with current techniques mutation detection rates of over 95% are reached, which imply that the contribution of molecular testing in MFS needs to be re-evaluated (13). It is likely that high throughput molecular techniques will further reduce the cost and labour-intensity of the test. Until then, the identification of "atypical patients" will likely remain restricted to those families in which the identification of a mutation in the proband allows extended molecular and clinical testing in the other family members.

Limitations

We are well aware that this manuscript should not be regarded as a scientific study since it does only contain material from three (large) families with MFS. Nevertheless, we believe that the observations in these families are very illustrative for the challenges encountered in daily clinical practice and could therefore contain useful information for clinicians.

Acknowledgements

This study is supported by a research mandate from the Ghent University (BOF 011D4701) to J. De Backer and by a research grant from the Fund for Scientific Research Belgium (FWO G029002 to A. De Paepe)

References

1. Dietz HC, Cutting GR, Pyeritz RE, Maslen CL, Sakai LY, Corson GM, et al. Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. Nature. 1991 Jul 25;352(6333):337-9.
2. De Paepe A, Devereux RB, Dietz HC, Hennekam RC, Pyeritz RE. Revised diagnostic criteria for the Marfan syndrome. Am J Med Genet. 1996;62(4):417-26.
3. Pereira L, Levran O, Ramirez F, Lynch JR, Sykes B, Pyeritz RE, et al. A molecular approach to the stratification of cardiovascular risk in families with Marfan's syndrome. N Engl J Med. 1994 Jul 21;331(3):148-53.
4. Silverman DI, Burton KJ, Gray J, Bosner MS, Kouchoukos NT, Roman MJ, et al. Life expectancy in the Marfan syndrome. Am J Cardiol. 1995;75(2):157-60.
5. Hutchinson S, Furger A, Halliday D, Judge DP, Jefferson A, Dietz HC, et al. Allelic variation in normal human FBN1 expression in a family with Marfan syndrome: a potential modifier of phenotype? Hum Mol Genet. 2003 Sep 15;12(18):2269-76.
6. Roman MJ, Devereux RB, Kramer-Fox R, O'Loughlin J. Two-dimensional echocardiographic aortic root dimensions in normal children and adults. American journal of cardiology, The. 1989;64(8):507-12.
7. Fattori R, Nienaber CA, Descovich B, Ambrosetto P, Reggiani LB, Pepe G, et al. Importance of dural ectasia in phenotypic assessment of Marfan's syndrome. Lancet. 1999 Sep 11;354(9182):910-3.
8. Loeys B, Nuytinck L, Van Acker P, Walraedt S, Bonduelle M, Sermon K, et al. Strategies for prenatal and preimplantation genetic diagnosis in Marfan syndrome (MFS). Prenat Diagn. 2002 Jan;22(1):22-8.

9. Hamosh A, Rosenstein BJ, Cutting GR. CFTR nonsense mutations G542X and W1282X associated with severe reduction of CFTR mRNA in nasal epithelial cells. Hum Mol Genet. 1992 Oct;1(7):542-4.
10. Dietz HC, Pyeritz RE, Puffenberger EG, Kendzior RJ, Jr., Corson GM, Maslen CL, et al. Marfan phenotype variability in a family segregating a missense mutation in the epidermal growth factor-like motif of the fibrillin gene. J Clin Invest. 1992 May;89(5):1674-80.
11. Bowers D. Marfan's syndrome and the Weill-Marchesani syndrome in the S. family. Ann Intern Med. 1959 Nov;51:1049-70.
12. Bowers D. Marfan's syndrome: the S. family re-visited. Can Med Assoc J. 1963 Aug 24;89:337-40.
13. Dagoneau N, Benoist-Lasselin C, Huber C, Faivre L, Megarbane A, Alswaid A, et al. ADAMTS10 mutations in autosomal recessive Weill-Marchesani syndrome. Am J Hum Genet. 2004 Nov;75(5):801-6.
14. Faivre L, Gorlin RJ, Wirtz MK, Godfrey M, Dagoneau N, Samples JR, et al. In frame fibrillin-1 gene deletion in autosomal dominant Weill-Marchesani syndrome. J Med Genet. 2003 Jan;40(1):34-6.
15. Cornier-Daire V. Weill-Marchesani syndrome is an heterogenous disorder due either to ADAMTS10 or FBN1 mutations 7th International Research Symposium on the Marfan Syndrome. Ghent, Belgium; 2005.
16. Corson GM. Regulation of growth factor signaling by fibrillin microfibrils. 7th International Research symposium on the Marfan Syndrome. Ghent, Belgium; 2005.
17. Collod-Beroud G, Le Bourdelles S, Ades L, Ala-Kokko L, Booms P, Boxer M, et al. Update of the UMD-FBN1 mutation database and creation of an FBN1 polymorphism database. Hum Mutat. 2003 Sep;22(3):199-208.
18. Putnam EA, Cho M, Zinn AB, Towbin JA, Byers PH, Milewicz DM. Delineation of the Marfan phenotype associated with mutations in exons 23-32 of the FBN1 gene. Am J Med Genet. 1996 Mar 29;62(3):233-42.
19. Weidenbach M, Brenner R, Rantamaki T, Redel DA. Acute mitral regurgitation due to chordal rupture in a patient with neonatal Marfan syndrome caused by a deletion in exon 29 of the FBN1 gene. Pediatr Cardiol. 1999 Sep-Oct;20(5):382-5.
20. Ades L. Personal Communication. 2001.
21. Loeys B, Nuytinck L, Delvaux I, De Bie S, De Paepe A. Genotype and phenotype analysis of 171 patients referred for molecular study of the fibrillin-1 gene FBN1 because of suspected Marfan syndrome. Arch Intern Med. 2001 Nov 12;161(20):2447-54.
22. Rantamaki T. Personal Communication. 2000.
23. Katzke S, Booms P, Tiecke F, Palz M, Pletschacher A, Turkmen S, et al. TGGE screening of the entire FBN1 coding sequence in 126 individuals with marfan syndrome and related fibrillinopathies. Hum Mutat. 2002 Sep;20(3):197-208.
24. Liu W, Qian C, Comeau K, Brenn T, Furthmayr H, Francke U. Mutant fibrillin-1 monomers lacking EGF-like domains disrupt microfibril assembly and cause severe marfan syndrome. Hum Mol Genet. 1996 Oct;5(10):1581-7.

25. Steinman B, Royce PM, Superti-Furga A. The Ehlers-Danlos Syndrome. In: Royce PM, Steinman B, editors. Connective Tissue and its Heritable Disorders. New York: Wiley-Lis; 2002.
26. Salim MA, Alpert BS, Ward JC, Pyeritz RE. Effect of beta-adrenergic blockade on aortic root rate of dilation in the Marfan syndrome. Am J Cardiol. 1994 Sep 15;74(6):629-33.
27. Black C, Withers AP, Gray JR, Bridges AB, Craig A, Baty DU, et al. Correlation of a recurrent FBN1 mutation (R122C) with an atypical familial Marfan syndrome phenotype. Hum Mutat. 1998;Suppl 1:S198-200.
28. Mizuguchi T, Collod-Beroud G, Akiyama T, Abifadel M, Harada N, Morisaki T, et al. Heterozygous TGFBR2 mutations in Marfan syndrome. Nat Genet. 2004 Jul 4.

Table and figure legends

Table 1: summary of clinical and molecular results from family 2. 0 indicates no involvement; P: pectus deformity; A: arachnodactyly; S: scoliosis; E: decreased elbow extension; FF: flat feet; jh: joint hypermobility; hp: high arched palate; ARD: aortic root dilatation; MPA: main pulmonary artery dilatation; MVP: mitral valve prolapse. NA: not available. + in the "skin" refers to stretch marks; + in *"FBN1"* indicates presence of the mutation

Table 2: Mean values of the obtained ratio's in relation to the presence or absence of skeletal features. WT: Wild Type; AS: Alternatively Spliced; ES: Exon Skipped.

Figure 1: pedigree of family 1. The proband is indicated with an arrow. Black symbols indicate affected subjects, fulfilling the Ghent criteria; Gray symbols indicate subjects with incomplete expression of the disease. Symbols with a vertical black bar indicate subjects affected by hearsay. Symbols with a "N" indicate subjects with normal clinical and molecular studies. The question mark indicates no clinical or molecular data are available.

Figure 2: pedigree of family 2. The proband is indicated with an arrow. Black symbols indicate affected subjects, fulfilling the Ghent criteria; Gray symbols indicate subjects with incomplete expression of the disease. Symbols with a vertical black bar indicate subjects affected by hearsay. Symbols with a "N" indicate subjects with normal clinical and molecular studies.

Figure 3: pedigree and molecular results of family 3. The proband is indicated with an arrow. Black symbols indicate affected subjects, fulfilling the Ghent criteria; Gray symbols indicate subjects with incomplete expression of the disease. The red bar indicates the mutated allele. ASO: allele-specific oligonucleotide hybridization analysis. 25x and 30x: value obtained after 25 and 30 PCR cycles respectively

Figure 4: expression of the normal allele in relation to the Z-score in family 2. WT: Wild Type; AS: Alternatively Spliced; ES: Exon Skipped.

Table 1

Patient ID (age/sex)	Skeletal	Cardiovascular	Ocular	Skin	DE	FBN1
VI:3 (0,5/M)	0	0	0	0	NA	+
VI:1 (11/M)	0	0	0	0	NA	+
V:6 (13/M)	0	ARD	0	0	NA	+
V:9 (15/M)	P, A, S, jh	0	0	0	NA	+
V:12(16/M)	0	MPA	0	0	NA	+
V:4 (16/M)	A, E	0	0	0	NA	+
V:3 (21/F)	0	MPA, MVP	mild myopia	+	-	+
V:10 (25/F)	P, A, E, FF, jh, hp	0	0	0	NA	+
V:8 (26/M)	P, A, E	0	0	0	NA	+
V:1 (27/M)	0	ARD	0	+	+	+
V:19 (28/F)	0	MVP	retinal detachment	+	-	+
V:6 (28/M)	0	MPA	0	0	NA	+
IV:6 (36/M)	P, A, E, S, FF, jh	ARD	mild myopia	+	NA	+
IV:16 (44/M)	P, FF	ARD	0	+	NA	+
IV:4 (45/M)	0	ARD	mild myopia	0	NA	+
IV:1 (47/M)	0	ARD	0	0	+	+
IV:12 (49/M)	0	ARD	0	+	NA	+
IV:10 (53/M)	0	ARD	0	0	NA	+
IV.21 (53/F)	0	0	mild myopia	0	NA	+
IV:9 (55/F)	0	0	0	0	-	+
VI:2 (8/F)	0	NA	NA	0	NA	-
V:15 (14/M)	S, P, hp	0	0	0	NA	-
V:14 (17/M)	0	NA	0	0	NA	-
V:5 (18/F)	0	NA	0	0	NA	-
V:11 (21/F)	0	0	0	0	NA	-
V:2 (24/M)	0	0	0	0	NA	-
IV:7 (37/F)	S	0	0	0	NA	-
IV:15 (45/F)	0	0	0	0	NA	-
IV:3 (46/M)	0	0	retinal detachment	0	NA	-

Table 2

Ratio	Present	Absent	p-value
WT/ES	1.05±0.15	0.8±0.28	0.14
WT/AS	2.14±0.11	2.03±0.16	0.31

Figure 1

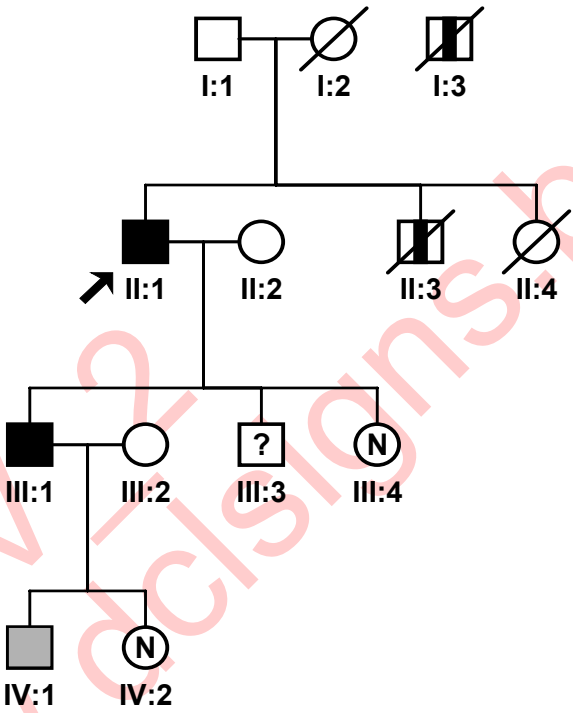


Figure 2

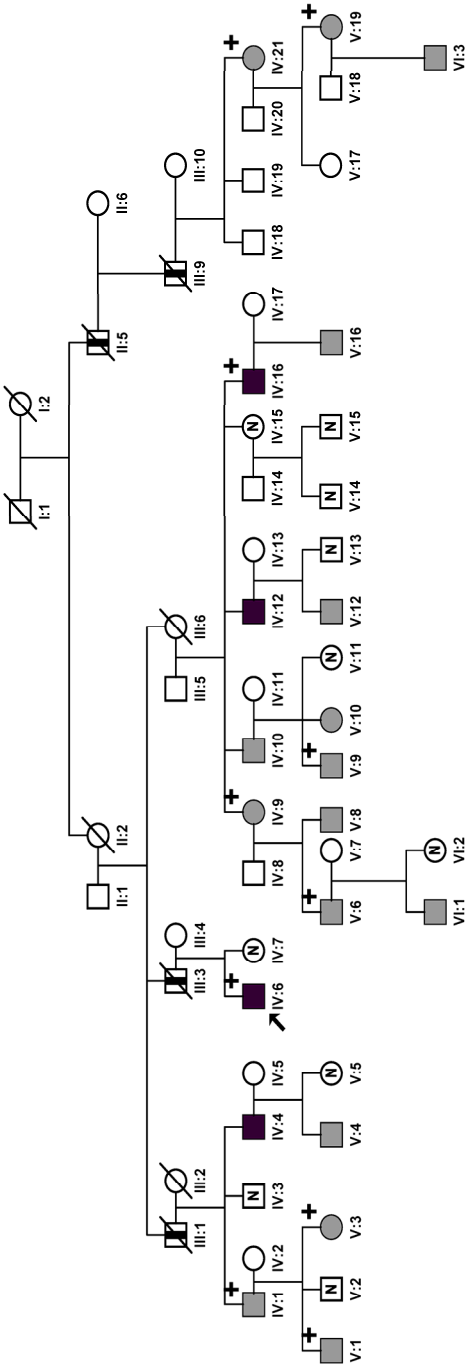

I:1
I:2
II:1
II:2
II:5
II:6
III:1
III:2
III:3
III:4
III:5
III:6
III:9
III:10
IV:1
IV:2
IV:3
IV:4
IV:5
IV:6
IV:7
IV:8
IV:9
IV:10
IV:11
IV:12
IV:13
IV:14
IV:15
IV:16
IV:17
IV:18
IV:19
IV:20
IV:21
V:1
V:2
V:3
V:4
V:5
V:6
V:7
V:8
V:9
V:10
V:11
V:12
V:13
V:14
V:15
V:16
V:17
V:18
V:19
VI:1
VI:2
VI:3

Figure 3

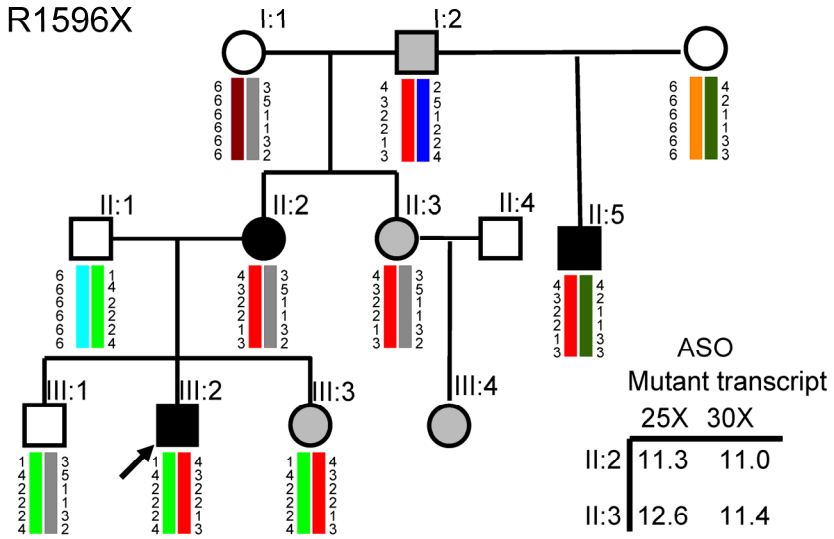
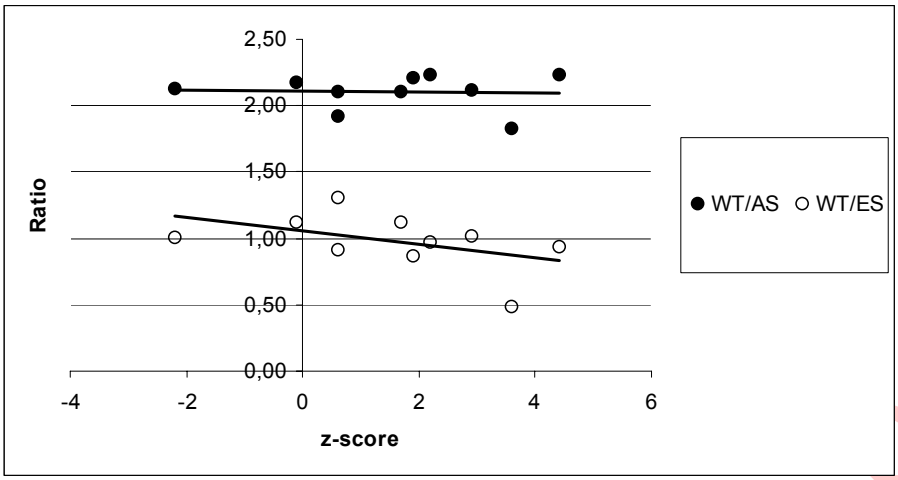


Figure 4



V. Discussion

Cardiovascular complications are the main determinants of life expectancy in Marfan syndrome (MFS). Thanks to improved medical and surgical treatment and follow-up, life expectancy has significantly increased over the past decades.

Despite this, significant morbidity and early mortality persist in MFS. This is related as well to inadequate or delayed diagnosis as to failure of medical or surgical treatments. Furthermore, the ageing MFS patient may develop cardiovascular problems that were unnoticed until now, such as left ventricular dysfunction.

Therefore, research aiming to improve the efficacy of cardiovascular evaluation and the identification of predictors of cardiovascular outcome in MFS is still needed and constitutes the focus of this thesis.

While guidelines for management of major cardiovascular manifestations of MFS such as aortic root dilatation are well established, the diagnosis and management of minor cardiovascular manifestations is still a challenge.

Mitral valve prolapse is the only minor criterion for which clear diagnostic guidelines are available (68). For the other minor criteria such as dilatation of the descending thoracic aorta, dilatation of the MPA and calcification of the mitral valve annulus validated techniques and reference values are lacking. This hampers the use of these criteria in the diagnosis of MFS.

In **chapter 1** of this thesis, we have assessed the different minor cardiovascular features of MFS in order to formulate practical guidelines for correct cardiovascular assessment in MFS patients.

Mitral valve prolapse, calcification of the mitral valve annulus and main pulmonary artery diameter were evaluated with echocardiography in 77 MFS patients (24 children < 14 years and 53 adults ≥ 14years). Diameters of the

descending thoracic and abdominal aorta were assessed with MRI in 29 adult patients.

We observed a high prevalence of MVP in MFS patients (66.2%) with equal distribution of classic and non-classic MVP, which is in line with findings from previous studies (72, 125).

Calcification of the mitral valve annulus has been reported as a cardiovascular manifestation of MFS. However, since correct quantification of calcification cannot be performed with echocardiography and would require computed tomography, we do not recommend the use of this criterion in a screening setting.

Echocardiographic evaluation of the MPA diameter was possible in all children and in all but 3 adult MFS patients. Main pulmonary artery diameter in adult MFS patients (≥ 14 years) was significantly higher than in de control population. This enabled us to define a cut-off value of 23 mm. According to this cut-off value, 94.2% of the MFS patients had MPA dilatation and 85% of the normal control subjects did not have MPA dilatation. In children (<14 years), we preferred the use of Z-scores, since age and BSA significantly influence MPA diameter in this age group. Z-scores according to age and BSA were calculated on the basis of normal values provided by Snider et al (124). Fifty percent of children had MPA dilatation, defined as a Z-score >2.

We observed a significant correlation between the MPA diameter and the diameter of the aortic root (r^2=0.54), although it is noteworthy that some MFS patients with normal aortic root diameters already presented MPA dilatation. This indicates the additional value of assessment of MPA dilatation, since it enables us to identify cardiovascular manifestations before the occurrence of significant aortic root dilatation.

Previously, Nollen and colleagues have measured the MPA diameter in 50 MFS patients using MRI (73). They also found significant differences between MFS patients and controls. Using a cut-off value of 28 mm at the level of the main pulmonary artery root, they reported a prevalence of MPA dilatation of 74% in MFS patients. When applying this cut-off value of 28 mm on our patient group, we would detect MPA dilatation in 80% of patients, which is within the same range as the data from Nollen et al. The small difference in obtained values may be attributable to the different techniques used for the evaluation. A study comparing echocardiography and MRI for this purpose would be very useful to confirm the figures.

The descending aorta, visualized with MRI was larger in MFS patients than in control subjects at all levels, but there was too much overlap to provide reliable cut-off values. Furthermore, imaging of the abdominal aorta requires either computed tomography or MRI scanning, which is not feasible in a screening setting.

We therefore do not recommend assessment the use of descending aortic diameters in the diagnostic assessment for MFS.

When calculating the Z-scores of the thoraco-abdominal aorta, we found that increased Z-scores (≥2) occur in only a minority of patients and are more common in patients who had previously undergone aortic root surgery. Similar findings were reported in earlier studies of the thoraco-abdominal aorta in MFS patients (75, 80). Therefore, we recommend regular imaging of the distal aorta in MFS patients with previous aortic surgery.

Based on our findings, we provide a practical flowchart for the cardiovascular assessment in patients referred for suspicion of MFS.

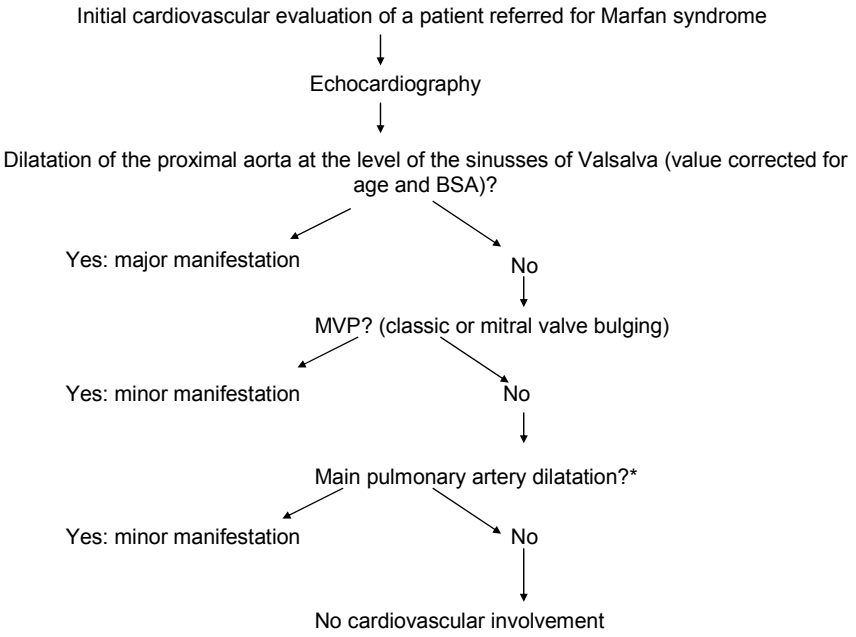


Figure 6: flow chart for the cardiovascular assessment of patients referred for the diagnostic evaluation of MFS.

A subset of patients with MFS develops significant LV dilatation and/or failure, not related to valvular heart disease. Evaluation of LV (dys)function in MFS is important in view of delineating adequate management strategies for follow-up and treatment and for predicting prognosis. Data on LV function in MFS are scarce. Our findings on this issue are provided in **chapter 2.**

The first study, conducted in 234 MFS patients demonstrated that LV dimensions and systolic function assessed with conventional echocardiography were normal in most MFS patients. However, a small proportion of patients presented increased LV dimensions (in 7%) or decreased fractional shortening (in 9%). Whether left ventricular dysfunction is a rare but severe complication of MFS or rather a widespread but generally asymptomatic finding could not be concluded from this study. Furthermore, diastolic function in MFS was not assessed in this study.

Therefore, we conducted a subsequent study of LV systolic and diastolic function in 26 MFS patients and 26 age- and sex matched control subjects, using Tissue Doppler Imaging (TDI) and Magnetic Resonance Imaging (MRI).

In line with the results of the previous study, conventional echocardiographic parameters of LV systolic function were not significantly different between MFS patients and control subjects. However, with MRI and TDI, we observed impairment of both systolic and diastolic function in MFS patients: ejection fraction was reduced, systolic myocardial tissue velocities and diastolic mitral annular velocities assessed with TDI were decreased and the deceleration time of the E wave was prolonged.

Left ventricular *diastolic* function in MFS has been evaluated in a few studies. A first study with MRI conducted by Savolainen and coworkers in 22 MFS children demonstrated impaired LV diastolic function with an increased DT_E and isovolumetric relaxation time ascribed to weakened elastic recoil (83). A subsequent echocardiographic study by Porciani and coworkers showed an unusual pattern of transmitral diastolic flow in which a decreased ventricular compliance (decreased DT_E) and reduced myocardial relaxation (increased isovolumetric relaxation time) coexist (84).

Studies on left ventricular *systolic* function in MFS are even scarcer and are limited to assessment of LV diameters and ejection fraction. Two studies investigated the incidence of LV dilatation and function in a small cohort of MFS patients without valvular regurgitation. Savoilanen and coworkers found no statistically significant difference in LV size and systolic function in 22 children with MFS (83). In a study by Chatrath and coworkers, 7 of the 36 adult patients (19%) showed increased LV dimension with normal LV systolic function (82).

Shortly after publication of our results, another study appeared which confirmed our results regarding diastolic dysfunction in MFS (85). It concerns a

study performed in 40 children and young adults with MFS using echocardiography. Prolonged DT_E and decreased E wave velocity were observed, again suggesting impaired LV relaxation in MFS.

The exact pathogenic mechanism by which microfibrillar deficiency leads to LV dysfunction is unclear at present. Immunohistochemical studies of the myocardium with fibrillin1 targeted antibodies have demonstrated that microfibrils form myofiber-collagen fiber linkages at sites where the power of myocardial contraction is being transmitted to the extracellular connective tissue framework in the myocardium (115). Mutations in the *FBN1* gene may cause structural and/or functional abnormalities in the microfibrils which lead to impairment of myocardial contraction. To prove this, myocardial biopsies would berequired for immunohistological testing in MFS patients – which is not feasible in view of associated risks.

Our findings also indicate the need for further follow up of LV function in MFS patients. Since aortic complications are now treatable to a large extent, it is likely that other problems such as LV dysfunction will gain importance. Our results may also have consequences for medical therapy in these patients who may benefit from drugs supporting myocardial contractile function such as Angiotensin Converting Enzyme inhibitors and/or Angiotensin II type-1 Receptor blockers.

In **chapter 3**, we studied elastic properties of the aorta in MFS, namely PWV and determinants of aortic wave reflection.

We applied a fully non-invasive protocol, combining ultrasound and MRI for wave reflection and central hemodynamics of the aorta. Early return of reflected pressure waves boosts systolic pressure and presents an extra load for the heart and the central vessels. In patients with MFS, this may increase the risk of aortic dilatation and rupture.

To assess whether wave reflection is elevated in MFS, we measured local reflection coefficients along the aorta (characteristic impedance) and their contribution to indices of global wave reflection (augmentation index, AIx). When compared to values obtained in a control population, we found no differences with regards to indices of local wave reflection (characteristic impedance) or global wave reflection (AIx). Our findings indicate that the morphological and functional changes in the (proximal part of the) aorta in patients with MFS do not lead to an increase in local characteristic impedance of the aorta.

These observations are in accordance with the findings from invasive studies by Yin and coworkers (126). A possible explanation is that both vessel calibre and stiffness counterbalance each other, with no net effect on characteristic impedance of the aorta.

We attempted to dissect the AIx into more specific determining factors and could demonstrate that the impact of local reflection properties was negligible. The main determinants of the AIx were PWV and the effective length of the arterial tree, which is in turn directly related to body length. Since both PWV and length of the arterial tree are elevated in MFS patients, they counterbalance each other, leading to no significant effect on the AIx. Our findings suggest that taller MFS patients have better indices of aortic stiffness. Unfortunately, the study was underpowered to detect significant differences according to length. Whether an increased length of the arterial tree and thus increased body length is related to a better prognosis is unknown and requires further study.

Having studied the cardiovascular phenotype in great detail in the first three chapters of this thesis, we studied the correlation between the cardiovascular phenotype and the underlying *FBN1* mutation in **chapter 4**.

Genotype-phenotype correlations are a challenging point of research in MFS. Identifying persons at risk by knowledge of their genotype would reinforce the role of molecular testing in the management of MFS patients. The issue of genotype/phenotype correlation in MFS was addressed in two different ways: firstly, we looked for a relationship between the *FBN1* phenotype and parameters of aortic stiffness and secondly, we performed a detailed phenotypic study of three large MFS families in which the *FBN1* mutation was known and which displayed striking variability in phenotypic severity. In these families, we have also studied the role of the expression level of both *FBN1* alleles in the determination of clinical severity.

Aortic stiffness was estimated through measurement of distensibility and pulse wave velocity with MRI. For the characterization of the genotype, we classified patients according to the type of *FBN1* mutation into two groups (missense and in frame deletions/insertions versus nonsense and out of frame deletions/insertions). Also, a subanalysis comparing nonsense mutations or out of frame deletions/insertions with cystein substitutions was performed. We found no association between the type of *FBN1* mutation and parameters of aortic stiffness. These findings are in line with previous observations from our group, which already indicated that no correlation could be found between the severity of the MFS phenotype and the position or the nature of the *FBN1* gene (35).

We also found that aortic stiffness varies widely among five members of the same family, with a *FBN1* splice site mutation, again showing that the *FBN1* genotype alone does not determine the severity of aortic stiffness.

We also looked for a correlation between parameters of aortic stiffness in MFS patients and a specific polymorphism in the *FBN1* gene (VNTR polymorphism

in exon 28) that had been shown to be associated to aortic stiffness in normal subjects as well as in patients with coronary artery disease (65), (64)

No association was found between any of the assessed aortic stiffness parameters and the *FBN1* polymorphism.

This indicates that other factors outside the *FBN1* gene account for the variation in aortic stiffness in MFS patients (such as modifying gene loci, or environmental factors).

In the second part of chapter 4, we reinforce the observations regarding the absence of genotype/phenotype correlations in MFS by providing detailed clinical findings in three unrelated families with a known *FBN1* mutation and striking intrafamilial variability. The clinical variability observed in these families is another illustration of the lack of genotype/phenotype correlations in MFS, since the same *FBN1* mutation causes a classic MFS phenotype in one subject, whereas his or her relatives, who also harbour this *FBN1* mutation, display only very mild or atypical features. To investigate one of the possible molecular mechanisms underlying the intrafamilial variability, we studied the relative expression of the mutant and the normal *FBN1* alleles in families 2 and 3. We could not demonstrate any correlation between the clinical severity and expression levels of either of the two *FBN1* alleles.

These families clearly demonstrate that the diagnosis in MFS may be challenging because of:

- Clinical overlap with other connective tissue disorders such as Ehlers-Danlos syndrome or Weill-Marchesani syndrome
- Age dependent expression of clinical features particularly relevant for diagnosis of children with MFS
- Atypical or very mild presentation of MFS, for example in patients who have a causal *FBN1* mutation with major involvement of only one or even no organ system and very mild or no involvement of other organ systems

From these studies several practical recommendations can be formulated to optimize the diagnostic process for MFS.

First, routine diagnostic assessment should include thorough clinical evaluation by a clinician with experience in connective tissue disorders in combination with echocardiographic examination and a slitlamp examination of the eyes. Repeated evaluation is necessary in all children with unclear diagnosis until they reach adulthood before definitely excluding a suspicion of MFS. Furthermore, it has previously been illustrated that clinical manifestations, especially in the cardiovascular system may be absent until late adulthood (127). We therefore

believe that lifelong cardiovascular screening is necessary in every patient suspected with MFS, such as patients presenting only one major manifestation.

Second, extensive family history taking, including clinical examination in first degree relatives can be highly contributive to the diagnosis, as illustrated in the first family described in the paper.

Third, additional molecular testing may be very helpful for confirmation or exclusion in patients with atypical presentation of MFS. Molecular testing enabled us to identify subjects with very mild phenotypes; it also enabled us to exclude MFS in family members displaying minor manifestations of MFS.

The molecular mechanisms underlying the striking intrafamilial variability remain largely unknown and require further study of environmental and epigenetic factors.

In conclusion, we have demonstrated that cardiovascular evaluation in patients with MFS should not be limited to measurement of the diameter of the aortic root. In a diagnostic setting, assessment of MVP and measurement of the MPA diameter can be very helpful. Careful study of LV function is needed in MFS in order to optimize treatment and follow-up. We also found that aortic wave reflection, a possible determinant of aortic dilatation, is not increased in MFS patients, due to the counterbalancing effect from increased aortic stiffness and increased length of the arterial tree. In our study, we found no correlation between the severity of the cardiovascular phenotype and the type of *FBN1* mutation, indicating that the *FBN1* genotype alone is not a major determinant of the clinical variability. Finally, we demonstrated the usefulness of additional molecular testing in patients with atypical or mild presentation of MFS.

VI. Future prospects

Several interesting topics for future research in MFS emerge from the results presented in this work

1. Based on our evaluation of minor diagnostic criteria for the cardiovascular organ system in MFS, we recommend further refinement of the current diagnostic criteria (Ghent nosology) for MFS. Our proposed guidelines have already been applied in the discussion at the international MFS nosology meeting in February 2007, and will be used in the finalized version of the revised Ghent nosology.

2. Our observation of impaired LV function in MFS patients needs further study and should be repeated over time in order to evaluate its prognostic value. It also means that development of new treatment strategies to support LV function in MFS are necessary. The findings by Habashi and co-workers demonstrating a clearly beneficial effect of losartan on aortic root dilatation in mice is very promising in this respect (128). Losartan is an angiotensin II type 1 receptor blocker that is already widely used in the treatment of patients with hypertension and heart failure. It is hypothesized that this agent modulates many of the phenotypes occurring in MFS mice through attenuation of TGFβ signalling, including aortic aneurysm formation, pulmonary emphysema and muscle hypotonia. If proven to be effective in MFS patients a beneficial effect of this drug on the LV function can be expected.

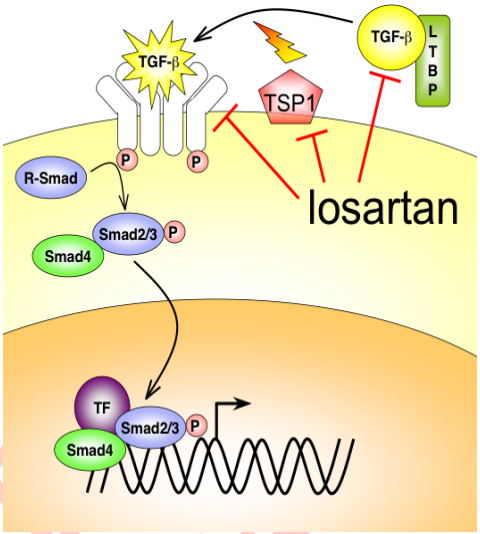
Immunohistochemic studies in MFS mice have demonstrated that losartan acts through modulation of the TGFβ pathway in addition to the known hemodynamic effects of the drug. When compared to β-blockers, similar hemodynamic effects were obtained, whereas the effect on aortic root growth and

histologic abnormalities were significantly more pronounced in the losartan treated mice. It was concluded that β-blockers work in this model as suggested in several studies in humans. They slow but do not halt abnormal aortic growth, and do not appear to directly interface with the underlying pathogenetic mechanism.

In contrast, angiotensin receptor blockers have the potential to completely arrest abnormal aortic growth and may even promote aortic wall remodelling. The similar hemodynamic effects of β-blockers and losartan therapy in this trial are suggestive that the particular protection afforded by angiotensin receptor blockers is not simply modification of the stress imposed on an inherently predisposed tissue, but rather relates to modification of the underlying predisposition presumably through antagonism of TGFβ.

The scheme in figure 7 illustrates the suggested modes of action of losartan.

Therapeutic potential of losartan
(angiotensin II type 1 receptor antagonist)



1. Decreases production of TGFβ
2. Decreases production of TGFβ receptor
3. Decreases production of thrombospondin-1 (TSP1), a potent activator of TGFβ

We have recently been enrolled in a multi-centre study on MFS patients, organized by the Pediatric Heart Disease Clinical Research Network. In this study, patients will be randomized to receive either losartan or atenolol (current standard treatment with β-blocker). The main endpoint of this study is aortic root growth, but an important secondary endpoint is LV function.

3. In the last part of this thesis, we demonstrated that the variation in clinical severity in MFS is not significantly influenced by the type of *FBN1* mutation. These observations clearly indicate the need for further studies to identify additional

modifiers of clinical variability in MFS. It has been demonstrated in several recent studies that fibrillin1 is an important modulator of the TGFβ signalling pathway. Upregulation of TGFβ signalling plays a role in the development of the pulmonary, muscular, cardiovascular and central nervous phenotype in MFS mice (129, 130). We intend to study the contribution of genetic variation in genes encoding components of the TGFβ signalling pathway to the clinical variability in MFS.

To this purpose a Single Nucleotide Polymorphism (SNP) platform will be constructed to study a panel of polymorphic markers in a large group of MFS patients. The correlation between different SNPs in genes involved in the TGFβ pathway and parameters of clinical severity and therapeutic response to β-blockers and losartan will be analysed.

Finally, since this work has clearly demonstrated the benefit of close collaboration between medical disciplines, we hope that further integration of genetics into the practice of cardiovascular medicine will benefit the daily care of MFS and other patients.

VII. Summary

Marfan syndrome (MFS) is a systemic disorder of connective tissue with autosomal dominant inheritance. The diagnosis of MFS is based on the identification of a combination of clinical manifestations in the ocular, musculoskeletal, and cardiovascular organ systems defined in the Ghent Nosology (De Paepe et al, 1996). Confirmation of the diagnosis in an individual requires the presence of major clinical manifestations in at least two organ systems associated with involvement of a third organ system. In relatives of an affected proband, major involvement of one organ system and involvement of a second organ system confirms the diagnosis.

Major clinical criteria are very specific for MFS and include a combination of (4 out of 8) skeletal manifestations, ectopia lentis, dural ectasia and dilatation or dissection of the ascending aorta. The prevalence of- and the guidelines for the assessment of each of these major criteria are well established. Minor clinical criteria are less typical, but their importance in the diagnostic process should not be underestimated. Unfortunately, figures on the prevalence as well as practical guidelines for the assessment of most minor criteria are lacking, especially for those involving the cardiovascular system.

The major cardiovascular manifestation in MFS is a progressive dilatation of the ascending aorta, leading to aortic aneurysm formation and eventually to fatal aortic rupture or dissection. Aortic dissection in early adult life is the leading cause of death in MFS. Early diagnosis of individuals at risk of the disease is extremely important as timely treatment of cardiovascular complications has greatly improved life expectancy in MFS. Despite progress in medical and surgical treatment of aortic aneurysms, MFS continues to be associated with significant morbidity and mortality. This may be related to inadequate diagnosis or treatment, but also to the occurrence of cardiovascular problems in ageing MFS patients that were unrecognised until now, such as left ventricular (LV) dysfunction.

This thesis is focused on the study of cardiovascular manifestations of MFS which localize beyond the aortic root and on the presently unknown relationship between the severity of the cardiovascular phenotype and the genotype.

In **the first part**, we have studied the prevalence and diagnostic value of the following cardiovascular manifestations of MFS: mitral valve prolapse (MVP) and calcification of the mitral valve annulus, dilatation of the main pulmonary artery (MPA) and dilatation or dissection of the descending aorta. We found a significantly higher prevalence of MVP in MFS patients compared to normal controls, indicating that this feature is useful in the diagnostic evaluation of the condition. In contrast, calcification of the mitral valve annulus appears to be very uncommon, difficult to quantify and therefore not useful in the diagnosis of MFS. We also studied the dimension of the MPA in a series of MFS patients and defined a cut-of value that can be used in the diagnostic evaluation of adult MFS patients. In addition, we showed that diameters of the aorta measured at different levels beyond the aortic root are increased in MFS patients compared to controls. Unfortunately, there was too much overlap with the values obtained in the normal control population to provide cut-off values for the descending aorta. Based on these findings, we developed practical guidelines for the cardiovascular evaluation of patients referred for MFS.

In **the second part**, we studied LV function in MFS patients free of valvular heart disease using a combination of echocardiography (both conventional echocardiography and tissue Doppler imaging) and Magnetic Resonance Imaging. We could demonstrate that MFS patients present a combination of systolic and diastolic dysfunction that is not related to valvular heart disease. This may be attributed to a primary contractile dysfunction of the myocardium and is likely related to the underlying alterations in the elastic features of the myocardium, resulting from the microfibrillar defect. This observation is important in the development of new therapeutic strategies for MFS. Affected individuals may benefit from a treatment with agents that support myocardial function such as angiotensin converting enzyme - inhibitors or angiotensin II type-1 receptor blockers. Furthermore, since MFS patients survive longer thanks to improved medical and surgical treatments, LV dysfunction may become an important issue in the follow-up of these patients.

In **the third part**, we have studied aspects of local and global wave reflection in the aorta of MFS patients. Early return of reflected waves boosts systolic pressure and presents an extra load for the heart and the central vessels. As such, these wave reflections are regarded as one of the important determinants of central blood pressure and can contribute to the development of aortic dilatation in MFS.

However, we were unable to demonstrate clear differences in both local and global parameters of wave reflection between MFS patients and normal controls. This could be explained by the fact that increased length of the aorta on the one hand and increased aortic stiffness on the other hand counterbalance each other in MFS patients without yielding any net effect on wave reflection.

In **the last part** of this thesis, we investigated the correlation between the severity of the cardiovascular phenotype in MFS and the type of *FBN1* mutation.

First, we investigated the correlation between parameters of aortic stiffness (distensibility and pulse wave velocity measured by Magnetic Resonance Imaging) and the type of *FBN1* mutation (missense or in-frame deletions/insertions versus nonsense or out-of-frame deletions/insertions). We could not demonstrate any significant differences between these different mutation types, indicating that the *FBN1* genotype is not the sole determinant of aortic stiffness.

Second, we provided a detailed description of clinical findings in three unrelated MFS families in which an *FBN1* mutation was identified and which demonstrate striking intrafamilial phenotypic variability as another illustration of the absence of genotype/phenotype correlations in MFS. This study also illustrated several important issues in MFS. First, repeated clinical examination of suspected patients can be necessary in order to establish a correct and final diagnosis. Second, extensive family history taking and clinical examination of first degree relatives can be highly contributory to the diagnosis. Third, patients with an 'atypical' MFS phenotype may show substantial clinical overlap with other connective tissue disorders such as Weill-Marchesani syndrome or Ehlers-Danlos syndrome and represent a diagnostic challenge. We demonstrated that additional mutational analysis of the *FBN1* gene can be a valuable aid to the diagnosis and help to outline medical management options in these challenging cases.

In conclusion, we have refined diagnostic guidelines for the assessment of minor cardiovascular manifestations in MFS, shown that LV dysfunction is part of the cardiovascular spectrum and should be followed in the management of MFS patients, and demonstrated that aortic wave reflection is not elevated in MFS. In this work, we also investigated genotype/phenotype correlations, illustrated the marked (intrafamilial) variability in phenotypic expression of the condition, and the value of molecular testing in the diagnosis of MFS.

Overall, this thesis nicely illustrates that close interaction and collaboration between cardiology and genetics is an added value to the study of disease pathogenesis of MFS and aortic aneurysms in general.

Samenvatting

Marfan syndrome (MFS) is een bindweefselaandoening die autosomaal dominant wordt overgeërfd. De diagnose van MFS is gebaseerd op specifieke criteria, samengevat in de "Ghent Nosology" (De Paepe et al 1996) en wordt gesteld in aanwezigheid van een combinatie van klinische kenmerken in het oculair, musculoskeletaal en cardiovasculair orgaansysteem. Bevestiging van de diagnose bij een patiënt vereist de aanwezigheid van majeure klinische manifestaties in ten minste twee orgaansystemen samen met betrokkenheid van een derde orgaansysteem. Bij een familielid van een aangetaste proband is de majeure betrokkenheid van één orgaansysteem en betrokkenheid van een tweede orgaansysteem noodzakelijk om de diagnose te stellen.

Majeure klinische criteria zijn zeer specifiek voor MFS en omvatten ectopia lentis, durale ectasie, dilatatie of dissectie van de aorta ascendens en een combinatie van minstens 4 uit een geheel van 8 skeletale manifestaties. De prevalentie van deze majeure manifestaties en de evaluatiemethode om ze vast te stellen zijn goed gekend. Mineure klinische criteria voor MFS zijn echter heel wat minder specifiek. Toch mag het belang van deze mineure criteria in de diagnostiek niet onderschat worden. Jammer genoeg zijn er weinig gegevens over de prevalentie van deze mineure criteria bij MFS en ontbreken ook vaak duidelijke richtlijnen om ze correct te diagnosticeren. Dit is zeker het geval voor de mineure criteria in het cardiovasculair system.

De voornaamste cardiovasculaire manifestatie van MFS is een progressieve dilatatie van de ascenderende aorta, die leidt tot aneurysmavorming en uiteindelijk ruptuur of dissectie van de aorta. Dit gebeurt reeds op jonge volwassen leeftijd en is de voornaamste doodsoorzaak in het MFS. Ondanks de verbetering van zowel de

medische als de chirurgische behandeling van aorta aneurysma kent het MFS nog steeds een belangrijke morbiditeit en mortaliteit. Dit heeft zeker ten dele te maken met een laattijdige diagnose en/of behandeling, maar ook met het optreden van specifieke medische complicaties bij de oudere MFS patënten die tot hier toe niet opgemerkt werden, zoals linker ventrikel (LV) dysfunctie.

Deze thesis beoogt de cardiovasculaire manifestaties van het MFS te bestuderen die buiten de proximale aorta gelocaliseerd zijn en de - op heden niet gekende - relatie tussen de ernst van het cardiovasculaire fenotype en het onderliggende genotype te evalueren.

In het **eerste deel** van deze thesis hebben we de prevalentie en diagnostische waarde van een aantal "mineure" cardiovasculaire manifestaties bestudeerd, met name mitraalklepprolaps en calcificatie van de mitraalklepring, dilatatie van de truncus pulmonalis en dilatatie of dissectie van de descenderende aorta. We vonden een significant hogere prevalentie van mitraalklepprolaps bij MFS patiënten in vergelijking met gezonde controlepersonen, wat erop wijst dat een evaluatie van de mitraalklep zeker zinvol is in de diagnostische evaluatie van MFS. Daarentegen werd calcificatie van de mitraalklepring niet teruggevonden bij onze patiënten en bleek deze manifestatie bovendien moeilijk objectief te evalueren. Daarom beschouwen we dit teken als niet bruikbaar in de diagnostiek van het MFS. De diameter van de truncus pulmonalis werd gemeten met echocardiografie in een reeks MFS patiënten. Op basis hiervan werd een cut-off waarde bepaald voor het definiëren van dilatatie bij volwassen MFS patiënten. Daarnaast hebben we ook aangetoond dat de diameter van de aorta op verschillende niveaus voorbij de proximale aorta toegenomen is bij MFS patiënten in vergelijking met normale controles. Omwille van een te grote overlap tussen beide groepen kon geen bruikbare cut-off waarde van de diameter van de descenderende aorta bepaald worden in MFS patiënten. Op basis van onze bevindingen hebben we praktische richtlijnen opgesteld voor de cardiovasculaire evaluatie bij patiënten die verwezen worden voor MFS.

In het **tweede deel** hebben we de LV functie bestudeerd bij patiënten met MFS met een combinatie van echocardiografie (zowel conventionele echocardiografie als tissue Doppler imaging) en Nucleair Magnetische Resonantie. We hebben aangetoond dat er bij MFS patiënten systolische en diastolische disfunctie aanwezig is die niet door kleplijden kan verklaard worden. We schrijven dit toe aan een primaire contractiele dysfunctie van het myocard die waarschijnlijk gerelateerd is aan de onderliggende veranderingen in de elastische eigenschappen van het myocard als gevolg van het microfibrillaire defect. Deze observatie is

belangrijk met het oog op de ontwikkeling van nieuwe behandelingen voor het MFS. Marfan patiënten zouden baat kunnen hebben bij een behandeling met medicatie die de myocardiale functie ondersteunt zoals angiotensine converting enzyme inhibitoren of angiotensine II receptor blokkers. Het valt te verwachten dat LV dysfunctie een belangrijke complicaties zal zijn bij de oudere MFS patiënt.

In het **derde deel** hebben we aspecten van lokale en globale golfreflectie in de aorta bestudeerd bij MFS patiënten. Vroegtijdige terugkeer van gereflecteerde golven verhoogt de systolische bloeddruk en veroorzaakt een extra belasting voor het hart en de centrale bloedvaten. Deze golfreflecties worden dus beschouwd als belangrijke determinanten van de centrale bloeddruk en zouden kunnen meespelen in het ontwikkelen van aortadilatatie bij MFS. Wij konden evenwel geen belangrijke verschillen aantonen in zowel lokale als globale parameters van golfreflectie tussen MFS patiënten en normale controlepersonen. Dit zou kunnen verklaard worden door het feit dat een toegenomen lengte van de aorta enerzijds en een toegenomen stijfheid van de aorta anderzijds mekaar opheffen, waardoor er geen netto effect bestaat op de golfreflectie.

In het **laatste deel** van deze thesis hebben we gezocht naar correlaties tussen het cardiovasculaire fenotype en de onderliggende *FBN1* mutatie.

Eerst hebben we de correlatie bestudeerd tussen parameters van aorta stijfheid (distensibiliteit en pulse wave velocity gemeten met nucleair magnetische resonantie) en het type *FBN1* mutatie (missense of in-frame deletie/insertie versus nonsens of out-of frame deletie/insertie). We konden geen significante verschillen aantonen wat betreft aortastijfheid tussen deze verschillende types mutatie, wat erop wijst dat het type *FBN1* mutatie niet de enige determinant is van aorta stijfheid.

Daarnaast hebben we een gedetailleerde beschrijving gegeven van de klinische bevindingen in drie niet-gerelateerde MFS families waarbij een onderliggende *FBN1* mutatie werd aangetoond. De opvallende intrafamiliale fenotypische variabiliteit in deze families illustreert de afwezigheid van genotype/fenotype correlaties in het MFS.

Deze studie brengt ook een aantal belangrijke management aspecten van MFS in aandacht. Ze toont duidelijk aan dat herhaald klinisch onderzoek soms noodzakelijk is alvorens de diagnose van MFS definitief kan bevestigd worden. In bepaalde gevallen is uitgebreide familiale anamnese en klinisch onderzoek bij eerstegraadsverwanten onontbeerlijk om de diagnose te kunnen stellen. Patiënten met een “atypisch” MFS fenotype kunnen belangrijke overlap tonen met andere bindweefselaandoeningen zoals Weill-Marchesani syndroom of Ehlers-Danlos syndroom. In deze gevallen kan aanvullend moleculair genetisch onderzoek een belangrijke additionele waarde hebben in de diagnostiek van het MFS.

Samenvattend hebben wij in dit werk de diagnostische richtlijnen voor evaluatie van de mineure cardiovasculaire manifestaties verfijnd; we hebben aangetoond dat LV dysfunctie deel uitmaakt van het cardiovasculair spectrum bij MFS patiënten en dient opgevolgd te worden; we hebben aangetoond dat golfreflectie in de aorta niet toegenomen is bij MFS patiënten, in tegenstelling tot de verwachtingen. Tenslotte hebben we ook genotype/fenotype correlaties bestudeerd, de uitgesproken (intrafamiliale) variabiliteit in de fenotypische expressie geillustreerd evenals de waarde van moleculair genetisch onderzoek in de diagnose van MFS.

Deze thesis illustreert zeer mooi de meerwaarde van een nauwe samenwerking tussen geneticus en cardioloog in de medische zorg voor Marfan patiënten en hun families.

Résumé

Le syndrome de Marfan (SMF) est une maladie des tissus conjonctifs qui se transmet sur un mode autosomique dominant. Le diagnostic du SMF se fait par identification de plusieurs manifestations cliniques dans différents systèmes : oculaires, musculo-squelettiques et cardiovasculaires définies dans le « Ghent Nosology » (De Paepe, 1996).

Pour faire le diagnostic du patient SMF il faut une atteinte de trois systèmes dont au moins deux avec des signes majeurs. Pour le parent d'un individu atteint, il suffit d'une atteinte de deux systèmes avec un seul signe majeur.

Pour faire le diagnostic du SMF il faut trouver des signes cliniques majeurs bien spécifiés et une combinaison d'au moins quatre des huit critères majeurs tels que une atteinte du squelette, une luxation du cristallin, une ectasie durale et une dilatation ou dissection de l'aorte ascendante. La fréquence ainsi que la façon d'évaluation de ces critères majeurs a été bien établie.

Les critères mineurs sont moins typiques, mais leur importance pour le diagnostic ne doit pas être sous-estimée. Malheureusement il n'y a pas de données sur leur fréquence ni de guide pratique pour l'évaluation de ces critères mineurs, en particulier de ceux concernant le système cardiovasculaire.

La manifestation cardiovasculaire majeure dans le SMF est une dilatation progressive de l'aorte ascendante, allant jusqu'à la formation d'un anévrysme aortique et éventuellement à une rupture ou une dissection aortique fatale. La dissection aortique chez le jeune adulte SMF est la cause principale de décès. Le diagnostic précoce d'individus à risque de cette maladie est très important car le traitement prompt des complications cardiovasculaires augmente considérablement l'espérance de vie. Malgré le progrès dans le traitement médical et chirurgical d'anévrysmes aortiques, le SMF reste associé à une morbidité et mortalité

significative. Ceci peut être relaté à un diagnostic difficile ou à traitement inadapté, mais aussi à la présence de problèmes cardiovasculaires inconnus jusqu'à présent, comme entre autre une dysfonction du ventricule gauche.

Cette thèse se concentre sur l'étude des manifestations cardiovasculaires qui sont en dehors de l'aorte proximale et sur la recherche d'une corrélation, jusqu'à ce jour inconnue, entre la gravité du phénotype cardiovasculaire et le génotype associé.

Dans une première partie, nous avons étudié la prévalence et la valeur diagnostique des manifestations cardiovasculaires telles que le prolapsus de la valve mitrale, la calcification de l'anneau de la valve mitrale, la dilatation de l'artère pulmonaire et enfin la dilatation ou la dissection de l'aorte descendante.

Nous avons trouvé une prévalence significativement élevée du prolapsus de la valve mitrale dans les patients SMF comparé à des contrôles normaux, indiquant que cette constatation est utile pour le diagnostic de la maladie.

Par contre, la calcification de l'anneau de la valve mitrale paraît très rare, difficile à quantifier et donc inutile pour le diagnostic.

Nous avons aussi étudié la dimension de l'artère pulmonaire dans une série de patients et défini une valeur limite utile pour l'évaluation des patients SMF adultes.

En plus, nous avons démontré que le diamètre de l'aorte mesuré à différents niveaux plus distaux est élevé chez les patients SMF comparé à un groupe contrôle. Malheureusement, il y avait trop de chevauchement des taux obtenus dans la population contrôle pour définir une valeur limite.

Ces résultats nous ont amenés à développer un guide pratique d'évaluation cardiovasculaire de patients supposés atteints du SMF.

Dans la seconde partie, nous avons étudié la fonction du ventricule gauche chez des patients SMF sans anomalies valvulaires en utilisant l'échocardiographie (conventionnelle et par Doppler tissulaire) et l'IRM. Nous avons pu démontrer que ces patients présentent une combinaison de dysfonction systolique et diastolique non liée à des anomalies valvulaires. Ceci peut être attribué à une dysfonction contractile primaire du myocarde qui est probablement relaté à des altérations sous-jacentes des tissus élastiques suite à un défaut dans le réseau microfibrillaire. Cette observation est importante pour le développement de nouvelles stratégies thérapeutiques du SMF.

Des individus atteints pourraient bénéficier d'un traitement avec des inhibiteurs de l'enzyme de conversion et antagonistes de récepteurs de l'angiotensine II. En plus, puisque les patients SMF survivent actuellement plus longtemps grâce au traitement médical et chirurgical amélioré, la dysfonction du ventricule gauche pourrait devenir un facteur important dans le suivi.

En troisième partie, nous avons étudié la réflexion d'ondes locales et globales dans l'aorte de patients SMF.

Le retour prématuré des ondes réfléchies augmente la pression systolique et représente une charge élevée pour le cœur et les vaisseaux centraux. Dans ce cas-ci, ces ondes réfléchies sont vues comme des déterminants importants de la pression centrale et ceci pourrait contribuer au développement de la dilatation aortique dans le SMF. En revanche, nous n'avons pas pu démontrer de différence nette dans les paramètres locaux et globaux de réflexion d'ondes entre des patients SMF et des contrôles normaux.

Ceci pourrait s'expliquer par la présence chez les patients SMF d'une aorte d'une part plus longue et d'autre part plus rigide qui se compensent sans produire d'effet net sur la réflexion d'ondes.

Dans la dernière partie de cette thèse, nous avons cherché une corrélation entre la sévérité du phénotype cardiovasculaire et le type de mutation du gène de la fibrilline1 (FBN1).

En premier lieu, nous avons étudié la corrélation entre les paramètres de rigidité aortique comme la « pulse wave » vélocité et la distensibilité artérielle mesurées en IRM et le type de mutation FBN1 : mutation non-sens ou délétions/insertions » in-frame » versus « out-of-frame ». Nous n'avons pu démontrer aucune différence entre ces différents types de mutation, indiquant que le génotype FBN1 n'est pas l'unique responsable de la rigidité aortique.

En second lieu, nous avons donné une description détaillée des résultats cliniques dans trois familles SMF non apparentées, ayant une mutation FBN1 mais ayant une hétérogénéité phénotypique intrafamiliale prononcée comme autre illustration de l'absence de corrélation génotype/phénotype dans le SMF.

Cette étude illustre aussi plusieurs autres aspects importants du suivi des patients SMF.

Primo : un examen clinique régulier des patients suspects peut être nécessaire pour faire un diagnostic correct et définitif.

Secundo : une anamnèse familiale approfondie et un examen clinique des parents du premier degré pourraient aider fortement au diagnostic.

Tercio : le patient au phénotype 'atypique' du SMF peut montrer des symptômes cliniques communs avec d'autres maladies comme le syndrome de Weill-Marchesani ou le syndrome d'Ehlers-Danlos, ce qui rend le diagnostic difficile.

Nous avons montré que l'analyse moléculaire du gène FBN1 peut être précieuse et aide à faciliter la gestion médicale de ces cas difficiles.

En conclusion, nous avons raffiné les critères diagnostiques pour l'évaluation des manifestations mineures cardiovasculaires dans le SMF ; nous avons démontré que la dysfonction du ventricule gauche fait partie du spectre cardiovasculaire et devrait être suivi dans les patients SMF, et que la réflexion des ondes aortiques n'est pas élevée chez les patients SMF. Nous avons aussi étudié les corrélations génotype/phénotype et illustré la variabilité (intrafamiliale) prononcée dans l'expression phénotypique de cette condition ainsi que la valeur de l'analyse moléculaire dans le diagnostic du SMF.

En résumé, cette thèse démontre bien que l'interaction minutieuse et la collaboration entre cardiologues et généticiens est une valeur ajoutée à l'étude de la pathogenèse du SFM et de l'anévrysme aortique en général.

VIII. REFERENCES

1. Nabel EG. Cardiovascular Disease. N Engl J Med. 2003 July 3, 2003;349(1):60-72.
2. Dietz HC, Cutting GR, Pyeritz RE, Maslen CL, Sakai LY, Corson GM, et al. Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. Nature. 1991 Jul 25;352(6333):337-9.
3. Wernly J. Thoracic aorta disease. In: Crawford M, DiMarco J, editors. Cardiology: Mosby International; 2001. p. 1.12.1-.
4. Lilienfeld DE, Gunderson PD, Sprafka JM, Vargas C. Epidemiology of aortic aneurysms: I. Mortality trends in the United States, 1951 to 1981. Arteriosclerosis. 1987 Nov-Dec;7(6):637-43.
5. Rosenbloom J, Abrams WR, Mecham R. Extracellular matrix 4: the elastic fiber. Faseb J. 1993 Oct;7(13):1208-18.
6. Robinson PN, Godfrey M. The molecular genetics of Marfan syndrome and related microfibrillopathies. J Med Genet. 2000 Jan;37(1):9-25.
7. Dobrin PB. Mechanics of normal and diseased blood vessels. Ann Vasc Surg. 1988 Jul;2(3):283-94.
8. Erdheim J. Medionecrosis aortae idiopathica cystica. Virchows Arch path Anat. 1930;276:187.
9. Guo D, Hasham S, Kuang SQ, Vaughan CJ, Boerwinkle E, Chen H, et al. Familial thoracic aortic aneurysms and dissections : genetic heterogeneity with a major locus mapping to 5q13-14. Circulation. 2001;103(20):2461-8.
10. Schlatmann TJ, Becker AE. Pathogenesis of dissecting aneurysm of aorta. Comparative histopathologic study of significance of medial changes. Am J Cardiol. 1977 Jan;39(1):21-6.
11. Carlson RG, Lillehei CW, Edwards JE. Cystic medial necrosis of the ascending aorta in relation to age and hypertension. Am J Cardiol. 1970 Apr;25(4):411-5.

12. Nataatmadja M, West M, West J, Summers K, Walker P, Nagata M, et al. Abnormal extracellular matrix protein transport associated with increased apoptosis of vascular smooth muscle cells in marfan syndrome and bicuspid aortic valve thoracic aortic aneurysm. Circulation. 2003 Sep 9;108 Suppl 1:II329-34.

13. Tan JL, Davlouros PA, McCarthy KP, Gatzoulis MA, Ho SY. Intrinsic histological abnormalities of aortic root and ascending aorta in tetralogy of Fallot: evidence of causative mechanism for aortic dilatation and aortopathy. Circulation. 2005 Aug 16;112(7):961-8.

14. Verloes A, Sakalihasan N, Koulischer L, Limet R. Aneurysms of the abdominal aorta: familial and genetic aspects in three hundred thirteen pedigrees. J Vasc Surg. 1995 Apr;21(4):646-55.

15. Norrgard O, Rais O, Angquist KA. Familial occurrence of abdominal aortic aneurysms. Surgery. 1984 Jun;95(6):650-6.

16. Hasham SN, Guo DC, Milewicz DM. Genetic basis of thoracic aortic aneurysms and dissections. Curr Opin Cardiol. 2002 Nov;17(6):677-83.

17. Biddinger A, Rocklin M, Coselli J, Milewicz DM. Familial thoracic aortic dilatations and dissections: a case control study. J Vasc Surg. 1997 Mar;25(3):506-11.

18. Coady MA, Davies RR, Roberts M, Goldstein LJ, Rogalski MJ, Rizzo JA, et al. Familial patterns of thoracic aortic aneurysms. Arch Surg. 1999 Apr;134(4):361-7.

19. Pyeritz RE, McKusick VA. The Marfan syndrome: diagnosis and management. N Engl J Med. 1979 Apr 5;300(14):772-7.

20. Pyeritz RE. The Marfan syndrome. Annu Rev Med. 2000;51:481-510.

21. Gray JR, Bridges AB, Faed MJ, Pringle T, Baines P, Dean J, et al. Ascertainment and severity of Marfan syndrome in a Scottish population. J Med Genet. 1994 Jan;31(1):51-4.

22. De Paepe A, Devereux RB, Dietz HC, Hennekam RC, Pyeritz RE. Revised diagnostic criteria for the Marfan syndrome. Am J Med Genet. 1996;62(4):417-26.

23. Hutchinson S, Furger A, Halliday D, Judge DP, Jefferson A, Dietz HC, et al. Allelic variation in normal human FBN1 expression in a family with Marfan syndrome: a potential modifier of phenotype? Hum Mol Genet. 2003 Sep 15;12(18):2269-76.

24. Collod-Beroud G, Le Bourdelles S, Ades L, Ala-Kokko L, Booms P, Boxer M, et al. Update of the UMD-FBN1 mutation database and creation of an FBN1 polymorphism database. Hum Mutat. 2003 Sep;22(3):199-208.

25. Biery NJ, Eldadah ZA, Moore CS, Stetten G, Spencer F, Dietz HC. Revised genomic organization of FBN1 and significance for regulated gene expression. Genomics. 1999 Feb 15;56(1):70-7.

26. Reinhardt DP, Ono RN, Sakai LY. Calcium stabilizes fibrillin-1 against proteolytic degradation. J Biol Chem. 1997 Jan 10;272(2):1231-6.

27. Biggin A, Holman K, Brett M, Bennetts B, Ades L. Detection of thirty novel FBN1 mutations in patients with Marfan syndrome or a related fibrillinopathy. Hum Mutat. 2004 Jan;23(1):99.

28. Robinson PN, Booms P, Katzke S, Ladewig M, Neumann L, Palz M, et al. Mutations of FBN1 and genotype-phenotype correlations in Marfan syndrome and related fibrillinopathies. Hum Mutat. 2002 Sep;20(3):153-61.

29. Loeys B, De Backer J, Van Acker P, Wettinck K, Pals G, Nuytinck L, et al. Comprehensive molecular screening of the FBN1 gene favors locus homogeneity of classical Marfan syndrome. Hum Mutat. 2004 Aug;24(2):140-6.

30. Tsipouras P, Del Mastro R, Sarfarazi M, Lee B, Vitale E, Child AH, et al. Genetic linkage of the Marfan syndrome, ectopia lentis, and congenital contractural arachnodactyly to the fibrillin genes on chromosomes 15 and 5. The International Marfan Syndrome Collaborative Study. N Engl J Med. 1992 Apr 2;326(14):905-9.

31. Kainulainen K, Steinmann B, Collins F, Dietz HC, Francomano CA, Child A, et al. Marfan syndrome: no evidence for heterogeneity in different populations, and more precise mapping of the gene. Am J Hum Genet. 1991 Sep;49(3):662-7.

32. Byers PH, Cole WG. Osteogenesis Imperfecta. In: Royce PM, Steinman B, editors. Connective Tissue and its inheritable disorders Molecular, Genetic, and medical aspects. Second ed. New York; 2002. p. 409.

33. Schrijver I, Liu W, Brenn T, Furthmayr H, Francke U. Cysteine substitutions in epidermal growth factor-like domains of fibrillin-1: distinct effects on biochemical and clinical phenotypes. Am J Hum Genet. 1999 Oct;65(4):1007-20.

34. Schrijver I, Liu W, Odom R, Brenn T, Oefner P, Furthmayr H, et al. Premature termination mutations in FBN1: distinct effects on differential allelic expression and on protein and clinical phenotypes. Am J Hum Genet. 2002 Aug;71(2):223-37.

35. Loeys B, Nuytinck L, Delvaux I, De Bie S, De Paepe A. Genotype and phenotype analysis of 171 patients referred for molecular study of the fibrillin-1 gene FBN1 because of suspected Marfan syndrome. Arch Intern Med. 2001 Nov 12;161(20):2447-54.

36. Kainulainen K, Karttunen L, Puhakka L, Sakai L, Peltonen L. Mutations in the fibrillin gene responsible for dominant ectopia lentis and neonatal Marfan syndrome. Nat Genet. 1994 Jan;6(1):64-9.

37. Putnam EA, Cho M, Zinn AB, Towbin JA, Byers PH, Milewicz DM. Delineation of the Marfan phenotype associated with mutations in exons 23-32 of the FBN1 gene. Am J Med Genet. 1996 Mar 29;62(3):233-42.

38. Tiecke F, Katzke S, Booms P, Robinson PN, Neumann L, Godfrey M, et al. Classic, atypically severe and neonatal Marfan syndrome: twelve mutations and

genotype-phenotype correlations in FBN1 exons 24-40. Eur J Hum Genet. 2001 Jan;9(1):13-21.

39. Palz M, Tiecke F, Booms P, Goldner B, Rosenberg T, Fuchs J, et al. Clustering of mutations associated with mild Marfan-like phenotypes in the 3' region of FBN1 suggests a potential genotype-phenotype correlation. Am J Med Genet. 2000 Mar 20;91(3):212-21.

40. Groenink M, Lohuis TA, Tijssen JG, Naeff MS, Hennekam RC, van der Wall EE, et al. Survival and complication free survival in Marfan's syndrome: implications of current guidelines. Heart. 1999;82(4):499-504.

41. Judge DP, Dietz HC. Marfan's syndrome. Lancet. 2005 Dec 3;366(9501):1965-76.

42. Roman MJ, Devereux RB, Kramer-Fox R, O'Loughlin J. Two-dimensional echocardiographic aortic root dimensions in normal children and adults. American journal of cardiology, The. 1989;64(8):507-12.

43. Dubois Da. A formula to estimate the approximate surface area if height and weight be known. Archives of Internal Medicine. 1916;17:863.

44. Sheil ML, Jenkins O, Sholler GF. Echocardiographic assessment of aortic root dimensions in normal children based on measurement of a new ratio of aortic size independent of growth. Am J Cardiol. 1995 Apr 1;75(10):711-5.

45. Rozendaal L, Groenink M, Naeff MS, Hennekam RC, Hart AA, van-der-Wall EE, et al. Marfan syndrome in children and adolescents: an adjusted nomogram for screening aortic root dilatation. Heart. 1998;79(1):69-72.

46. Apter JT. Correlation of visco-elastic properties with microscopic structure of large arteries. IV. Thermal responses of collagen, elastin, smooth muscle, and intact arteries. Circ Res. 1967 Dec;21(6):901-18.

47. Pyeritz RE. Marfan syndrome: current and future clinical and genetic management of cardiovascular manifestations. Semin Thorac Cardiovasc Surg. 1993 Jan;5(1):11-6.

48. Roman MJ, Rosen SE, Kramer-Fox R, Devereux RB. Prognostic significance of the pattern of aortic root dilation in the Marfan syndrome. J Am Coll Cardiol. 1993;22(5):1470-6.

49. Dapunt OE, Galla JD, Sadeghi AM, Lansman SL, Mezrow CK, de Asla RA, et al. The natural history of thoracic aortic aneurysms. J Thorac Cardiovasc Surg. 1994 May;107(5):1323-32; discussion 32-3.

50. Pyeritz RE. Genetics and Cardiovascular Disease. In: Braunwald E, editor. Cardiovascular Disease. Philadelphia: Saunders; 1992. p. 1641-3.

51. Legget ME, Unger TA, O'Sullivan CK, Zwink TR, Bennett RL, Byers PH, et al. Aortic root complications in Marfan's syndrome: identification of a lower risk group. Heart. 1996 Apr;75(4):389-95.

52. Silverman DI, Gray J, Roman MJ, Bridges A, Burton K, Boxer M, et al. Family history of severe cardiovascular disease in Marfan syndrome is associated with

increased aortic diameter and decreased survival. Journal of the American College of Cardiology. 1995;26(4):1062-7.

53. Jondeau G, Boutouyrie P, Lacolley P, Laloux B, Dubourg O, Bourdarias JP, et al. Central pulse pressure is a major determinant of ascending aorta dilation in Marfan syndrome. Circulation. 1999;99(20):2677-81.

54. Adams JN, Brooks M, Redpath TW, Smith FW, Dean J, Gray J, et al. Aortic distensibility and stiffness index measured by magnetic resonance imaging in patients with Marfan's syndrome. Br Heart J. 1995;73(3):265-9.

55. Groenink M, de Roos A, Mulder BJ, Verbeeten B, Jr., Timmermans J, Zwinderman AH, et al. Biophysical properties of the normal-sized aorta in patients with Marfan syndrome: evaluation with MR flow mapping. Radiology. 2001;219(2):535-40.

56. Hirata K, Triposkiadis F, Sparks E, Bowen J, Boudoulas H, Wooley CF. The Marfan syndrome: cardiovascular physical findings and diagnostic correlates. Am Heart J. 1992;123(3):743-52.

57. Jeremy RW, Huang H, Hwa J, McCarron H, Hughes CF, Richards JG. Relation between age, arterial distensibility, and aortic dilatation in the Marfan syndrome. Am J Cardiol. 1994;74(4):369-73.

58. Nollen GJ, Groenink M, Tijssen JG, Van Der Wall EE, Mulder BJ. Aortic stiffness and diameter predict progressive aortic dilatation in patients with Marfan syndrome. Eur Heart J. 2004 Jul;25(13):1146-52.

59. Baumgartner D, Baumgartner C, Matyas G, Steinmann B, Loffler-Ragg J, Schermer E, et al. Diagnostic power of aortic elastic properties in young patients with Marfan syndrome. J Thorac Cardiovasc Surg. 2005 Apr;129(4):730-9.

60. Blacher J, Guerin AP, Pannier B, Marchais SJ, Safar ME, London GM. Impact of aortic stiffness on survival in end-stage renal disease. Circulation. 1999 May 11;99(18):2434-9.

61. Laurent S, Boutouyrie P, Asmar R, Gautier I, Laloux B, Guize L, et al. Aortic stiffness is an independent predictor of all-cause and cardiovascular mortality in hypertensive patients. Hypertension. 2001 May;37(5):1236-41.

62. Meaume S, Benetos A, Henry OF, Rudnichi A, Safar ME. Aortic pulse wave velocity predicts cardiovascular mortality in subjects >70 years of age. Arterioscler Thromb Vasc Biol. 2001 Dec;21(12):2046-50.

63. Cruickshank K, Riste L, Anderson SG, Wright JS, Dunn G, Gosling RG. Aortic pulse-wave velocity and its relationship to mortality in diabetes and glucose intolerance: an integrated index of vascular function? Circulation. 2002 Oct 15;106(16):2085-90.

64. Powell JT, Turner RJ, Henney AM, Miller GJ, Humphries SE. An association between arterial pulse pressure and variation in the fibrillin-1 gene. Heart. 1997 Oct;78(4):396-8.

65. Medley TL, Cole TJ, Gatzka CD, Wang WY, Dart AM, Kingwell BA. Fibrillin-1 genotype is associated with aortic stiffness and disease severity in patients with coronary artery disease. Circulation. 2002 Feb 19;105(7):810-5.

66. Yasmin, Wilkinson IB, O'Shaughnessy KM, Lanne T, De Basso R, Powell JT. Influence of fibrillin-1 genotype on aortic stiffness in men: a note of caution. J Appl Physiol. 2006 April 1, 2006;100(4):1431-2.

67. Yasmin, O'Shaughnessy KM, McEniery CM, Cockcroft JR, Wilkinson IB. Genetic variation in fibrillin-1 gene is not associated with arterial stiffness in apparently healthy individuals. J Hypertens. 2006 Mar;24(3):499-502.

68. Freed LA, Levy D, Levine RA, Larson MG, Evans JC, Fuller DL, et al. Prevalence and clinical outcome of mitral-valve prolapse. N Engl J Med. 1999;341(1):1-7.

69. Levine R, Handschumacher M, Sanfilippo A, Hagege A, Harrigan P, Marshall J, et al. Three-dimensional echocardiographic reconstruction of the mitral valve, with implications for the diagnosis of mitral valve prolapse. Circulation. 1989 September 1, 1989;80(3):589-98.

70. Pyeritz RE, Wappel MA. Mitral valve dysfunction in the Marfan syndrome. Clinical and echocardiographic study of prevalence and natural history. Am J Med. 1983 May;74(5):797-807.

71. van Karnebeek CD, Naeff MS, Mulder BJ, Hennekam RC, Offringa M. Natural history of cardiovascular manifestations in Marfan syndrome. Arch Dis Child. 2001 Feb;84(2):129-37.

72. Yetman AT, Bornemeier RA, McCrindle BW. Long-term outcome in patients with Marfan syndrome: is aortic dissection the only cause of sudden death? J Am Coll Cardiol. 2003 Jan 15;41(2):329-32.

73. Nollen GJ, van Schijndel KE, Timmermans J, Groenink M, Barentsz JO, van der Wall EE, et al. Pulmonary artery root dilatation in Marfan syndrome: quantitative assessment of an unknown criterion. Heart. 2002 May;87(5):470-1.

74. Dietz HC, Pyeritz RE. Marfan syndrome and related disorders. In: Scriver CR BA, Sly WS, Valle D, editor. The metabolic and molecular bases of inherited diseases; 1995. p. 5287-311.

75. Finkbohner R, Johnston D, Crawford ES, Coselli J, Milewicz DM. Marfan Syndrome : Long-term Survival and Complications After Aortic Aneurysm Repair. Circulation. 1995 February 1, 1995;91(3):728-33.

76. Engelfriet PM, Boersma E, Tijssen JG, Bouma BJ, Mulder BJ. Beyond the root: Dilatation of the distal aorta in the marfan syndrome. Heart. 2006 Feb 17.

77. van Ooijen B. Marfan's syndrome and isolated aneurysm of the abdominal aorta. Br Heart J. 1988 Jan;59(1):81-4.

78. Pruzinsky MS, Katz NM, Green CE, Satler LF. Isolated descending thoracic aortic aneurysm in Marfan's syndrome. Am J Cardiol. 1988 May 1;61(13):1159-60.

79. Finkbohner R, Johnston D, Crawford ES, Coselli J, Milewicz DM. Marfan syndrome. Long-term survival and complications after aortic aneurysm repair. Circulation. 1995 Feb 1;91(3):728-33.

80. Kawamoto S, Bluemke DA, Traill TA, Zerhouni EA. Thoracoabdominal aorta in Marfan syndrome: MR imaging findings of progression of vasculopathy after surgical repair. Radiology. 1997 Jun;203(3):727-32.

81. Nollen GJ, Meijboom LJ, Groenink M, Timmermans J, Barentsz JO, Merchant N, et al. Comparison of aortic elasticity in patients with the marfan syndrome with and without aortic root replacement. Am J Cardiol. 2003 Mar 1;91(5):637-40.

82. Chatrath R, Beauchesne LM, Connolly HM, Michels VV, Driscoll DJ. Left ventricular function in the Marfan syndrome without significant valvular regurgitation. Am J Cardiol. 2003 Apr 1;91(7):914-6.

83. Savolainen A, Nisula L, Keto P, Hekali P, Viitasalo M, Kaitila I, et al. Left ventricular function in children with the Marfan syndrome. Eur Heart J. 1994;15(5):625-30.

84. Porciani MC, Giurlani L, Chelucci A, Pepe G, Giusti BH, Brunelli T, et al. Diastolic subclinical primary alterations in Marfan syndrome and Marfan-related disorders. Clin Cardiol. 2002 Sep;25(9):416-20.

85. Das BB, Taylor AL, Yetman AT. Left ventricular diastolic dysfunction in children and young adults with Marfan syndrome. Pediatr Cardiol. 2006 Mar-Apr;27(2):256-8.

86. McKusick VA. The cardiovascular aspects of Marfan's syndrome: a heritable disorder of connective tissue. Circulation. 1955;11:321-42.

87. Murdoch JL, Walker BA, Halpern BL, Kuzma JW, McKusick VA. Life expectancy and causes of death in the Marfan syndrome. N Engl J Med. 1972 Apr 13;286(15):804-8.

88. Marsalese DL, Moodie DS, Vacante M, Lytle BW, Gill CC, Sterba R, et al. Marfan's syndrome: natural history and long-term follow-up of cardiovascular involvement. J Am Coll Cardiol. 1989 Aug;14(2):422-8; discussion 9-31.

89. Silverman DI, Burton KJ, Gray J, Bosner MS, Kouchoukos NT, Roman MJ, et al. Life expectancy in the Marfan syndrome. Am J Cardiol. 1995;75(2):157-60.

90. Silverman DI, Burton KJ, Gray J, Bosner MS, Kouchoukos NT, Roman MJ, et al. Life expectancy in the Marfan syndrome. Am J Cardiol. 1995 Jan 15;75(2):157-60.

91. Gott VL, Greene PS, Alejo DE, Cameron DE, Naftel DC, Miller DC, et al. Replacement of the aortic root in patients with Marfan's syndrome. N Engl J Med. 1999 Apr 29;340(17):1307-13.

92. Silverman DI, Gray J, Roman MJ, Bridges A, Burton K, Boxer M, et al. Family history of severe cardiovascular disease in Marfan syndrome is associated with increased aortic diameter and decreased survival. J Am Coll Cardiol. 1995 Oct;26(4):1062-7.

93. Hasham SN, Willing MC, Guo DC, Muilenburg A, He R, Tran VT, et al. Mapping a locus for familial thoracic aortic aneurysms and dissections (TAAD2) to 3p24-25. Circulation. 2003 Jul 1;107(25):3184-90.

94. Shores J, Berger KR, Murphy EA, Pyeritz RE. Progression of aortic dilatation and the benefit of long-term beta-adrenergic blockade in Marfan's syndrome. N Engl J Med. 1994;330(19):1335-41.

95. Haouzi A, Berglund H, Pelikan PC, Maurer G, Siegel RJ. Heterogeneous aortic response to acute beta-adrenergic blockade in Marfan syndrome. Am Heart J. 1997;133(1):60-3.

96. Groenink M, de Roos A, Mulder BJ, Spaan JA, van der Wall EE. Changes in aortic distensibility and pulse wave velocity assessed with magnetic resonance imaging following beta-blocker therapy in the Marfan syndrome. Am J Cardiol. 1998 Jul 15;82(2):203-8.

97. Rios AS, Silber EN, Bavishi N, Varga P, Burton BK, Clark WA, et al. Effect of long-term beta-blockade on aortic root compliance in patients with Marfan syndrome. Am Heart J. 1999;137(6):1057-61.

98. Salim MA, Alpert BS, Ward JC, Pyeritz RE. Effect of beta-adrenergic blockade on aortic root rate of dilation in the Marfan syndrome. Am J Cardiol. 1994 Sep 15;74(6):629-33.

99. Rossi-Foulkes R, Roman MJ, Rosen SE, Kramer-Fox R, Ehlers KH, O'Loughlin JE, et al. Phenotypic features and impact of beta blocker or calcium antagonist therapy on aortic lumen size in the Marfan syndrome. Am J Cardiol. 1999;83(9):1364-8.

100. Yetman AT, Bornemeier RA, McCrindle BW. Usefulness of enalapril versus propranolol or atenolol for prevention of aortic dilation in patients with the Marfan syndrome. Am J Cardiol. 2005 May 1;95(9):1125-7.

101. Davies RR, Goldstein LJ, Coady MA, Tittle SL, Rizzo JA, Kopf GS, et al. Yearly rupture or dissection rates for thoracic aortic aneurysms: simple prediction based on size. Ann Thorac Surg. 2002 Jan;73(1):17-27; discussion -8.

102. Bentall H, De Bono A. A technique for complete replacement of the ascending aorta. Thorax. 1968 Jul;23(4):338-9.

103. Sarsam MA, Yacoub M. Remodeling of the aortic valve anulus. J Thorac Cardiovasc Surg. 1993 Mar;105(3):435-8.

104. David TE, Feindel CM. An aortic valve-sparing operation for patients with aortic incompetence and aneurysm of the ascending aorta. J Thorac Cardiovasc Surg. 1992 Apr;103(4):617-21; discussion 22.

105. de Oliveira NC, David TE, Ivanov J, Armstrong S, Eriksson MJ, Rakowski H, et al. Results of surgery for aortic root aneurysm in patients with Marfan syndrome. J Thorac Cardiovasc Surg. 2003 Apr;125(4):789-96.

106. Birks EJ, Webb C, Child A, Radley-Smith R, Yacoub MH. Early and long-term results of a valve-sparing operation for Marfan syndrome. Circulation. 1999 Nov 9;100(19 Suppl):II29-35.

107. Bethea BT, Fitton TP, Alejo DE, Barreiro CJ, Cattaneo SM, Dietz HC, et al. Results of aortic valve-sparing operations: experience with remodeling and reimplantation procedures in 65 patients. Ann Thorac Surg. 2004 Sep;78(3):767-72; discussion -72.

108. Loeys BL, Chen J, Neptune ER, Judge DP, Podowski M, Holm T, et al. A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in TGFBR1 or TGFBR2. Nat Genet. 2005 Mar;37(3):275-81.

109. Mizuguchi T, Collod-Beroud G, Akiyama T, Abifadel M, Harada N, Morisaki T, et al. Heterozygous TGFBR2 mutations in Marfan syndrome. Nat Genet. 2004 Jul 4.

110. Loeys BL, Schwarze U, Holm T, Callewaert BL, Thomas GH, Pannu H, et al. Aneurysm syndromes caused by mutations in the TGF-beta receptor. N Engl J Med. 2006 Aug 24;355(8):788-98.

111. Pannu H, Fadulu VT, Chang J, Lafont A, Hasham SN, Sparks E, et al. Mutations in Transforming Growth Factor-{beta} Receptor Type II Cause Familial Thoracic Aortic Aneurysms and Dissections. Circulation. 2005 July 26, 2005;112(4):513-20.

112. Pepin M, Schwarze U, Superti-Furga A, Byers PH. Clinical and genetic features of Ehlers-Danlos syndrome type IV, the vascular type. New England journal of medicine, The. 2000;342(10):673-80.

113. Beighton P, De Paepe A, Steinmann B, Tsipouras P, Wenstrup RJ. Ehlers-Danlos syndromes: revised nosology, Villefranche, 1997. Ehlers-Danlos National Foundation (USA) and Ehlers-Danlos Support Group (UK). Am J Med Genet. 1998;77(1):31-7.

114. Wenstrup RJ, Murad S, Pinnell SR. Ehlers-Danlos syndrome type VI: clinical manifestations of collagen lysyl hydroxylase deficiency. J Pediatr. 1989 Sep;115(3):405-9.

115. Coucke PJ, Willaert A, Wessels MW, Callewaert B, Zoppi N, De Backer J, et al. Mutations in the facilitative glucose transporter GLUT10 alter angiogenesis and cause arterial tortuosity syndrome. Nat Genet. 2006 Mar 19.

116. Milewicz DM, Michael K, Fisher N, Coselli JS, Markello T, Biddinger A. Fibrillin-1 (FBN1) mutations in patients with thoracic aortic aneurysms. Circulation. 1996 Dec 1;94(11):2708-11.

117. Francke U, Berg MA, Tynan K, Brenn T, Liu W, Aoyama T, et al. A Gly1127Ser mutation in an EGF-like domain of the fibrillin-1 gene is a risk factor for ascending aortic aneurysm and dissection. Am J Hum Genet. 1995 Jun;56(6):1287-96.

118. Zhu L, Vranckx R, Van Kien PK, Lalande A, Boisset N, Mathieu F, et al. Mutations in myosin heavy chain 11 cause a syndrome associating thoracic aortic aneurysm/aortic dissection and patent ductus arteriosus. Nat Genet. 2006 Mar;38(3):343-9.

119. Vaughan CJ, Casey M, He J, Veugelers M, Henderson K, Guo D, et al. Identification of a chromosome 11q23.2-q24 locus for familial aortic aneurysm disease, a genetically heterogeneous disorder. Circulation. 2001;103(20):2469-75.

120. Hahn RT, Roman MJ, Mogtader AH, Devereux RB. Association of aortic dilation with regurgitant, stenotic and functionally normal bicuspid aortic valves. J Am Coll Cardiol. 1992 Feb;19(2):283-8.

121. Huntington K, Hunter AG, Chan KL. A prospective study to assess the frequency of familial clustering of congenital bicuspid aortic valve. J Am Coll Cardiol. 1997 Dec;30(7):1809-12.

122. Emanuel R, Withers R, O'Brien K, Ross P, Feizi O. Congenitally bicuspid aortic valves. Clinicogenetic study of 41 families. Br Heart J. 1978 Dec;40(12):1402-7.

123. Freed LA, Benjamin EJ, Levy D, Larson MG, Evans JC, Fuller DL, et al. Mitral valve prolapse in the general population: the benign nature of echocardiographic features in the Framingham Heart Study. Journal of the American College of Cardiology. 2002 2002/10/2;40(7):1298-304.

124. Snider AR, Enderlein MA, Teitel DF, Juster RP. Two-dimensional echocardiographic determination of aortic and pulmonary artery sizes from infancy to adulthood in normal subjects. Am J Cardiol. 1984 Jan 1;53(1):218-24.

125. van Karnebeek CDM, Naeff MSJ, Mulder BJM, Hennekam RCM, Offringa M. Natural history of cardiovascular manifestations in Marfan syndrome. Arch Dis Child. 2001 February 1, 2001;84(2):129-37.

126. Yin FC, Brin KP, Ting CT, Pyeritz RE. Arterial hemodynamic indexes in Marfan's syndrome. Circulation. 1989;79(4):854-62.

127. Black C, Withers AP, Gray JR, Bridges AB, Craig A, Baty DU, et al. Correlation of a recurrent FBN1 mutation (R122C) with an atypical familial Marfan syndrome phenotype. Hum Mutat. 1998;Suppl 1:S198-200.

128. Habashi JP, Judge DP, Holm TM, Cohn RD, Loeys BL, Cooper TK, et al. Losartan, an AT1 antagonist, prevents aortic aneurysm in a mouse model of Marfan syndrome. Science. 2006 Apr 7;312(5770):117-21.

129. Ng CM, Cheng A, Myers LA, Martinez-Murillo F, Jie C, Bedja D, et al. TGF-beta-dependent pathogenesis of mitral valve prolapse in a mouse model of Marfan syndrome. J Clin Invest. 2004 Dec;114(11):1586-92.

130. Neptune ER, Frischmeyer PA, Arking DE, Myers L, Bunton TE, Gayraud B, et al. Dysregulation of TGF-beta activation contributes to pathogenesis in Marfan syndrome. Nat Genet. 2003 Mar;33(3):407-11.

Appendix

Diagnostic criteria for Marfan syndrome (De Paepe et al, Gent nosology (22))

System	Major Criteria	Minor Criteria
Skeletal System	Presence of at least four of the following manifestations: • Pectus carinatum • Pectus excavatum requiring surgery • Decreased upper to lower segment ratio or arm span to height ratio greater than 1.05 • Positive wrist sign or thumb sign • Scoliosis of greater than 20 degrees, or spondylolisthesis • Reduced elbow extension • Flat feet • Protrusuio acetabuli	• Pectus excavatum of moderate severity • Joint hypermobility • Highly arched palate • Facial appearance: dolichocephaly; malar hypoplasia; enophthalmos; retrognathia; downslanting palpebral fissures

System	Major Criteria	Minor Criteria
Ocular System	• ectopia lentis	• Abnormally flat cornea (as measured by keratometry) • Increased axial length of globe (as measured by ultrasound) • Hypoplastic iris or hypoplastic (under-developed) ciliary muscle causing decreased constriction of pupil (myosis)
Cardiovascular System	• Dilatation of the ascending aorta with or without aortic regurgitation and involving at least the sinuses of Valsalva; or • Dissection of the ascending aorta	• Mitral valve prolapse (MVP) with or without mitral valve regurgitation (MVR) • Dilatation of the main pulmonary artery, in the absence of valvular or peripheral pulmonic stenosis or any other obvious cause, below the age of 40 • Calcification of the mitral annulus below the age of 40 • Dilatation or dissection of the descending thoracic or abdominal aorta below the age of 50
Pulmonary System	None	• Spontaneous pneumothorax: • Apical blebs:
Skin and Integument	None	• Striae not associated with marked weight changes, pregnancy or repetitive stress • Recurrent or incisional herniae
Dura (the covering of spinal cord and brain)	• lumbosacral dural ectasia determined by CT or MRI	None

System	Major Criteria	Minor Criteria
Family/Genetic History	• Having a parent, child or sibling who meets these diagnostic criteria independently • Presence of a mutation in FBN1 (the fibrillin 1 gene), known to be associated with Marfan syndrome • Presence of a haplotype around FBN1, inherited by descent, known to be associated with unequivocally diagnosed Marfan syndrome in the family	None

Dankwoord

In het tot stand komen van dit doctoraat ben ik door tal van mensen bijgestaan. Liefst zou ik iedereen die ik op weg hiernaartoe heb ontmoet bedanken voor hun bijdrage – maar dat is een onmogelijke opdracht. Toch zijn er een aantal mensen die ik hier in het bijzonder wil danken.
In de eerste plaats gaat mijn dank uit naar de patiënten en vrijwilligers die bereid zijn geweest deel te nemen aan dit onderzoek. Zonder hun bereidwillige medewerking was dit werk onmogelijk geweest. Ik hoop dat de resultaten hiervan een steentje zullen bijdragen aan een betere toekomst voor de Marfan patiënten.

Mijn promotor Prof. Dr. De Paepe, u wil ik van harte danken voor alle mogelijkheden die u mij geboden heeft. U gaf me op een cruciaal moment de kans om op op de dienst Medische Genetica te komen werken, dat was de vervulling van een oude droom. U heeft mij alle kansen gegeven om mij verder te ontwikkelen in de genetica en ik ben u daar bijzonder erkentelijk voor. Uw zin voor perfectie, uw constructieve opmerkingen en uw toewijding voor de basis wetenschappelijke research zijn voor mij van onschatbare waarde geweest.

Prof. De Sutter, beste Johan, zonder jouw enthousiasme om mij in dit project te ondersteunen had ik hier niet gestaan. Je schrok er niet voor terug om je in de materie van de genetica te verdiepen en zinvolle aangrijpingspunten met de cardiale beeldvorming te zoeken. Je bent binnen de cardiologie altijd een groot voorbeeld geweest voor mij en ik heb je toewijding voor zowel kliniek als research altijd bijzonder geapprecieerd. De toekomst zag er ooit anders uit, ik kan alleen maar hopen dat we toch nog een mogelijkheid zullen vinden om verder samen te werken.

I want to thank the members of the jury of this thesis, Prof. Dr. T. Gillebert, Prof. Dr. G. Jondeau, Prof. Dr. D. Matthys, Prof. Dr. L. Van Bortel, Prof. Dr. G. Van

Camp, Prof. Dr. J. Vandewalle en Prof. Dr. G. Van Nooten for their constructive comments that have helped to improve the quality of this work.

Dr. B. Loeys, beste Bart, zoveel jaren terug heb jij me geïntroduceerd op de genetica en daar ben ik je heel dankbaar voor. Ondertussen heb je een wetenschappelijke carrière uitgebouwd waar de meesten onder ons alleen maar kunnen van dromen. Toch ben je altijd met beide voeten op de grond blijven staan en was geen enkele vraag je ooit teveel. Je hebt mijn projecten altijd postitief ondersteund en ik beschouw het als een hele eer om samen met jou te kunnen verder werken.

Prof. P. Segers, beste Patrick, jij hebt me bij het prille begin van dit project ingewijd in de wonderlijke wereld van de arteriële mechanica, gezien vanuit het standpunt van een ingenieur. Jouw nuchtere visie op de feiten en je kritische analyse zijn van bijzondere waarde geweest in dit project en hebben mijn gezichtsveld ver verruimd – dank voor alles.

Dr. D. Devos, beste Dan, jouw bereidwilligheid en enthousiasme om het MRI luik van dit doctoraat uit te bouwen zijn van onschatbare waarde geweest. Ik zal nooit die week-end dagen vergeten die we samen hebben doorgebracht in de kelders van K12 om dit werk te kunnen realiseren – mijn oprechte dank daarvoor.

Prof. P Coucke, beste Paul, dankzij jou kreeg ik de kans de essentie van de genetica van dichtbij te leren kennen. Ik moet in alle eerlijkheid bekennen dat ik bij de aanvang van dit project het onderscheid tussen moleculaire- en cytogenetica maar half begreep – daar is dankzij jou gelukkig verandering in gekomen. Ook buiten het werk – op de skipiste of op de dansvloer – heb ik jouw enthousiasme altijd sterk geapprecieerd.

Prof. H. Dietz, dear Hal, my gratitude goes out to you and your wonderful team at Johns Hopkins. Despite the 6000 miles between Ghent and Baltimore, you were always the first to send my manuscripts back with very constructive comments. Your contribution to this thesis and ever positive encouragement are of great value to me – thank you so much.

Prof. Dr. B. Mulder, beste Barabara, jou wil ik in het bijzonder danken voor de aangename en vlotte samenwerking in dit project. Jouw aanstekelijke enthousiasme voor Marfan en congenitale cardiologie in de bredere zin hebben een grote indruk op mij gemaakt. Ook dank aan de mensen van jouw team, in het bijzonder Dr. Maarten Groenink, Dr. Gijs Nollen en Dr. Lilian Meijboom.

Beste Fransiska, ik kan me geen betere “room-mate” voorstellen dan jij. We zijn ongeveer samen begonnen aan dit avontuur en hebben het ook samen kunnen afronden. Je hebt - gewild of ongewild - veel hoogtes en laagtes van mij van nabij meegemaakt. Ik ben je erg dankbaar voor je luisterend oor, voor je relativeringsvermogen en voor de vele fijne momenten buiten het werk.

Mme Jousten, chère Yvonne, je vous remercie de tout mon cœur pour tout ce que vous avez fait pour moi. Vôtre enthousiasme et vôtre courage, même aux moments les plus difficiles, m’ont inspiré très profondément dans la réalisation de ce projet.

Mijn collega’s op de cardiologie, Tine De Backer, Sofie Gevaert, Katarina Van Beeumen en Nico Van de Veire wil ik hartelijk danken voor hun interesse in mijn werk en hun bereidwilligheid om op momenten dat het nodig was mijn klinische taken over te nemen. Voor het delen van patient gegevens wil ik Michel De Pauw danken.
De verpleegkundigen op de echo en de poli cardio, Sofie, Petra, An, Martine en Frida dank voor jullie hulp bij de uitgevoerde onderzoeken. Frank, dank voor de hulp bij de organisatie van de poli en de echo.
De kindercardiologen, Katya De Groote, Daniel De Wolf, Dirk Matthys, Seppie Panzer, Rik Verhaaren, en de cardiochirurgen Thierry Bové en Katrien François allen hartelijk dank voor de vlotte samenwerking en de interessante discussies.
Op de genetica gaat mijn dank ook uit naar Bert Callewaert en Olivier Van Akker, dankzij jullie heb ik het laatste jaar meer tijd gehad om dit project af te maken.
De mensen van het bindweefsellabo, Sophie Deleyn, Sofie Symoens, Karen Wettinck, Petra Van Akker en Inge Vereecke, dank voor jullie hulp en geduld bij mijn experimenten in het labo.
De medewerkers op de polikliniek genetica, Sandra Janssens, Prof. Jules Leroy, Bart Leroy, Geert Mortier, Bruce Poppe, Ariane Van Tongerlo en Philippe De Wilde, dank voor de vele leerrijke momenten.
Alle mensen van het secretariaat genetica, Katia, Mieke, Leen, Isabelle, Nathalie, Liesbeth en Virginie, en Karlien Geenens van het secretariaat cardio, dank voor de “technische” ondersteuning.

Colette Storme en Marie-Rose Verschraegen-Spae wil ik danken voor hun hulp bij de franse vertaling.

Mijn ouders, papa en mama, aan jullie heb ik zoveel te danken. Het zijn jullie die de basis van dit doctoraat hebben gelegd. Jullie onvoorwaardelijke liefde, steun en vertrouwen in al mijn projecten zijn essentieel geweest in de realisatie van dit doctoraat. Ook mijn bijzondere dank voor de ondersteuning op het thuisfront. Op

goede en kwade momenten heb ik altijd op jullie mogen rekenen, zonder jullie was dit nooit gelukt.

Bernard en Charlotte, ook jullie wil ik danken voor jullie interesse en vertrouwen. Jullie zijn me al lang voorgegaan in het afleggen van een doctoraat en dat heeft me zeker gestimuleerd om door te zetten.

Jan, lieve Jan, mijn dankbaarheid voor jou is moeilijk onder woorden te brengen. Er zijn zoveel dingen waarvoor ik je wil danken – we hebben de afgelopen jaren nog zoveel meer gerealiseerd dan elk een doctoraat bijeen schrijven. Velen hebben zich hierbij de vraag gesteld hoe we hierin konden slagen – het antwoord hierop is nochtans eenvoudig: dit dubbel-project is tot een goed einde gekomen dankzij jouw relativeringsvermogen, je organisatorisch talent en je morele steun. Dank voor alles!

Tenslotte wil ik jullie danken, mijn liefste Jules en Louise. Jullie bijzondere gave om als bliksemafleider te fungeren, zelfs in het zwaarste onweer is bewonderenswaardig. Een lach op jullie gezichtje en de lichtjes in jullie ogen hebben mij meermaals doen beseffen dat er zoveel meer is dan dit doctoraat. Er is veel tijd in dit project gekropen, tijd die ik liever met jullie had doorgebracht, maar ik beloof jullie dat ik er alles zal aan doen om dat goed te maken!

All's is well that ends well (William Shakespeare).

Gent, juni 2007

Curriculum Vitae

Personalia

Last name	De Backer
First name	Julie
Date of birth	october 14 1970
Citizenship	Belgian
Marital status	married to Jan De Waele
	Two children Jules
	Louise
Home adress	D. Van Monckhovenstraat 28, 9000 Gent, Belgium
Office address	University Hospital Ghent, Belgium
	Dept of Cardiology and Medical Genetics
	De Pintelaan 185, 9000 Ghent, Belgium
	Phone: 0032 9 240 36 03
	Fax: 0032 9 240 49 70
Email	julie.debacker@UGent.be

Education

1982 – 1988 Secondary school - Latin Science
Koninklijk Atheneum – Gent Voskenslaan

1988 – 1995 Medicine
University of Ghent, Faculty of Medicine
Graduated suma cum laude

1995 - 2001 Specialisation in Cardiology
Ghent University

Research activities

2001-2007 Preparation of PhD in Medical Sciences
Promotor: Prof. Dr. A. De Paepe
Co-Promotor: Prof. Dr. J. De Sutter
Funded by:
Research mandate from the Ghent University (BOF 011D4701)
Research grant from the Fund for Scientific Research Belgium (FWO G029002) (Promotor: A. De Paepe)

Membership

Belgian Society of Cardiology
Belgian Society of Human Genetics
European Society of Cardiology
Belgian Working Group of Non Invasive Cardiac Imaging (board member)

Current activity

Cardiology staff member (University Hospital Ghent), specialized in Adult Congenital Heart Disease and echocardiography
Staff member at the Medical Genetics Department (University Hospital Ghent), specialized in heritable connective tissue disorders and cardiogenetics

Publications - international, peer reviewed

1. De Sutter J, Tavernier R, Van de Wiele C, De Backer J, De Backer G, Dierckx R, Jordaens L. QT dispersion does not predict inducibility in patients with coronary artery disease and documented life-threatening ventricular arrhythmias. PACE 1998;21:885
2. De Sutter J, Tavernier R, Van de Wiele C, De Backer J, Jordaens L, Dierckx R. 201-Thallium SPECT and QT dispersion as predictors of inducibility during electrophysiological testing in patients with life –threatening ventricular arrhythmias. J Nucl Med 1998;39:155P
3. De Sutter J, Van de Wiele C, De Backer J, Voet J, De Buyzere M, Gheeraert P, Dierckx R, Taeymans Y. Admission Fibrinogen concentration is a marker of final myocardial infarct size in patients treated with primary angioplasty. Eur Heart J 1998;19:565
4. De Sutter J, Tavernier R, Van de Wiele C, De Backer J, Kazmierczak J, De Backer G, Dierckx RA, Jordaens L. QT dispersion is not related to infarct size or inducibility in patients with coronary artery disease and life-threatening ventricular arrhythmias. Heart 1999; 81:533-538
5. De Sutter J, Tavernier R, Van de Wiele C, De Backer J, Jordaens L , Dierckx R. Residual ischemia does not influence recurrences of ventricular arrhythmias after defibrillator (ICD) implantation. J Nucl Cardiol 1999;6:S25
6. De Backer J, Mak R, De Bacquer D, Van Renterghem L, Verbraekel E, Kornitzer M, De Backer G - Parameters of inflammation and infection in a community based case-control study of coronary heart disease. Atherosclerosis. 2002 Feb;160(2):457-63
7. De Sutter J, De Mey S, De Backer J, De Winter O, De Maeseneire S, De Buyzere M, Dierckx R, Gillebert T, Verdonck P – Diastolic dysfunction, Infarct size, and exercise capacity in remote myocardial infarction: a combined approach of mitral E-wave deceleration time and color M-mode flow propagation velocity. Am J Cardiol 2002;89:593
8. Segers P, Rabben SI, De Backer J, De Sutter J, Gillebert TC, Van Bortel L and Verdonck P "Functional Analysis of the Common Carotid Artery : Relative Distension Differences over the Vessel Wall Measured In Vivo". J Hypertens 2004 May;22(5):973-81.
9. Loeys B, De Backer J, Van Acker P, Wettinck K, Pals G, Nuytinck L, Coucke P and De Paepe A Comprehensive Molecular Screening of the FBN1 gene favours locus homogeneity of Classical Marfan syndrome.. Human Mutation 24: 140 – 146 (2004)
10. Vermeulen SJ, Menten B, De Bie S, Coucke P, Malfait F, De Backer J, Speleman F, De Paepe A, Loeys B. DUP25 remains unconfirmed. Am J Med Genet. 2004 Dec 15;131A(3):320-1.

11. Loeys B, Chen J, Neptune ER, Judge DP, Podowski M, Holm T, Meyers J, Leitch CC, Katsanis N, Sharifi N, XuFL, Myers LA, De Backer J, Hellemans J, Chen Y, Davis EC, Webb CL, Kress W, Spevak PJ, Coucke P, Rifkin DB, De Paepe AM, Dietz HC. A syndrome of altered cardiovascular, craniofacial, neurocognitive and skeletal development caused by mutations in TGFBR1 or TGFBR2. Nature Genetics 2005 Mar;37(3):275-81

12. Meijboom LJ, Timmermans J, van Tintelen JP, Nollen GJ, De Backer J, van den Berg MP, Boers GH, Mulder BJM. Evaluation of Left Ventricular Dimensions and Function in the Marfan Syndrome without Significant Valvular Regurgitation. American Journal of Cardiology Am J Cardiol 2005;95:795-797

13. De Sutter J, De Backer J, Velghe A, Van de Veire N, De Buyzere M, Gillebert T Determinants of septal mitral annulus velocity (E') and the ratio of transmitral early peak velocity to E' (E/E'): effects of age, gender and left ventricular mass. American Journal of Cardiology . 2005 Apr 18; 513 (1-2): 35-45

14. De Backer J, Matthys D, Gillebert TC, De Paepe A, De Sutter J. The use of Tissue Doppler Imaging for the assessment of changes in myocardial structure and function in inherited cardiomyopathies. European Journal of Echocardiography 2005 Aug;6(4):243-50

15. De Backer J, Devos D, Segers P, Matthys D, François K, Gillebert TC, De Paepe A, De Sutter J. Primary Impairment of left ventricular function in Marfan syndrome. Int J Cardiol. 2006 Oct;112(3):353-8

16. Segers P, De Backer J, Devos D, Rabben SI, Gillebert TC, Van Bortel LM, De Sutter J, De Paepe A, Verdonck PR. Aortic reflection coefficients and their association with global indices of wave reflection in healthy controls and patients with Marfan disease. Am J Physiol Heart Cir Physiol 2006 Jun;290(6):H2385-92

17. Van de Veire N, De Backer J, Ascoop AK, Middernacht B, Velghe A, De Sutter J. Echocardiographically estimated left ventricular end-diastolic and right ventricular systolic pressure in normotensive healthy individuals. Int J of Cardiovasc Imaging 2006 Oct;22(5):633-41

18. De Backer J, NollenGJ, Devos D, Pals G, Coucke P, Verstraete K, van der Wall EE, De Paepe A, Mulder BJM. Variability of aortic stiffness is not associated with the fibrillin1 genotype in patients with Marfan's syndrome. Heart 2006 Jul;92(7):977-8

19. De Backer J, Loeys B, Devos D, Dietz H, De Sutter J, De Paepe A. A critical analysis of minor cardiovascular criteria in the diagnostic evaluation of patients with Marfan syndrome. Genetics in Medicine. 2006 Jul;8(7):401-8

20. Coucke PJ, Willaert A, Wessels MW, Callewaert B, Zoppi N, De Backer J, Fox JE, Mancini GM, Kambouris M, Gardella R, Facchetti F, Willems PJ, Forsyth R, Dietz HC, Barlati S, Colombi M, Loeys B, De Paepe A. Mutations in the facilitative glucose transporter GLUT10 alter angiogenesis and cause arterial tortuosity syndrome.Nat Genet. 2006 Apr;38(4):452-7

21. Loeys BL, Schwarze U, Holm T, Callewaert BL, Thomas GH, Pannu H, De Backer, J. F., Oswald, G. L., Symoens, S., Manouvrier, S., Roberts, A. E., Faravelli, F., Greco, M. A., Pyeritz, R. E., Milewicz, D. M., Coucke, P. J., Cameron, D. E. , Braverman, A. C., Byers, P. H., De Paepe, A. M. , Dietz, H. C.. Aneurysm syndromes caused by mutations in the TGF-beta receptor. N Engl J Med. 2006 Aug 24;355(8):788-98.

22. Malfait F, Symoens S, De Backer J, Hermanns-Le T, Sakalihasan N, Lapiere CM, Coucke P, De Paepe A. Three arginine to cysteine substitutions in the pro-alpha (I)-collagen chain cause Ehlers-Danlos syndrome with a propensity to arterial rupture in early adulthood. Hum Mutat. 2007 Apr,28(4):387-95

23. Williams JA, Loeys BL, Nwakanma LU, Dietz HC, Spevak PJ, Patel ND, François K, Gott VL, Vrivella LA, Cameron DE. Early surgical experience with Loeys-Dietz: a new syndrome of aggressive thoracic aortic aneurysm disease. Ann Thorac Surg. 2007 Feb; 83 (2):S757-63

24. De Backer J, Loeys B, Leroy B, Coucke P, Dietz H, De Paepe A. Utility of molecular analyses in the interrogation of extreme intrafamilial variability in the Marfan syndrome. Clinical Genetics 2007 – in press

25. Faivre L., Collod-Beroud G., Loeys B., Child A., Binquet C., Gautier E., Callewaert B., Arbustini E., Mayer K., Arslan-Kirchner M., Kiotsekoglou A. , Comeglio P., Marziliano N., Dietz HC., Halliday D., Beroud C., Bonithon-Kopp C., Claustres M., Muti C., Plauchu H., Robinson PN., Adès LC., Biggin A., Benetts B., Brett M., Holman KJ., De Backer J., Coucke P., Francke U., De Paepe A., Jondeau G., Boileau C. Effect of mutation type and location on clinical outcome in 1013 probands with Marfan syndrome or related phenotypes with FBN1 mutations: an international study. American Journal of Human Genetics – in press

Publications – National

1. Loeys B., De Backer J., Matthys D., De Paepe A. Moleculaire genetica bij de vasculaire vorm van het syndroom van Ehlers-Danlos. Percentiel, Vol7, Nr2 2002
2. De Sutter J, De Backer J, Gillebert T. Actuele klinische toepassingen van Tissue Doppler Imaging. De agenda Cardio. Nr2, april 2003
3. De Backer J, De Paepe A. Het syndroom van Marfan bij kinderen: diagnostiek en behandeling. Tijdschrift van de Belgische kinderarts. 2004. P74
4. De Backer J, De Sutter J, Devos D, François K, Gillebert TC, De Paepe A. Familiale aandoeningen van de thoracale aorta. Tijdschr. Voor Geneeskunde, 60, nr 12, 2004, 840-849
5. Loeys B, De Backer J, De Paepe A. Het Marfan syndroom. De Agenda Cardio; Nr 13; Januari 2006
6. De Paepe A, Loeys B, De Backer J, Coucke P. Nieuwe inzichten in de genetische basis van aneurysmevorming – De Agenda Cardio Juli 2006