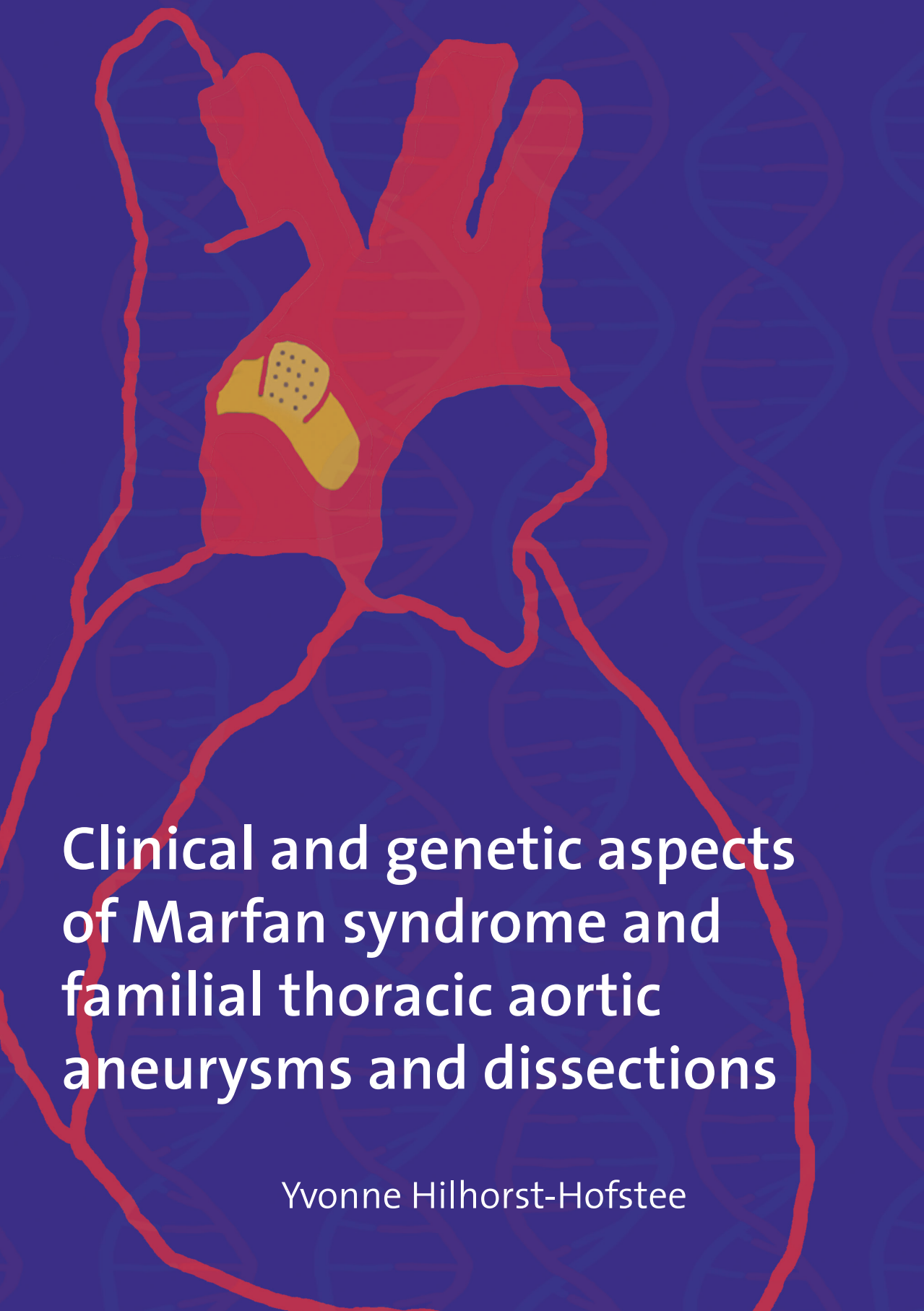

Clinical and genetic aspects of Marfan syndrome and familial thoracic aortic aneurysms and dissections

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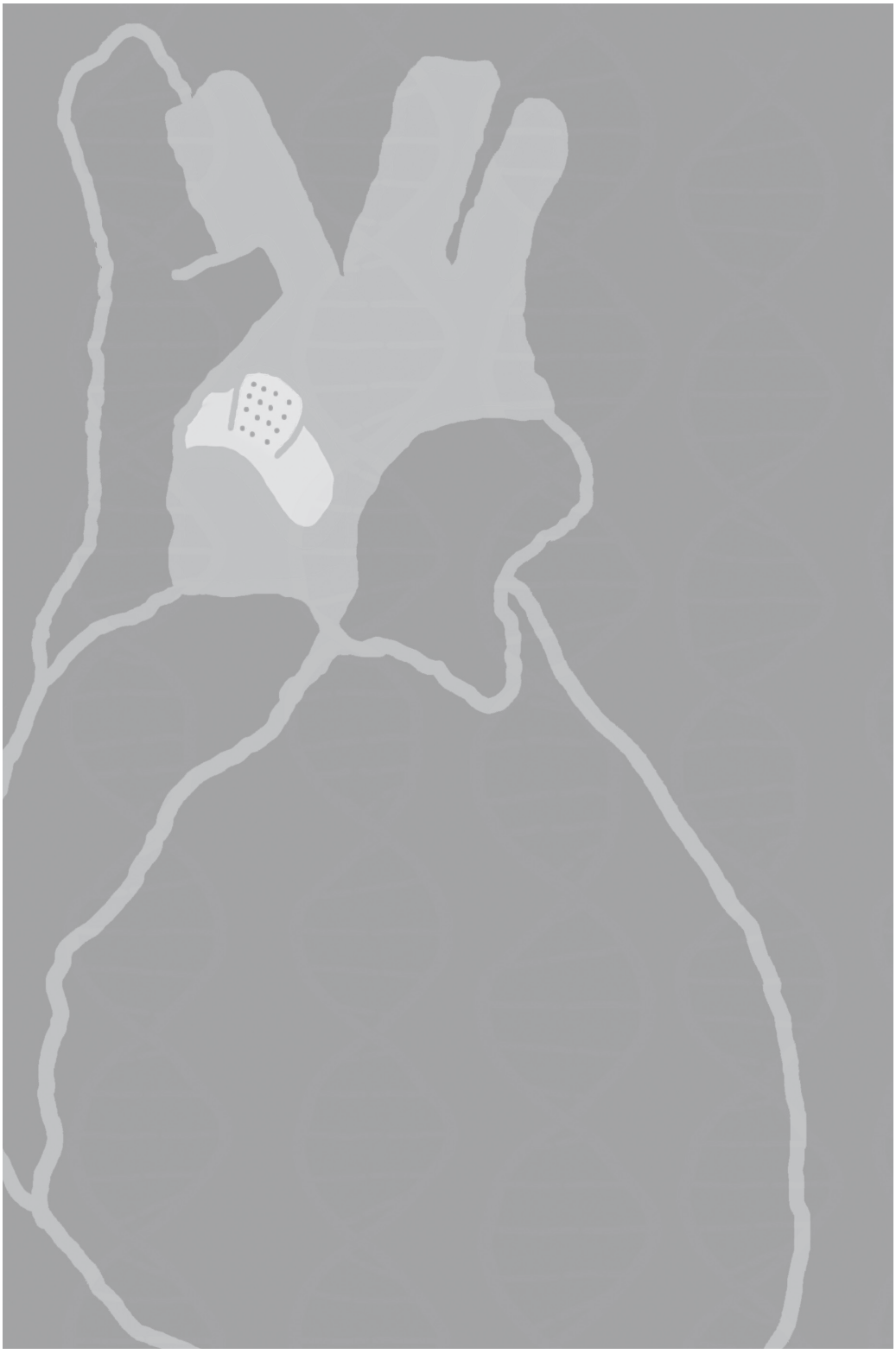

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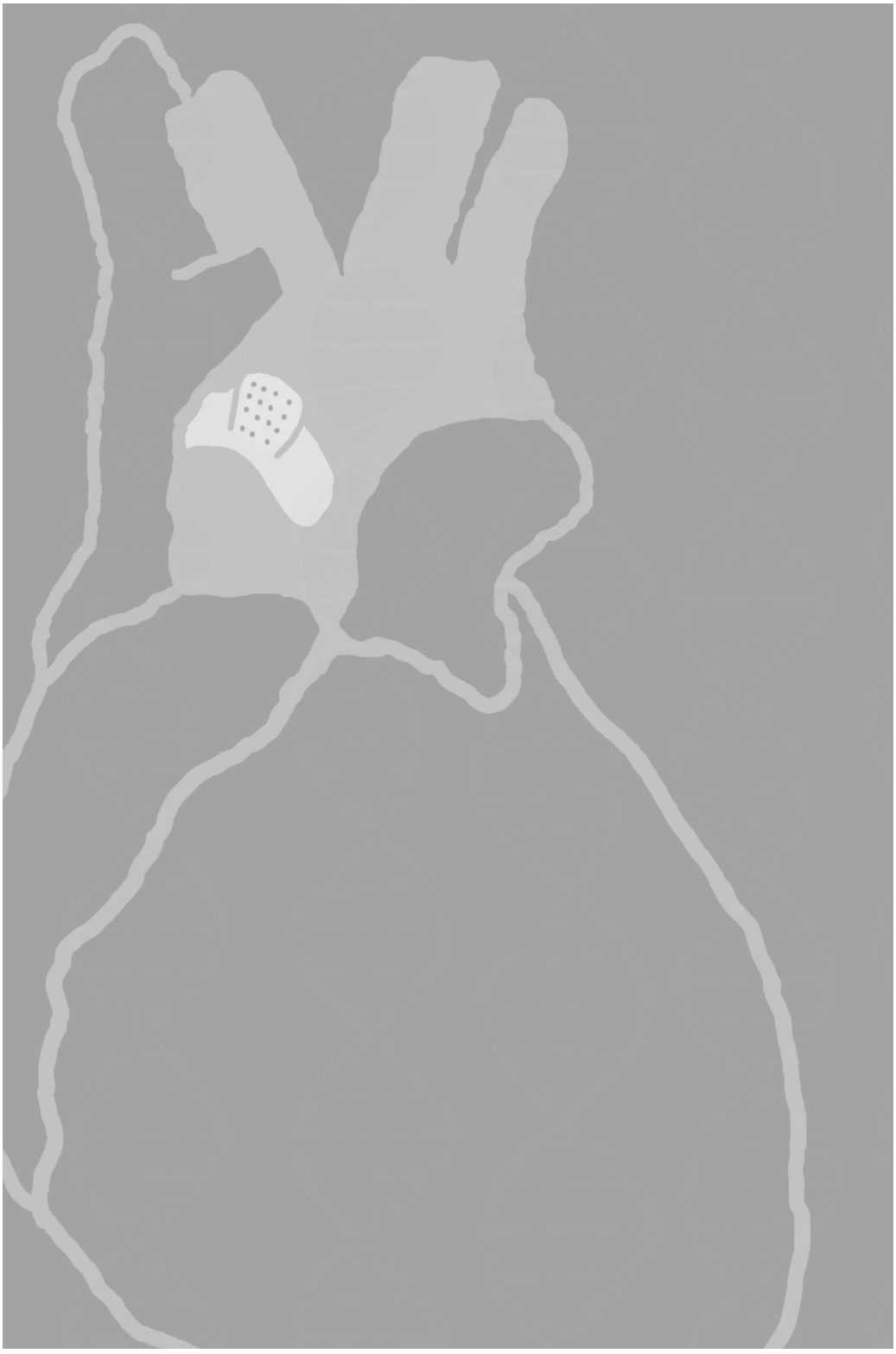

1

Introduction

Introduction

1 General introduction

Since the recognition of Marfan syndrome, a wide variety of case reports have been published illustrating diverse aspects of the disease. The 50 pedigrees studied by Victor McKusick, which included more than 100 affected patients, enabled him to describe the syndrome and its variability in detail. McKusick hypothesised that the underlying problem was a heritable defect of the elastic fibres connecting to smooth muscle cells. Furthermore, he discussed a possible explanation for the skeletal overgrowth, which could not be easily explained by a structural defect of connective tissue (McKusick, 1955a; McKusick, 1955b). It has been clear for more than half a century that patients with Marfan syndrome have a predisposition for thoracic aortic aneurysms and dissections. However, it became clear only recently that nonsyndromic thoracic aortic aneurysms and dissections may also have an important genetic component (Biddinger et al., 1997; Coady et al., 1999; Milewicz et al., 1998; Nicod et al., 1989).

In many countries, including the Netherlands, diagnosis and treatment of Marfan syndrome is organised in multidisciplinary centres of expertise. An increasing number of referrals to these centres are potentially attributably to familial thoracic aortic aneurysms and dissections (FTAAD) rather than to Marfan syndrome. This thesis focuses on the clinical and genetic aspects of both Marfan syndrome and FTAAD.

2 Marfan syndrome

2.1 Clinical manifestations and diagnostic criteria

Marfan syndrome is named after Antoine Bernard-Jean Marfan, a Parisian paediatrician who, in 1896, described a five-year-old girl named Gabrielle P., taking particular note of her extraordinarily long limbs and fingers. It was only later appreciated that Gabrielle P.

might have actually been affected with congenital contractural arachnodactyly (CCA), one of the syndromes included in the differential diagnosis of Marfan syndrome (Beals and Hecht, 1971; Hecht and Beals, 1972). Clinical descriptions of patients potentially affected by Marfan syndrome can be found in early medical records. Literary descriptions of potentially affected individuals also occur, such as the character described by Edgar Allan Poe in 1844, in his *Tales of the Ragged Mountains*, as 'He was singularly tall and thin. He stooped much. His limbs were exceedingly long and emaciated. His forehead was broad and low. His complexion was absolutely bloodless. His mouth was large and flexible, and his teeth were more wildly uneven, although sound, than I had ever before seen teeth in a human head'. This man died as a consequence of a venomous leech used to relieve 'the great determination of blood to the head'. The latter feature may be interpreted as aortic regurgitation caused by progressive dilatation of the ascending aorta (Battle, 2011).

Marfan syndrome is a genetic, multisystem disorder with a minimal birth prevalence of 1 in 10,000 live births and a minimal prevalence of 1 in 14,000 (Gray et al., 1994). However, later estimates assume a prevalence of 2-3 in 10,000 (Judge and Dietz, 2005; Pyeritz, 2000). The cardiovascular, skeletal, and ophthalmologic organ systems are affected and patients have a high risk for aortic root aneurysm and dissection. This life-threatening aspect of Marfan syndrome forms the cardinal feature and has been the subject of numerous studies aimed at influencing aortic growth and preventing aortic dissection. Not only is the ascending aorta affected, patients are also prone to develop aneurysms and dissections in the remaining part of the aorta. Mitral valve prolapse is also very common. Though not generally life-threatening, ophthalmologic and skeletal disorders have a major impact on a patient's negative perception of the severity of the disease and their own well-being (De Bie et al., 2004; Peters et al., 2001). More than half of all patients with Marfan syndrome have ectopia lentis (Maumenee, 1981). Other less specific ocular features are myopia, retinal degeneration and detachment, iris hypoplasia, iris transillumination, irididonesis, and a flat cornea. Patients with Marfan syndrome often have a tall and slender build, with dolichostenomelia (long arms and legs) and arachnodactyly. Other skeletal features include pectus deformities, scoliosis, joint hypermobility, protrusio acetabuli, and pes planus with hindfoot deformity.

Clinical criteria required to diagnose the syndrome were defined by an international panel of experts in 1986, and revised in 1996 and 2010 (Beighton et al., 1988; De Paepe A. et al., 1996; Loeys et al., 2010). In the more recent criteria, the revised Ghent nosology, greater weight has been given to aortic root dilatation or dissection and ectopia lentis as cardinal features (**Chapter 3**). In the absence of one of these two cardinal features, further supporting evidence such as the presence of a pathogenic *FBN1* mutation or a combination

of systemic features is required. A scoring system has been developed for the mainly skeletal systemic features. Box 1 shows a summary of the current international diagnostic criteria, with some alternative diagnoses that show overlapping features.

Box 1. Summary of the revised Ghent criteria (Loeys et al., 2010)

Diagnostic criteria for Marfan syndrome
In the absence of an independently diagnosed family member with Marfan syndrome:
1. Aortic root Z-score ≥2 **AND** lens(sub)luxation
2. Aortic root Z-score ≥2 **AND** pathogenic mutation in FBN1
3. Aortic root Z-score ≥2 **AND** systemic score ≥7
4. Lens(sub)luxation AND FBN1 mutation that has been identified in an individual with an aortic aneurysm

In the presence of an independently diagnosed family member with Marfan syndrome:
5. Lens(sub)luxation **AND** an independently diagnosed family member
6. Systemic score ≥7 **AND** an independently diagnosed family member
7. Aortic root (Z-score ≥2 above 20 years old, ≥3 below 20 years) **AND** an independently diagnosed family member

Systemic score
- Wrist and thumb sign – 3 (wrist OR thumb sign – **1**)
- Pectus carinatum deformity – 2 (pectus excavatum or chest asymmetry – **1**)
- Hindfoot deformity – 2 (plain pes planus – **1**)
- Pneumothorax – **2**
- Durale ectasia – **2**
- Protrusio acetabuli – **2**
- Reduced upper versus lower segment ratio AND increased arm span versus height ratio AND no severe scoliosis – **1**
- Scoliosis or thoracolumbar kyphosis – **1**
- Reduced elbow extension – **1**
- Facial features (3/5) – 1 (dolichocephaly, enophtalmos, downslanting palpebral fissures, malar hypoplasia, retrognathia)
- Skin striae – **1**
- Myopia > 3 diopters – **1**

The maximum score is 20. A score of ≥7 indicates systemic involvement

Diagnostic criteria for alternative diagnoses
The alternative diagnoses of Ectopia Lentis syndrome (ELS), Myopia-Mitral valve prolapse-borderline and non-progressive Aortic root dilatation-Skeletal findings-Striae (MASS) and Mitral Valve Prolapse Syndrome (MVPS) are based on the following criteria :

ELS: Lens(sub)luxation, with or without systemic features **AND** a mutation in FBN1 that has not previously been associated with aortic root aneurysm/dissection or no mutation in FBN1

MASS : Aortic root Z-score <2 **AND** systemic score ff15 with at least one skeletal feature **WITHOUT** lens(sub)luxation

MVPS: Mitral valve prolapse AND aortic root Z-score <2 AND systemic score <5 **WITHOUT** lens(sub) luxation

2.2 Molecular genetics

Marfan syndrome is an autosomal dominant disorder with an extremely variable expression, both within and between families. A mutation in the *FBN1* gene is present in the majority of Marfan patients, and the first missense mutations were demonstrated in Marfan patients in 1991 (Dietz et al., 1991). *FBN1* encodes fibrillin-1, a large protein that forms the major component of extracellular microfibrils in both elastic and nonelastic connective tissue throughout the body. Fibrillin-1 is characterised by a highly conserved modular domain organisation (Figure 1a), with the Epidermal Growth Factor-like (EGF) domain as the most prominent. This domain is present 47 times and contains six highly conserved cysteine residues which stabilise the structure through three disulfide bonds (Downing et al., 1996). Of the EGF domains, 43 have a consensus sequence for calcium binding (cb) in the N-terminal pocket of the domain. The binding of calcium to cbEGF domains is necessary for stabilisation of the tertiary structure, prevention of proteolytic degradation and for protein-protein interaction (Cooke et al., 1987; Dietz et al., 1993; Handford et al., 1991). The EGF domains are interrupted by seven transforming growth factor (TGF)-binding protein domains, which are characterised by eight cysteine residues involved in intra-domain disulfide bonds (Figure 1a) (Pereira et al., 1993; Robinson et al., 2006).

In a study of *FBN1* mutations and clinical correlations in 1,013 probands with Marfan syndrome and related phenotypes, the mutations were classified as missense in 56% of cases, frameshift in 17%, nonsense in 14%, splicing in 11%, and in-frame deletions or insertions in 2% of cases. The majority of *FBN1* missense mutations are cysteine substitutions, modifying the tertiary structure of the protein and predicted to have a mainly dominant negative effect. Excluding 29 erroneous splicing events that could not be determined unambiguously, the remaining 984 mutations included 68% in-frame and 32% that lead to a premature termination codon (PTC). Nonsense or frameshift mutations are expected to result in nonsense mediated decay (NMD) of mutant mRNA and consequently to haploinsufficiency. However, it cannot be excluded that a truncated protein escaping NMD results in a dominant negative effect.

While a prediction of the phenotype based on the type of mutation is not possible on an individual basis, the following phenotype-genotype correlations have been established: Missense mutations substituting or creating a cysteine show a higher correlation with ectopia lentis than other missense mutations. Patients with major skeletal and skin involvement show a PTC mutation more frequently than patients with an in-frame mutation. Mutations in exons 24-32 are more often associated with a neonatal onset of the disease or a significantly higher probability of a more severe cardiovascular, ocular and/or skeletal phenotype, with a younger age at diagnosis and a shorter overall survival. Patients with mutations located in the 5' region of the gene have a higher probability of

ectopia lentis (Biggin et al., 2004; Faivre et al., 2007; Loeys et al., 2004; Rommel et al., 2005; Schrijver et al., 1999; Schrijver et al., 2002).

2.3 Pathophysiology

Fibrillin monomers polymerise into the microfibrils that form the outer sheet of elastic fibres over a core of elastin (Figure 1b). These fibres provide elastic properties and mechanical stability to both tissues and organs. The architecture of a mature elastic fibre is tissue specific. This is seen in the tunica media of the aorta and elastic arteries, where the elastic fibres form concentric fenestrated lamellae, separated by smooth muscle cell (SMC) layers, while in other tissues, for example in the ciliary zone of the eye, microfibrils are found in the absence of elastin (Raviola, 1971). Fibrillin-1 molecules have a head-to-tail orientation in the microfibrils and display a typical beads-on-a-string ultrastructure. Six to eight fibrillin molecules are included between two beads with periodicities of 50-55 nm in a relaxed state, which can be stretched to more than 100 nm (Figure 1b). Irreversible deformation occurs at higher periodicities (Baldock et al., 2001; Haston et al., 2003; Sakai et al., 1991). In addition to the linear interactions, lateral intermolecular cross-links are formed to provide mechanical stability (Kielty et al., 2002).

It was thought that impairment of the structural function of the microfibrils is the main determinant of the phenotype of Marfan syndrome. However, features such as bone overgrowth, facial characteristics, lung abnormalities, myxomatous changes of mitral valves, and hypoplasia of muscle and fat tissue could not be explained by loss of tissue integrity. It is now appreciated that fibrillin-1 not only has a structural function, but is also involved in the homeostasis of elastic matrix, in matrix-cell attachments and in regulating growth factors. The extracellular matrix serves as a modulator of cell signalling through receptors such as the integrins, and as a storage place for several growth factors including TGFβ (Hubmacher et al., 2006). TGFβ and its binding protein LTBP1 are known to be components of the extracellular matrix (Taipale et al., 1996). An important function of fibrillin-1 is to control the bioavailability of TGFβ. TGFβ is kept in an inactive form through attachment to the latency associated peptide (LAP), a peptide formed when immature TGFβ is proteolytically cleaved into LAP and mature TGFβ. In turn, LAP binds to one of four large latent TGFβ-binding proteins (LTBP). This complex, named the large latent complex (LLC), is linked to fibrillin-1 (Figure 2). Degradation of microfibrils by inflammatory proteolytic enzymes will initiate the release of TGFβ from the LLC, and TGFβ consequently becomes available for binding to the receptors TGFBR2 and TGFBR1 (ALK5) (Ten Dijke and Arthur, 2007). Activation of TGFBR2 leads to the phosphorylation and activation of TGFBR1, which in turn phosphorylates the receptor-activated SMAD proteins (R-SMAD2 and 3) that form a heterotrimer with SMAD4 (two R-SMADs to one SMAD4). This complex

binds to DNA in the nucleus, together with transcription factors to activate or repress gene transcription (Massague et al., 2005). It was reported in 2003 that mice deficient in fibrillin-1 exhibit dysregulation of TGFβ activation and signalling, showing impairment of distal alveolar septation during development and rescue of the phenotype by perinatal antagonism of TGFβ (Neptune et al., 2003). Similar observations were reported for the myxomatous changes of the mitral valves, histological changes in the lumbosacral dura and muscle regeneration in fibrillin-1 deficient mice (Cohn et al., 2007; Jones et al., 2005; Ng et al., 2004). Furthermore, SMAD2 phosphorylation was shown to be increased in the aortic media of a Marfan mouse model, and aortic dilatation could be prevented by either a TGFβ neutralising antibody or the angiotensin II type 1 receptor (AT1) blocker, losartan (Habashi et al., 2006). Recent results in mouse models of Marfan syndrome showed that TGFβ also induces other (noncanonical) signalling pathways (non-Smad dependent). It was demonstrated that extracellular signal-regulated kinase (ERK) 1/2 activation through angiotensin II type 2 (AT2) receptor is an important contributor to aortic disease and that this signalling cascade may be a new therapeutic target (Habashi et al., 2011; Holm et al., 2011). Overall, it is now thought that mutation of *FBN1* and the consequent reduction in fibrillin-1 leads to incorrect LLC sequestration and excessive activation of TGFβ signalling, which may then result in several of the phenotypic manifestations of Marfan syndrome (Figure 2).

This increased understanding of the role of TGFβ signalling in the pathogenesis of Marfan has led to new treatment strategies aimed at the aortic manifestations of the disease. In humans, therapy with AT1 blockers in a small paediatric cohort slowed down the rate of aortic dilatation in children with Marfan syndrome (Brooke et al., 2008). The results of several larger prospective clinical trials of AT1 blocker therapy on the aortic manifestations of the disease in humans are now awaited (Forteza et al., 2011; Gambarin et al., 2009; Moberg et al., 2011; Radonic et al., 2010; Brooke et al., 2008; Habashi et al., 2006).

3 Familial thoracic aortic aneurysms and dissections (FTAAD)

It has long been recognised that thoracic aortic aneurysms and dissections without other features of Marfan syndrome can be inherited as a dominant disorder with variable expression and reduced penetrance. Following the discovery of a pathogenic mutation in *TGFBR2* in a large French family with thoracic aortic aneurysms and dissections (Mizuguchi et al., 2004), several other genes have been shown to cause FTAAD (Milewicz and Tran-Fadulu, 1993). Mutations in *TGFBR2* lead either to FTAAD or to a more severe phenotype known as Loeys-Dietz syndrome (LDS) (Loeys et al., 2005). FTAAD caused by *TGFBR2* mutations, but without additional features typical of LDS, is also known as Marfan syndrome type II. While some affected FTAAD family members may indeed show minor

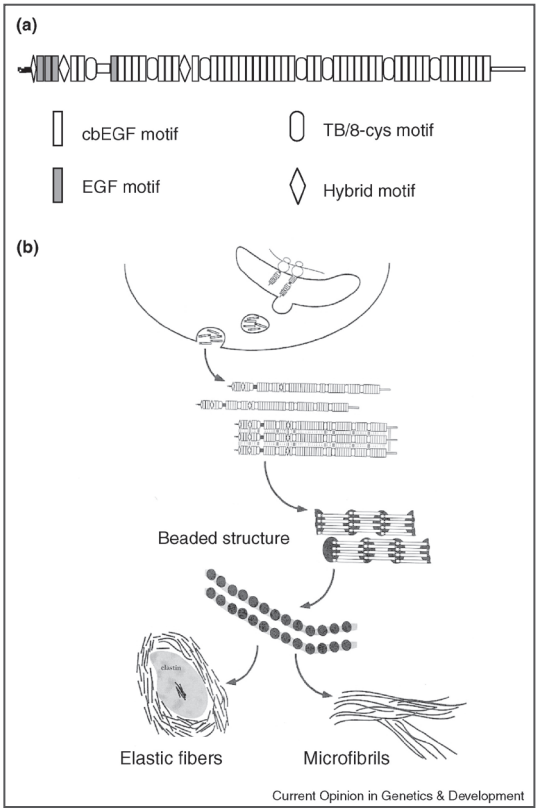

Figure 1. The fibrillin-1 protein from gene to microfibrils. (a) Schematic diagram showing the modular structure of *FBN1*, the gene encoding fibrillin-1. The Epidermal Growth Factor-like (EGF) domain is present 47 times and each domain contains six highly conserved cysteine residues that stabilise domain structure via three disulfide bonds. Of the EGF domains, 43 have a consensus sequence for calcium binding (cbEGF) in the N-terminal pocket of the domain that may mediate protein-protein interactions. The EGF domains are interrupted by seven transforming growth factor (TGF)-binding protein domains, characterised by eight cysteine residues involved in intra-domain disulfide bonds (TB/8-cys). (b) Schematic representation of the synthesis and secretion of fibrillin-1 and the assembly in the elastic fibres. After translation in the endoplasmic reticulum, the fibrillin-1 molecules are secreted and incorporated into the extracellular matrix (ECM). The fibrillin-1 molecules form a network with a head-to-tail orientation and intermolecular cross-links. Through specific folding, a beads-on-a-string structure with characteristic elastic properties is formed. Depending on the location, the microfibrils either form elastic fibres with a core of elastin or occur independently in the ECM. Reprinted with permission from Elsevier (Ramirez and Dietz, 2007).

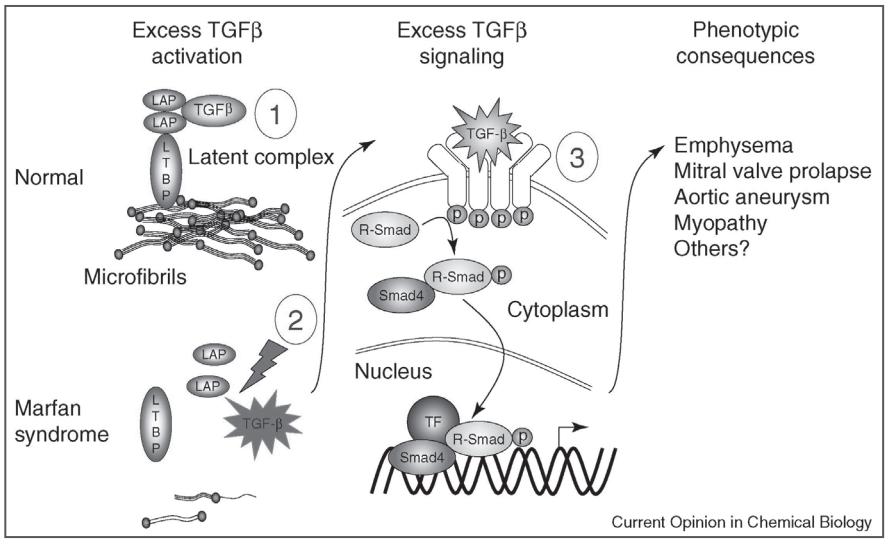

Figure 2. Schematic representation of the TGFβ signalling pathway regulated by microfibrils. (1) Microfibrils in the ECM bind the LLC (large latent complex) composed of LTBP (latent transforming growth factor-b binding protein), LAP (latency associated protein) and TGFβ. (2) TGFb may be released from its inactive form by perturbations of the microfibrillar network, for example, in the case of fibrillin-1 deficiency caused by a mutation in FBN1. The active form of TGFβ interacts with the TGFBR1/2 receptor complex, with consequent phosphorylation of receptor-activated Smad proteins (R-Smads) that in turn form a heterotrimeric complex with Smad4. After translocation from the cytoplasm to the nucleus, the R-Smad/Smad4 complex binds to DNA and is, in combination with transcription factors (TF), capable of gene transcription or repression during development and tissue remodelling. This might explain several features of Marfan syndrome.
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Marfan features, ectopia lentis has never been described in these families.
Mutations in known genes are found in up to 20% of FTAAD and genetic testing is available for *FBN1, TGFBR1, TGFBR2, ACTA2, MYH11, MYLK*, *SMAD3, TGFB2* and *NOTCH1*. Table 1 shows the clinical characteristics of FTAAD for the genes identified to date. These genes encode structural proteins involved in connective tissue, members of the TGFβ pathway, or components or regulators of the contractile unit of vascular smooth muscle cells (SMCs). A recent example is the discovery of mutations in *SMAD3*, which is another

member of the TGFβ pathway essential for TGFβ signal transduction. Using genome-wide linkage analysis, this gene was discovered in a large family with arterial aneurysms and dissections.

In a study of a group of 99 individuals with thoracic aortic aneurysms and dissections without mutations in *FBN1*, *TGFBR1* and *TGFBR2*, heterozygous mutations in *SMAD3* could be identified in two other families (van de Laar et al., 2011). Extensive clinical and imaging studies of affected individuals showed aneurysms of the thoracic and abdominal aorta, medium-sized arteries and arterial tortuosity. Furthermore, aortic dissections occurred in some family members at a young age and affected only mildly dilated aortas. Apart from aspecific dysmorphic and cutaneous features, most of the family members presented with early onset osteoarthritis of the knees, spine and thumbs, and intervertebral disc degeneration. Based on the vascular and skeletal phenotype, the name aneurysms-osteoarthritis syndrome (AOS) was proposed. A further five families with similar features have since been described and intracranial aneurysms or subarachnoid haemorrhage were reported as an additional feature (Regalado et al., 2011).

Recently, two independent studies showed that haploinsufficiency for *TGFB2* causes FTAAD with overlapping features of Marfan and Loeys-Dietz syndrome (Boileau et al., 2012; Lindsay et al., 2012). In *TGFB2*, which encodes a TGFβ ligand, loss of function mutations affecting the TGFβ signalling pathway were again found. As with mutations in *FBN1*, *TGFBR1, TGFBR2* and *SMAD3*, mutations in *TGFB2* lead to paradoxical (late) activation of the TGFβ signalling pathway. Several hypotheses have been presented to explain this effect, including an overshoot of a negative feedback loop, signalling through alternative pathways or the effects of activated inflammatory cells (Akhurst, 2012). This particular TGFβ paradox remains to be elucidated.

The genes *ACTA2*, *MYH11* and *MYLK* are all involved in smooth muscle cell (SMC) contraction within the media of the aorta. *ACTA2* encodes the SMC-specific α-actin, which is the most abundant protein in vascular SMCs and forms the thin filaments of the SMC contractile unit (Guo et al., 2007). *MYH11* encodes the SMC-specific β-myosin heavy chain, which forms the thick filaments of the SMC contractile unit (Khau Van et al., 2005; Pannu et al., 2007). This contractile unit is linked to the fibrillin microfibrils in the extracellular matrix through reversible association with integrin receptors (Milewicz et al., 2008). Mutations in *MYLK*, the myosin light chain kinase (MLCK) that controls SMC contractile function, can also cause FTAAD (Wang et al., 2010). Experiments in aortic tissue of carriers of a mutation in *ACTA2* or *MYH11* have shown that defective fibronectin fibril assembly results in activation of TGFβ signalling (Renard et al., 2011).

Table 1. Overview of FTAAD or syndromes associated with thoracic aortic aneurysms or dissections

Syndrome	Gene	Chromosome location	Protein name	Protein function	Phenotype	% of FTAAD
TAAD as a main feature						
Marfan syndrome	*FBN1*	15q21.1	fibrillin-1	structural component of elastic fibres; binding inactive TGFβ	Marfan syndrome; ectopia lentis syndrome; acromicric and geleophysic dysplasia; Weill-Marchesani syndrome; stiff skin syndrome; Shprintzen-Goldberg syndrome	syndromic
FTAAD/LDS	*TGFBR2*	3p24.1	transforming growth factor receptor type 2	transmembrane receptor for TGFβ, together with TGFBR1 regulation of cell proliferation and differentiation, extracellular matrix production	FTAAD; aortic aneurysms and dissections, arterial tortuosity and aneurysms, skeletal, craniofacial and cutaneous features (LDS)	4%
FTAAD/LDS	*TGFBR1*	9q22.33	transforming growth factor receptor type 1	as TGFBR2	FTAAD; aortic aneurysms and dissections, arterial tortuosity and aneurysms, skeletal, craniofacial and cutaneous features (LDS)	1%
FTAAD4	*MYH11*	16p13.11	myosin light chain kinase	part of contractile unit of SMC	FTAAD with PDA	1%
FTAAD6	*ACTA2*	10q23.31	actin, alpha2, smooth muscle aorta	part of contractile unit of SMC	FTAAD with tortuosity, premature stroke and coronary artery disease; multisystemic smooth muscle dysfunction	10-14%
FTAAD7	*MYLK*	3q21.1	myosin light chain kinase, smooth muscle	controlling SMC contractile function	FTAAD	1%
AOS	*SMAD3*	15q22.33	mothers against decapentaplegic homolog 3	signal transducer downstream from TGFBR 1 and 2	Tortuosity, aneurysms and dissections of arteries and aorta, osteoarthritis, intervertebral disc degeneration	2%
FTAAD/MFS-like	*TGFB2*	1q41	transforming growth factor beta-2	cytokine regulating multiple aspects of cellular behaviour	FTAAD with tortuosity, cerebrovascular disease and some features shared with LDS and MFS	2%
Frequently associated with TAAD						
BAV/FTAAD	*NOTCH1*	9q34.3	notch homolog 1, translocation-associated (Drosophila)	transmembrane receptor that regulates cell fate decisions during development	Bicuspid aortic valve with ascending aortic aneurysms and dissections	10% of familial cases
ATS	*SLC2A10*	20q13.12	glucose transporter 10	glucose homeostasis; regulation of TGFβ pathway	Tortuosity, stenosis and aneurysms of arteries and aorta, cutis laxa, joint laxity, contractures	syndromic
ARCL1	*FBLN4*	11q13.1	fibulin-4	extracellular matrix proteins regulating elastic fibre formation and connective tissue development	Cutis laxa, arterial tortuosity, (aortic) aneurysms and stenosis, retrognathia, joint laxity and arachnodactyly	syndromic
ADCL1	*ELN*	7q11.23	elastin	amorphous component of elastic fibres	Cutis laxa, gastrointestinal diverticula, hernia, genital prolapse, pulmonary artery stenosis, bicuspid aortic valve, aortic aneurysm, emphysema	syndromic

Table 1. Overview of FTAAD or syndromes associated with thoracic aortic aneurysms or dissections

Syndrome	Gene	Chromosome location	Protein name	Protein function	Phenotype	% of FTAAD
Occasionally associated with TAAD						
EDS vascular type	*COL3A1*	2q31	collagen type III	structural component in extracellular matrix	Thin translucent skin, easy bruising, characteristic facial appearance (in some individuals), and arterial, intestinal, and/or uterine fragility	syndromic
SGS	*SKI*	1p36	V-SKI avian sarcoma viral oncogene homolog	regulating TGFβ pathway by binding at Smad4	Severe marfanoid habitus, intellectual disability, camptodactyly, typical facial dysmorphism, craniosynostosis, aortic aneurysm	syndromic
ADPKD1	*PKD1*	14q12	polycystin 1	regulation of multiple signalling pathways to maintain normal renal tubular structure and function	Progressive renal cystic disease, extra-renal cysts, intracranial aneurysms, aneurysms and dissections of aorta or other arteries	syndromic
ADPKD2	*PKD2*	4q22.1	polycystin 2	nonselective cat ion channel involved in Ca(2+) transport and Ca(2+) signalling in renal epithelial cells	Progressive renal cystic disease, extra-renal cysts, intracranial aneurysms, aneurysms and dissections of aorta or other arteries	syndromic
PH EDS variant	*FLNA*	Xq28	filamin A	actin binding protein, interacts with the actin cytoskeleton and thereby regulates various aspects of cell shape, motility and function	periventricular heterotopia, joint and skin hyperextensibility, aortic dilatation and dissection	syndromic
Turner syndrome	na	X	na	na	Short stature, gonadal dysgenesis, dysmorphic features	syndromic
Noonan	*PTPN11 and others*	12q24.13	protein-tyrosine phosphatase, nonreceptor-type, 11	regulating the responses of eukaryotic cells to extracellular signals in the RAS/MAPK signalling pathway	short stature, a short neck with webbing or redundancy of skin, cardiac anomalies, deafness, motor delay, bleeding diathesis, dysmorphic features	syndromic
NF1	*NF1*	17q11.2	neurofibromin 1	cytoplasmic protein, regulatory function in several intracellular processes including RAS/MAPK signalling pathway	café-au-lait spots, Lisch noduli in the iris, neurofibromas, axillary freckling, intracerebral tumors, scoliosis	syndromic
OI	*COL1A1, COL1A2 and others*	17q31.33, 7q21.3	collagen, type 1, alpha-1; collagen, type 1 alpha-2	two alpha -1 and one alpha-2 chains form the triple helical structure of collagen type I ion the extracellular matrix	susceptibility to bone fractures with a wide range in severity	syndromic
Alagille syndrome	*JAG1*	20p12.2	jagged-1	ligand of Notch receptor, their binding play a role in cell differentiation and morphogenesis through transcription factors	cholestasis, cardiac disease, skeletal abnormalities, ocular abnormalities, and a characteristic facial phenotype	syndromic
HHT	*ENG, ACVRL1, SMAD4*	9q34.11, 12q13.13, 18q21.2	endoglin; activin a receptor, type II-like kinase 1; mothers against decapentaplegic homolog 4	endoglin is a homodimeric glycoprotein and component of the TGFBR complex; activin a receptor, type II-like kinase 1 is a type I cell surface receptor for the TGFβ ligands; SMAD4 is a signal transducer downstream from TGFBR 1 and 2	arteriovenous malformations in liver, lung and brain; smal telangiectases on lips, tongue, buccal mucosa, face, chest, and fingers; juvenile polyposis (in case of mutations in *SMAD4*)	syndromic
XL Alport syndrome	*COL4A5*	Xq23.3	collagen, type 4, alpha-5	alpha-5 forms together with alpha-3 and -4 heterotrimers of type IV collagen that form networks that serve as scaffolding for the deposition of other matrix glycoproteins and for cell attachment	progressive renal insufficiency, progressive sensorineurla hearing loss, ocular lesions, aortic aneurysms	syndromic

ADPKD autosomal dominant polycystic kidney disease; AOS aneurysms-osteoarthritis syndrome; ADCL1 autosomal dominant cutis laxa type 1; ARCL1 autosomal recessive cutis laxa type 1; ATS arterial tortuosity syndrome; BAV bicuspid aortic valve; Ca calcium; CCA congenital contractural arachnodactyly; EDS Ehlers-Danlos syndrome; FTAAD familial thoracic aortic aneurysms and dissections; HHT Hereditary Hemorrhagic Telangiectasia; LDS Loeys-Dietz syndrome; MFS Marfan syndrome; na not applicable; NF1 Neurofibromatosis type 1; OI osteogenesis imperfecta; PDA patent ductus arteriosus; PH periventricular heterotopia; SGS Shprintzen-Goldberg syndrome; SKS stiff skin syndrome; SMC smooth muscle cell; TGFβ transforming growth factor β; TGFBR1 transforming growth factor, β receptor 1; WMS Weill-Marchesani syndrome; XL -linked

A very common congenital valve abnormality, bicuspid aortic valve, has an incidence of 1.4% (Larson and Edwards, 1984) and leads to an increased risk of dilatation of the ascending aorta and eventually, to dissection (Michelena et al., 2011). In 2005, mutations in the signalling and transcriptional regulator *NOTCH1* were discovered in dominant pedigrees and were associated with a spectrum of developmental aortic valve anomalies, including bicuspid aortic valves and severe valve calcification (Garg et al., 2005; Garg, 2006). Mutations in *NOTCH1* have also been found in familial and sporadic cases with bicuspid aortic valves (McKellar et al., 2007; Mohamed et al., 2006).

4 Other syndromes associated with thoracic aortic aneurysms and dissections

Some other syndromic disorders that may be associated with thoracic aortic disease are listed in Table 1. One example is the vascular type of Ehlers-Danlos syndrome (EDS) caused by a mutation in *COL3A1*, encoding collagen type 3. Typical features are a thin translucent skin, characteristic facial appearance, and arterial, intestinal and uterine fragility. Patients have a high risk of rupture of internal organs and dissections of medium-sized arteries, and the thoracic aorta is involved in a minority of cases.

A very rare autosomal recessive connective disorder, arterial tortuosity syndrome (ATS), is characterised by severe tortuosity, stenosis and aneurysms of the aorta and medium-sized arteries, in addition to skin and skeletal involvement (Cine et al., 2011; Callewaert et al., 2008; Coucke et al., 2006; Wessels et al., 2004). To the best of our knowledge, dissections of the aorta have not been described. ATS is caused by mutations in the *SLC2A10* gene that encodes the facilitative glucose transporter 10 (GLUT10). Mutations in *EFEMP2* (also known as *FBLN4*) cause a subtype of autosomal recessive cutis laxa (ARCL) with features similar to ATS and characterised by a severe vascular phenotype with arterial tortuosity, aneurysm and stenosis, including ascending aortic aneurysms (Al-Hassnan et al., 2012; Callewaert et al., 2008; Dasouki et al., 2007; Huang et al., 2010; Hucthagowder et al., 2006; Kappanayil et al., 2012; Loeys et al., 1993; Renard et al., 2010). Also in autosomal dominant cutis laxa (ADCL1) caused by mutations in the elastin gene (*ELN*), progressive aortic dilatation was described in four of five probands, with bicuspid aortic valves in two of them (Callewaert et al., 2011). In Shprintzen-Goldberg syndrome (SGS), a recognisable phenotype with severe marfanoid features, intellectual disability and craniosynostosis, some cases were found to be due to mutations in *FBN1*, *TGFBR1* and *TGFBR2*, but very recently mutations in *SKI* were shown to be the cause of most cases of SGS. This gene encodes a protein that is associated with SMAD4 and has a regulatory function in the TGFβ pathway (Carmignac et al., 2012; Doyle et al., 2012).

Autosomal dominant polycystic kidney disease (ADPKD) is a relatively common chronic kidney disease. The majority of ADPKD patients carry mutations in *PKD1* or *PKD2*, encoding

polycystin 1 and polycystin 2, respectively (The European Polycystic Kidney Disease Consortium, 1994; Peters et al., 1993). ADPKD is associated with vascular manifestations, the most prevalent of which are intracranial aneurysms and subarachnoid haemorrhage, but aortic aneurysms, dissections and aneurysms of other arteries have also been described (Adeola et al., 2001; Harris and Torres, 1993; Lee et al., 2004; Pirson et al., 2002). Polycystin 1 and 2 are expressed in vascular SMCs in arteries and are thought to play a role in the interaction between SMCs and adjacent elastic tissue - and may be involved in the vascular complications seen in ADPKD (Griffin et al., 1997; Hassane et al., 2007; Torres et al., 2001).
FLNA mutations cause an X-linked dominant disorder characterised by periventricular heterotopia with seizures and dyslexia, but also including features of EDS such as joint and skin hyperextensibility. Patients have also been reported to show cardiovascular abnormalities including aortic dilatation and dissection (Sheen and Walsh, 2005). Turner syndrome is a well-known chromosomal disorder and is most commonly caused by monosomy of the X-chromosome in females. Bicuspid aortic valve, coarctation of the aorta and hypertension are common abnormalities in Turner syndrome and predispose to aortic dilatation and dissection. Aortic complications have been described in patients with Turner syndrome even in the absence of these risk factors (Carlson and Silberbach, 2007; Elsheikh et al., 2002; Gravholt et al., 2006). Other syndromes such as Noonan syndrome, neurofibromatosis type 1, osteogenesis imperfecta, Alagille syndrome, hereditary hemorrhagic telangiectasia and X-linked Alport syndrome seem to be associated with a slightly increased risk of thoracic aortic disease (Andersen et al., 2010; Ashraf et al., 1993; Hsi et al., 2003; Kamath et al., 2004; Kashtan et al., 2010; Lin et al., 1987; Moriyama et al., 1995; Oderich et al., 2007; Power et al., 2006; Radunovic et al., 2011; Shachter et al., 1984).

5 Scope and outline of this thesis

Marfan syndrome is one of the most thoroughly investigated syndromic forms of thoracic aortic aneurysms. Patients with Marfan syndrome not only have an increased risk of aortic aneurysms and dissections but also suffer from other pathology affecting the eyes, the skeletal system and the skin, leading to significant morbidity. The management of this multisystemic disorder is a challenge for the clinicians involved. **Chapter 2** presents the Dutch multidisciplinary guidelines for the diagnosis and management of Marfan syndrome, based upon current knowledge and recently revised clinical criteria. The revised Ghent criteria for the diagnosis of Marfan syndrome are presented in **Chapter 3**. During the process of developing clinical guidelines and criteria for Marfan syndrome, it became clear that several aspects lacked scientific support and were solely based upon expert opinion. The following chapters discuss the presentation of Marfan syndrome and FTAAD

in relation to specific mutations. It has become increasingly clear that genetic factors play an important role in the pathogenesis of thoracic aortic aneurysms. With the shift from a structural defect of connective tissue to a signalling defect, insight into the underlying pathogenetic mechanism of specific mutations is of utmost importance. Novel insights may allow the development of therapeutic strategies to prevent the life-threatening dissections that can occur in genetically-determined aneurysms, even in those of a very young age. The clinical and genetic study of a series of missense *FBN1* mutations, leading to the substitution of the first aspartic acid in the cbEGF domain for a valine, is described in **Chapter 4. F**indings in a large family, with three Marfan patients carrying homozygous mutations of *FBN1*, suggested that the non-penetrance of the specific mutation in heterozygous form could be explained by a haploinsufficiency model. A threshold model could go someway to explaining a proportion of the intra- and extra-familial variability of Marfan syndrome. **Chapter 5** presents ten patients with a complete *FBN1* gene deletion. The clinical features of these patients illustrate that pure haploinsufficiency of *FBN1* is sufficient for the expression of the complete phenotype of Marfan syndrome. **Chapter 6** reports an unexpected result of a microarray analysis performed in a boy with learning difficulties and behavioural problems. The identification of an interstitial deletion of chromosome 15 that disrupts the *SMAD3* gene was a finding with major health implications for family members, and revealed a significant familial risk for aortic and arterial aneurysms and dissections. In **Chapter 7,** two patients with Marfan syndrome are described with unexplained intracranial hypertension.

Chapter 8 includes a general summary, together with a discussion of the implications of the studies in this thesis, and future prospects for diagnosis and treatment of thoracic aortic aneurysms and dissections. This is followed by a Dutch summary in **Chapter 9**.

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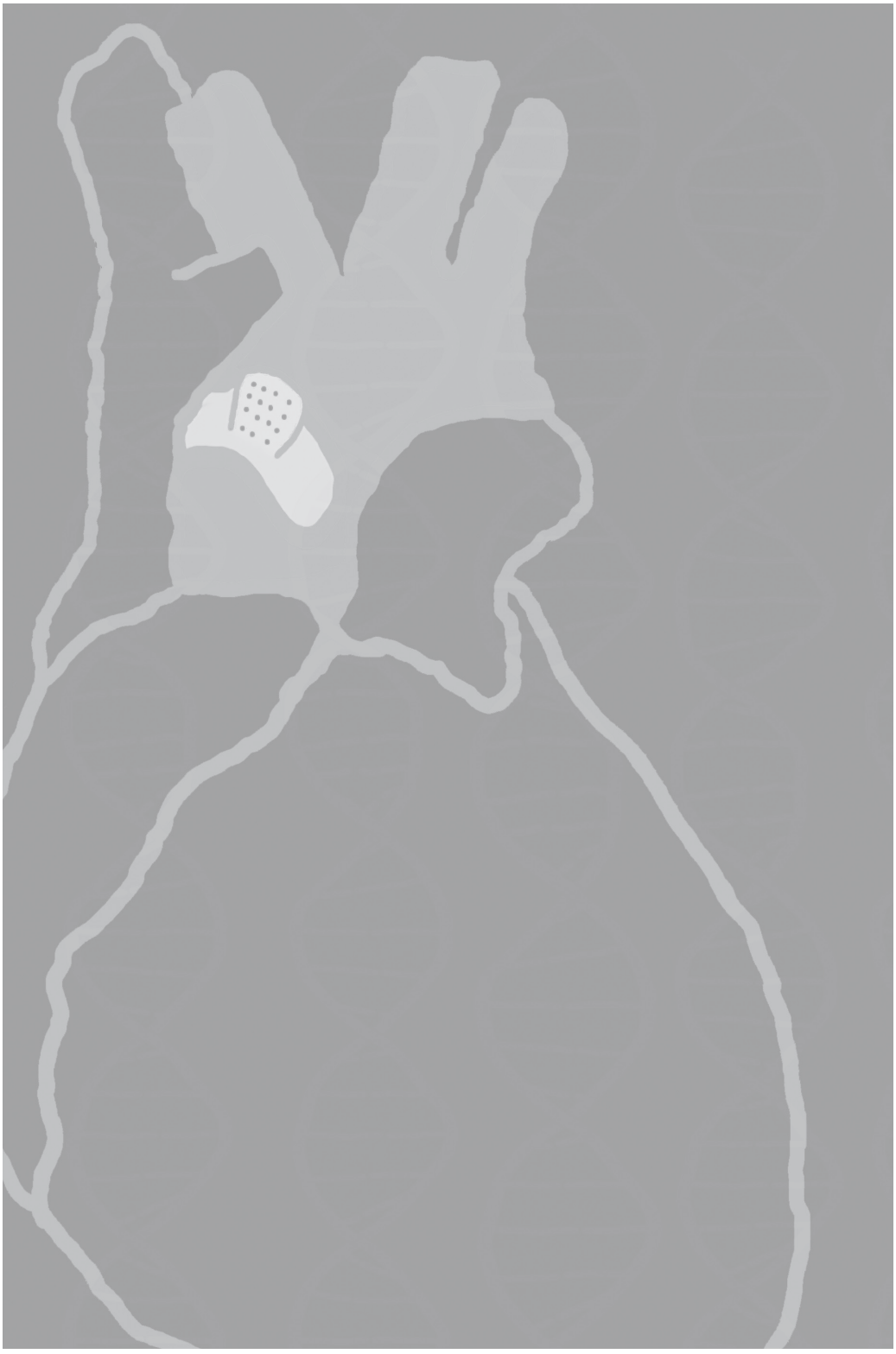

2

Practical clinical guidelines for the diagnosis and management of Marfan syndrome

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Section 1 General Introduction

1.1 Motivation for guideline development

Few original data on the birth prevalence of Marfan syndrome are available in the medical literature. The prevalence at birth was originally estimated at around 1/10,000 and the prevalence at 1/14,217 (Gray et al, 1994), but later estimates assume a prevalence of 2-3 per 10,000 (Judge & Dietz, 2005; Pyeritz, 2000), although the basis for these higher estimates is unclear. Based on the figures of Gray (1994) and a life expectancy with Marfan syndrome of 70 years (a figure based on little data), one arrives at an estimate of around 1,200 to 1,300 patients in the Netherlands, with 18 newborns per year.

Marfan syndrome is an autosomal dominant, multisystem disorder in which the heart and aorta, skeleton and eyes are the major affected organs. Due to the variety of disease expression, the wide variability and the hereditary nature of the condition, there is often a range of specialists involved in the care of a patient with Marfan syndrome.

Because the disease is relatively rare, 4 expert centres (Marfan clinics) have been established in the Netherlands. These centers provide Marfan syndrome diagnostics when a diagnosis is suspected on clinical grounds and ensure adequate clinical assessment and treatment. While the Marfan clinics are in close contact with one another, there are nevertheless differences in the approach to and organisation of care.

Moreover, there is currently no uniform policy for referral from primary and secondary centres to a Marfan clinic. In order to develop a uniform policy regarding the referral, diagnosis and treatment of Marfan patients, the scientific societies involved in the care for Marfan patients have decided, following an initiative of the Dutch Society of Clinical Genetics (VKGN), to develop a guideline with uniform, and as far as possible, evidence-based recommendations.

The development of the guideline was funded by the Stichting Kwaliteitsgelden Medisch Specialisten (SKMS). The Department of Professional Quality Support of the Dutch Order of Medical Specialists provided methodological support.

1.2 Aim of the guideline

The guideline provides recommendations for physicians regarding referral policy, which amongst others provides guidelines regarding the characteristics required to indicate a referral to a Marfan clinic. In addition, recommendations are made for care providers at Marfan clinics regarding the diagnostic procedure, the logistics thereof, the clinical assessment and treatment of Marfan patients and family studies. Specific recommendations regarding prenatal diagnosis, pregnancy and childbirth are also made.

No recommendations are provided for the treatment of disorders or problems that occur more frequently in Marfan syndrome than in the normal population, but which do not require treatment other than that appropriate in non-Marfan patients.
Patients with Marfan syndrome, organised in the Contactgroup Marfan Netherlands, were involved in the creation of this guideline and made recommendations for the organisation of care.
With this guideline, the working group hopes to create a tool for the provision of uniform care in Marfan syndrome.

1.3 Delineation of the guideline

Description of the problem and clinical questions.
The working group has formulated a number of clinical questions (see Appendix 1) that form the basis for the various sections of this guideline. The guideline does not aim to describe the entire process of clinical care, but focuses on specific bottlenecks.
The guideline covers the diagnosis and treatment of patients with Marfan syndrome and is based on new criteria established in 2010, "the revised Ghent nosology for the Marfan syndrome" (Loeys et al, 2010), subsequently referred to as the Ghent II criteria. The guideline refers to the original Ghent criteria as Ghent I (De Paepe et al, 1996).

1.4 The intended users of the guideline

The guideline is primarily aimed at all healthcare professionals involved in the detection, diagnosis, monitoring and treatment of patients with Marfan syndrome: GPs, (paediatric) cardiologists, paediatricians, thoracic surgeons, clinical geneticists, ophthalmologists, gynaecologists, orthopaedic surgeons, midwives, paediatricians and doctors at health clinics. The directive is therefore not only intended for specialists linked to a Marfan clinic. The secondary target group is that of patients with Marfan syndrome.

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Section 2 Methodology of Guideline Development

2.1 AGREE

This guideline has been prepared on the basis of the "Appraisal of Guidelines for Research & Evaluation II" (AGREE II) instrument (www.agreecollaboration.org). This is a widely accepted international instrument for the assessment of the quality of guidelines.

2.2 Working group

For the development of the guideline, a multidisciplinary working group was established in 2010, consisting of representatives from all relevant specialties involved in the assessment and care of Marfan syndrome. The working group consisted of clinical geneticists, cardiologists, a cardiothoracic surgeon, ophthalmologists, a gynaecologist, a paediatrician/-cardiologist, orthopaedic surgeons, a molecular geneticist and an anaesthetist (see Members of the Working Group).

The working group members were mandated to participate by their professional associations. The group worked for two years on the creation of the guideline.

2.3 Declarations of conflicts of interest

Members of the working group have declared in writing whether they have maintained, over the last five years, a (financially supportive) relationship with commercial enterprises, organisations or institutions related to the subject of the guideline.

2.4 Patient participation

During the development of the guideline, a specific focus was to appraise the perspective of the patient. The working group included a representative of the patient organisation Contact Group Marfan Netherlands. In addition, a patient focus group was organised. The discussions within the focus group were summarised in a report that was made available to members for verification and possible additional comments. A summary of this report (Appendix 3) was used by the working group in the preparation of the guideline, and finally, members of the focus group were asked to comment on the draft guideline.

2.5 Analysis of critical issues

Particularly difficult issues were identified during the preparatory phase, in cooperation with the chair of the working group. These issues were discussed in the working group, and on this basis clinical questions were formulated.

2.6 Clinical questions and outcome measures

Clinical questions were formulated by the working group based on critical issues. When possible, the most important and patient-relevant outcome measures were identified for each clinical question.

2.7 Literature search strategy

The basis for the guideline is provided by evidence from published scientific research. During the exploratory search, the Cochrane Library was searched and existing guidelines were specifically sought in (inter)national guideline clearinghouses that allow online searches.

Because not all clinical questions lend themselves to a systematic literature search, prior agreement was made to limit systematic literature searches to questions regarding treatment and to those related to pregnancy and childbirth.

Based on specific search terms, the electronic databases Medline and Embase were searched for published scientific studies relevant to the clinical questions formulated. If necessary, searching for additional studies was carried out. Initial searching focused on (systematic reviews or meta-analyses of) randomised controlled trials (RCTs). In the absence of RCTs, searching was resumed; now focusing on prospective comparative controlled trials and prospective non-comparative studies. Languages were limited to English, Dutch and German.

The working group members selected items by relevance. In addition, studies were extracted from reference lists of retrieved literature. For certain clinical questions, this resulted in additional relevant articles. The selected articles were used to answer the clinical question.

For the remaining clinical questions, the available scientific evidence proved to be insufficient. To answer these questions, the expertise of the working group members was elicited, supported by scientific literature when available. Because Marfan syndrome is a rare disorder, the available literature is often limited to case series and small patient groups.

2.8 Strategy for assessing literature

Literature reviews were carried using the EBRO methodology. Individual studies were assessed for study approach/design. Following this assessment, the level of study evidence was determined according to the classification described in Table 2.1 and Table 2.2. A summary of the literature and the level of evidence of the relevant studies can be found in the guideline text under the headings, ‘summary of the literature’ and ‘conclusion’.

Table 2.1. EBRO classification of individual study quality

Evidence level	Diagnostic accuracy of research	Injury or side effects, etiology, prognosis
A1	Meta-analysis of at least 2 independently conducted studies of A2-level	
A2	Research conducted in relation to a reference test (gold standard) with predefined cut-off values and independent assessment of results, with a sufficiently large series of consecutive patients, all of whom have undergone both the index and reference tests	Prospective cohort study of sufficient size and follow-up, which adequately controlled for confounding and with sufficient exclusion of selective follow-up
B	Research relative to a reference test, but not with all the features listed under A2	Prospective cohort study, but not with all the features listed under A2 or a retrospective cohort or case-control study
C	Non-comparative study	

Table 2.2. Level of evidence supporting a conclusion

Conclusion based on	
1	Study of level A1 or at least two independently conducted studies of level A2
2	One study of level A2 or at least two independently conducted studies of level B
3	One study of level B or C
4	Expert opinion

2.9 Considerations

Before making a specific recommendation, in addition to the scientific evidence, other factors must be considered including the expertise of the working group members, patient preferences, costs, availability of facilities or organisational aspects. These factors are, in sofar as they have not been scientifically investigated, listed under the heading 'considerations'.

2.10 Recommendations

The recommendations provide an answer to the central clinical question and are based on both the available scientific evidence and on the most important considerations.

2.11 Comment and authorisation phase

The draft guideline was presented to the relevant (scientific) associations for comments. The comments were gathered and discussed within the working group. In response to the comments, the draft guideline was adapted and finalised by the working group. The final guideline was then sent to the directors of the (scientific) associations for authorisation.

2.12 Implementation

During the development of the guideline, the implementation and feasibility of the recommendations were already taken into account. Consideration was given to factors that could either promote or hinder the introduction of the guideline in practice. The guideline has been distributed to all relevant professional groups and institutions, and an electronic version of the guideline will be published. The electronic version will be available for download from the VKGN website and from other participating organisations, and from the Kwaliteitskoepel: www.kwaliteitskoepel.nl. A summary of the guideline will be submitted to the Netherlands Journal of Medicine and to other relevant journals. In order to bring the guideline to the attention of the target audience, a symposium will also be organised.

2.13 Legal implications of the guideline

Guidelines are not laws, but are based on evidence-based insights and recommendations that caregivers must satisfy in order to provide high-quality care. Since these recommendations are mainly based on 'general evidence for optimal care of the average patient', the professional autonomy of healthcare providers allows them deviate from the guidelines in individual cases, where necessary. Even deviation from the guidelines may be necessary in certain situations. In cases where the guideline is not followed, any deviation should be justified and documented and, where relevant, this should be following consultation with the patient.

2.14 Revision

The board of the VKGN will decide whether the guideline is still current, not later than 2017. If necessary, a new working group will be formed to revise the guideline. The validity of the guideline will be earlier curtailed if new developments necessitate the initiation of a revision process.

The Dutch Society of Clinical Genetics (VKGN) is the originator of this guideline and is as such primarily responsible for the topicality of the guideline. Other scientific associations participating in the guideline or users of the guideline share responsibility, and should inform the VKGN on relevant developments within their field.

Section 3 Diagnostics

Introduction

Marfan syndrome is a multisystem disorder with a highly variable expression, leading to difficulties in detection and diagnosis. A diagnosis of Marfan syndrome is made on the basis of clinical and genetic findings, whereby use is made of the Ghent II criteria (Loeys et al, 2010). These criteria are a revision of the Ghent I criteria (Chapter 3 of this thesis, De Paepe et al, 1996). Based on these criteria and related critical comments, recommendations are made which form the basis for a diagnosis of Marfan syndrome.
From the question of the selection of an appropriate diagnostic procedure, the question arises as to the minimum characteristics indicating diagnostics. Again, recommendations are made, including the most appropriate diagnostic procedure.

Summary of the literature

What are the diagnostic criteria for Marfan syndrome?
The possible characteristics of Marfan syndrome are listed in Appendix 2. This table shows the characteristics used in the Ghent I (De Paepe et al, 1996) and Ghent II criteria (Loeys et al, 2010). The list is not comprehensive, and although not all features are included in the Ghent criteria, they may nevertheless contribute to the recognition of Marfan syndrome.
Sponseller et al. (2010) have compared the frequency of occurrence of a number of Ghent criteria (mainly facial and skeletal abnormalities) in genetically confirmed Marfan patients (n = 183) and in a control group (n = 1257) and based on this, determined the sensitivity and specificity of the different characteristics (Sponseller et al, 2010).

1. *Facial features*
   All facial features are subjective and are almost never objectified in size or number. Despite this, facial features show a high sensitivity. A high palate with irregular dentition is no longer included as a diagnostic criterion in Ghent II, due to a low specificity.
2. *Skeletal features*
   Skeletal features can be partly objectively determined using measurements and radiographic examination. This is the case for arachnodactyly, in which use is made of the wrist and thumb sign. The thumb sign is positive if the distal phalanx of the thumb protrudes beyond the ulnar border of the hand at maximum adduction. The wrist sign is considered positive if the top of the thumb overlaps the entire nail of the little finger when circling the wrist.

Bodily proportions can be measured, with a span/length ratio of 1.05 or more being considered abnormal in the absence of scoliosis. When determining relatively long legs using the Ghent II criteria, normal values for the upper and lower ratios are given but these lack literature references. For acetabular protrusion and scoliose, standardised measurements are applied to a radiograph of either the pelvis or the spine (Sponseller et al, 2006).

3. *Cardiovascular features*

   In the most recent criteria, the greatest weight is given to a dilatation of the aortic root or aortic dissection type A. The combination of a dilated aortic root and a dilatation of the pulmonary artery is a strong indicator of Marfan syndrome (Nollen et al, 2002). Mitral valve prolapse is included in the systematic score. In a study of 52 relatively young patients with Marfan syndrome, aged between 3 and 28 years, 43 patients (83%) showed an aortic root dilatation and 46 (88%) showed a mitral valve prolapse (van Karnebeek et al, 2001).

   For the implementation and interpretation of cardiovascular imaging, see section 4.

4. *Ocular features*

   Until recently, diagnosis based on the Ghent I criteria included scoring for both major and minor criteria in which various ophthalmologic abnormalities were attributed a diagnostic value. The number of ophthalmologic abnormalities that contribute to the diagnosis of Marfan syndrome is greatly reduced in the Ghent II criteria, and only lens (sub)luxation and myopia of more than three diopters are still included. Lens (sub) luxation is the only major ophthalmologic criterion in both the Ghent I and Ghent II criteria.

   A literature review by Nemet et al. (2006) describes the main ocular features of Marfan syndrome. Maumenee (1981) described eye diseases in a population of 160 Marfan patients from 0-60 years of age. Lens (sub)luxation or *ectopia lentis* is a known feature of Marfan syndrome, with a reported incidence varying from 50 to 87% (Nemet et al, 2006). The luxation is usually (but not always) bilateral and towards temporal-top (Maumenee, 1981; Nemet et al, 2006). In rare cases (2-3%) full *luxatio lentis* may occur, resulting in a lens free in the vitreous humourand (non-iatrogenic) aphakia (Nemet et al, 2006). A literature review by Dureau (2008) described the pathophysiology of lens (sub) luxation and attributed it to poor quality zonular fibres. In the case of Marfan syndrome, these fibres are stretched or even broken, permitting the lens to luxate (Dureau, 2008). Myopia greater than 3D is poorly indicative and is thus considered a less important criterion in the systematic score for the Ghent II criteria. Myopia is believed to occur in 34-44% of Marfan patients, compared with 4.8% of the normal population (Nemet et al, 2006). In a study of the level of myopia in Marfan syndrome patients, 50% of patients

showed myopia of 3D or more (Nemet et al, 2006).

Other ophthalmic characteristics that often affect the eyes of Marfan patients include iris hypoplasia, iris transillumination and iridodonesis. A flat cornea has also been described in the eyes of patients with Marfan syndrome. Heur (2008) compared the results of keratometry and central cornea thickness (CCT) from 62 Marfan patients (mean age 22.3 years) with those of 98 controls (mean age 19.3 yrs). The Marfan patients had a significantly lower outcome on keratometry and CCT than the controls. Peripheral retinal degeneration and retinal detachments occur in between 5 to 25.6% of patients with Marfan syndrome. These complications are more common in eyes with lens(sub) luxation and/or a long axis length (Nemet et al, 2006).

5. *Pulmonary*

   Spontaneous pneumothorax contributes to the systematic score. In a retrospective study of 166 patients aged 13 or older, 4.8% had experienced pneumothorax one or more times. Two of the 8 patients had experienced a pneumothorax twice or even several times (Karpman et al, 2011).

6. *Skin*

   Conspicuous striae at unusual locations count towards the systematic score, under the condition that the striae are not the result of significant weight change or pregnancy. Locations defined as unusual under the Ghent II criteria include the middle of the back, the lumbar region, upper arm, axilla region and hips (Loeys et al, 2010).

7. *Dura*

   Under the latest criteria, lumbosacral dural ectasia are now included in the systematic score. The method commonly used in the Netherlands to determine dural ectasia is that of Oosterhof (Oosterhof et al, 2001); this method has a high sensitivity and specificity, of 95% and 98%, respectively. The gold standard used was the presence (n = 44) or absence (n = 44) of Marfan syndrome based on clinical and genetic criteria. However, when comparing the method of Oosterhof with two other methods, a strikingly high level of dural ectasia was found in the control group. Using the Oosterhof method, Weigang found a sensitivity of 94% and a specificity of 57% (Weigang et al, 2006). In an observational study of 33 patients with features of Marfan or Loeys-Dietz but without genetic abnormalities, Sheikzadeh reported high levels of dural ectasia in individuals with non-specific connective tissue abnormalities but without Marfan syndrome (Sheikhzadeh et al, 2010).

8. *Other organ systems*

   Recurrent inguinal or umbilical hernia, incisional hernia, and reduced fat and muscle are not specific to Marfan syndrome but are very common. These features are no longer part of the diagnostic criteria.

9. *Family*

   Having a first-degree relative with Marfan syndrome carries significant weight in the Ghent II criteria.

10. *DNA*

    Marfan syndrome is usually caused by mutations in the *FBN1* gene, which codes for the protein, fibrillin-1. In a recent publication by Sheikzadeh, a mutation in *FBN1* was detected in 80% of the patients who met the original, the new or both Ghent criteria (Sheikhzadeh et al, 2011). The Ghent II criteria attributes considerable weight to causal mutations in the *FBN1* gene.

Neonatal Marfan syndrome

The debate in literature as to whether neonatal Marfan syndrome should be seen as a separate entity, or as a very severe form of Marfan syndrome, remains unresolved. Hennekam provided arguments supporting the limitation of the term 'neonatal Marfan syndrome' to newborns with severe mitral and/or tricuspid valve insufficiency and infantile emphysema (Hennekam, 2005). These children almost always die before the 2nd year of life due to progressive valve dysfunction and heart failure. Similarly to children with neonatal Marfan syndrome, children with a severe expression of Marfan syndrome may display serious skeletal abnormalities, lens (sub)luxation, aortic root dilatation and elastic skin, but almost never show life-threatening valve dysfunction. Neonatal Marfan syndrome (as defined by Hennekam) is almost always de novo (Stheneur et al, 2011).

What is the policy in the case of a newborn with 50% risk of Marfan syndrome?

This question leads to the question of what is the earliest age at which Marfan syndrome can present with features that may require treatment. The literature indicates that it is extremely rare for a child with classic Marfan syndrome to undergo cardiovascular surgery in the first year of life. Everitt et al. (2009) conducted a retrospective study in children with Marfan syndrome, and of the 196 patients, 18 (9%) had surgery in childhood. The youngest age at the time of the first cardiovascular surgery was 8 years. This is in contrast to the age of 1 in cases of neonatal Marfan syndrome and Loeys-Dietz syndrome (Everitt et al., 2009).

Conclusions

Level 4	The clinical diagnostic criteria for Marfan syndrome include abnormalities of the face, the skeleton, the cardiovascular system, the eyes, lungs, skin and the dura. In addition, DNA abnormalities (pathogenic mutations) and family history are also important. Aortic root dilatation, lens (sub)luxation, a first-degree relative with Marfan syndrome and a pathogenic mutation are the main criteria on which the diagnosis is based. D Loeys et al., 2010

Considerations

In this guideline the diagnostics of Marfan syndrome are based on the Ghent II criteria. In addition, the following considerations are taken into account:

1. General

   In order to avoid unnecessary diagnostics in cases of individuals with non-specific features of Marfan syndrome, an initial examination by a clinical geneticist affiliated to a Marfan clinic should be considered. The geneticist can assess whether additional diagnosis is necessary. Specific characteristics, such as aortic root dilatation/dissection without an obvious cause or a lens (sub)luxation, warrant extensive investigation.

2. Physical examination

   The physical examination is preferably carried out by a specialist with experience in Marfan syndrome. Most of the external characteristics of Marfan syndrome are non-specific and can only be subjectively determined. Appendix 2 describes the physical characteristics of Marfan syndrome.

   The Ghent II criteria assume a specific upper/lower segment ratio when determining relative leg length, but this is challenging to measure. The preference of the working group is for the measurement of the sitting height/length ratio, as robust normal values are available (Talma, 2010).

   The working group notes that striae are common in the middle of the back, the hips and lumbar region in the general population, and therefore expresses reservations regarding the inclusion of this criterion in the Ghent II criteria. The working group therefore recommends the inclusion of striae as a systematic criterion only when

present in places other than the abovementioned locations and when not associated with weight change. Marfan syndrome is characterised by striae particularly on the shoulders, upper arms and around the axilla.

3. Ophthalmological examination

Only two ophthalmological features score points on the diagnosis under the Ghent II criteria, lens (sub)luxation and myopia of more than three diopters.

Assessing lens position should occur at maximum mydriasis, using slit lamp examination. This is the only way in which lens (sub)luxation can be determined with certainty, as the condition can manifest itself in a very subtle manner. In some cases, only an irregular lens edge (notching) due to a single stretched zonula fibre is noted.

A refraction test should take place in all patients. The strength of existing spectacles or contact lenses is often the starting point in such cases. In young children, where subjective visual acuity and refraction measurement is not possible, the objective refraction should be determined using cycloplegic retinoscopy.

In order to calculate the degree of myopia, the value determined for the spherical equivalent of the cylinder is added to the value of the spherical refraction.

4. DNA research

The pathogenic effect of mutations, and thus the causal relation to Marfan syndrome, is insufficiently treated in the Ghent II criteria.

If the presence of an *FBN1* mutation is important for the diagnosis, this can only be taken into account if, in the opinion of an appropriately qualified clinical molecular geneticist, the mutation is pathogenic. When in doubt as to the pathogenicity of the mutation, it should not be included in the diagnostic criteria. When a mutation is not clearly pathogenic, additional research can be considered such as analysis of mRNA or protein from cultured fibroblasts, or DNA analysis of the mutation in the family.

If no clearly pathogenic mutation in *FBN1* is found, this should give rise to serious doubts regarding the diagnosis of Marfan syndrome and invoke a differential diagnostic consideration of whether there are grounds to investigate other genes.

5. X-ray diagnosis

X-ray diagnosis for acetabular protrusion and scoliosis is rarely necessary in the context of diagnosis, but can be indicated in the case of complaints or as an adjunct to therapy.

6. Diagnosis in children

The exclusion of a diagnosis of Marfan syndrome in children on clinical grounds alone is either not possible or difficult, especially in young children, as symptoms may arise later in life.

a. Child with a parent with both Marfan syndrome and a mutation in *FBN1*. DNA testing can determine whether the children of a parent carrying a pathogenic mutation in the *FBN1* gene will themselves develop Marfan syndrome. DNA testing can be offered in the first year of life so that, following confirmation of a mutation, an initial paediatric cardiological examination can be carried out to exclude rare aortic or valve problems that already require care in the first year of life. If possible, the first physical and ophthalmic examinations can also be conducted by a Marfan clinic. An initial ophthalmological examination in the first year of life is indicated for the early detection of cases at risk for serious lens luxation with a danger of amblyopia. The child will be monitored more or less frequently, depending on the findings.

b. Child with a parent with Marfan syndrome, but without a known *FBN1* mutation. In children with a 50% chance of developing Marfan syndrome but with an affected parent in whom no *FBN1* gene mutation was found, it is recommended that the initial study take place in the first year of life (physical, paediatric cardiological and ophthalmological examinations). If no indications are found, it is recommended that the child be re-examined at around the ages of 5, 12, and 17 years (Canadas et al, 2010). If there is still no evidence of Marfan syndrome, then after multidisciplinary consultation, the child can be discharged from follow-up and depending on the presentation in the family, a cardiologist at a peripheral centre can monitor the individual every five years. If evidence of Marfan syndrome is noted by the first birthday, more frequent monitoring can be agreed, depending on the findings.

c. Child with a negative family history.

In non-familial cases of children suspect for Marfan syndrome, without a DNA abnormality and with adequate clinical suspicion, it is recommended that the clinical examination be repeated at the ages of 5, 12 and 17 years. Given the high sensitivity of DNA analysis in the diagnosis of Marfan syndrome, only a very small number of children will fall into this group. If an *FBN1* mutation is found, the child will be treated as a confirmed Marfan syndrome case even if the clinical criteria are not yet met. This situation is referred to as 'potential Marfan syndrome' in Ghent II, a rather confusing term.

Recommendations

I. *Refer all patients with the following features for assesment by a specialist in Marfan syndrome, preferably in association with a Marfan clinic:*
- Aortic root dilatation or dissection of the thoracic aorta without obvious cause, or
- (Sub)luxation of the lens, or
- First-degree relative with Marfan syndrome.

II. *Refer* patients with less specific features of Marfan syndrome to an experienced specialist in Marfan syndrome, assessing the appropriate diagnostic route based on the features.

III. *Criteria used to diagnose Marfan syndrome:*
The Ghent II criteria are applied but with the following reservations:
- Striae in the middle of the back, the hips and lumbar region are not counted as systematic criteria. Striae on the shoulders, upper arms and around the axilla are counted if not associated with weight change.
- In the adult population, absolute values are used for diagnosis of aortic root dilatation. See section 4.

IV. *Standard diagnostic examination in cases of suspected Marfan syndrome:*
The following tests are advised in cases with an adequate suspicion of syndrome:
- Complete physical examination and family history;
- Cardiological examination including ultrasound of the heart and thoracic aorta;
- Eye examinations, including slit lamp examination in full mydriasis and measurement of the refractive error;
- DNA-analysis.

V. *Diagnosis in children*
- Perform DNA-analysis in the first year of life, in a child with a 50% chance of Marfan syndrome and a known FBN1 mutation in the father or mother, to exclude or confirm the diagnosis Marfan syndrome.
- If the diagnosis cannot be established or excluded with certainty in a child with a 50% chance of Marfan syndrome, re-examine the child around the ages of 5, 12, and 17 years.
- A neonate with pronounced physical characteristics of Marfan syndrome should be examined by a paediatric cardiologist, for suspected neonatal Marfan syndrome, at the first suitable moment.

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Section 4 Diagnostic Imaging of Aortic Root Dilatation

Introduction

The presence and extent of aortic root dilatation plays a central role in the diagnosis, monitoring and treatment of patients with Marfan syndrome. Various non-invasive techniques can be used for imaging of the aorta, with echocardiography as the standard approach, and MRI and CT providing additional information where necessary. Since absolute cut-off values are used in adults, both for the diagnosis of aortic root dilatation and as an indicator for preventive aortic root replacement, standardised methods of measurement and uniform normal values of great importance.

Summary of the literature

Echocardiography

International guidelines for the performance of echocardiography are available for both adults and children (Lang et al., 2005; Lopez et al., 2010).

The proximal portion of the ascending aorta is imaged in a parasternal long-axis view. Diameters are measured at the level of the aortic annulus, aortic root (largest diameter at the level of the sinus of Valsalva), sinotubular (ST)-junction and the ascending aorta half a centimetre above the ST-junction. Confusion arises because, according to the guidelines of the American Society of Echocardiography (Lang et al., 2005), these measurements should be taken at end diastole in adults, while the paediatric guideline (Lopez et al., 2010) of the same American Society advises the measurement of the dimensions during mid-systole.

Discussion is still ongoing as to whether the front wall of the aorta should or should not be included. This will be concurrently measured during the *leading edge to leading edge* measurement, but not by the *inner edge to inner edge* measurement technique. Commonly used normal curves, such as those of Roman (Roman et al., 1989), are based on leading edge to leading edge measurements and this seems to be the main reason why this measurement is recommended in the existing echocardiography guidelines for adults (Lang et al., 2005).

In contrast, the paediatric guidelines recommend the inner edge to inner edge measurement technique (Lopez et al., 2010).

The international guidelines (Lang et al., 2005, Lopez et al., 2010) favour two-dimensional aortic measurements rather than M-mode measurements because the aortic root moves

relative to the cursor line during the cardiac cycle, resulting in a systematic underestimation (of 2 mm) of the maximum diameter of the aortic root at the level of the sinus of Valsalva (Roman et al., 1989). It is worth noting that this observation was not confirmed in a study by Rozendaal et al. (1998).

The normal aortic root dimensions in adults differ between males and females, and are also influenced by posture and age. Nevertheless, absolute cut-off values are generally used for the diagnosis of aortic root dilatation or as indications for aortic root replacement. Biaggi et al. (2009) analysed ultrasound data (leading edge to leading edge in end systole) for 1,799 patients without cardiac disease and found that a size of ≥46 mm in men and ≥43 mm in women was abnormal. According to the curves developed by Biaggi, the maximum P95 diameter is 42 mm for men (age 70 years, BSA 2.1) and 38 mm for women (age 70 years, BSA 1.9).

Depending on age and BSA, smaller diameters can already be considered abnormal. Pelliccia et al. (2010) examined 2,317 athletes and found that a diameter of >40 mm in men and >34 mm in women fell outside the normal range (leading edge to leading edge in end diastole). Radonic et al. (2011) studied 38 healthy subjects with a large BSA and found a maximal aortic root diameter of 38 mm (echo, leading edge to leading edge in end diastole). Based on these findings and the literature, these authors suggested that an aortic root of >40 mm is always dilated. According to Radonic et al. the use of Z-scores, as recommended in the Ghent II criteria, may therefore lead to the diagnosis of Marfan syndrome being missed in patients with a large BSA.

A 1993 study of 182 tall men and women showed that the aortic root diameter does not increase linearly with a length of >P95, and that the aortic root diameter no longer increases with a larger BSA. The P95 does not exceed 39 mm (Reed et al., 1993). Kinoshita et al. (2000) investigated the prevalence of aortic dilatation amongst 1,929 athletes (particularly basketball players) and also found that the relationship between aortic root diameter in relation to the BSA does not increase linearly. The measured P95 was smaller than 39 mm. They considered an aortic root of >40 mm to be pathological and this was confirmed in 7 of the 1,929 athletes, including two for whom a diagnosis of Marfan syndrome was established. All other measurements were <40 mm.

In children, the measured dimensions are normally expressed as Z-scores. Standardisation by body surface is common, although the aortic root dimensions also seem to show a good correlation with height (Sheil et al., 1995). Separate nomograms have also been developed for tall children and adolescents, adapted to the specific build of this group (Rozendaal et al., 1998).

The nomograms for children recently published by Gautier and by Pettersen are based on

measurements in larger groups of children than the original Roman nomograms (353 or 782 versus 53) (Gautier et al., 2010; Pettersen et al., 2008). The Roman and Gautier nomograms were developed from leading edge to leading edge measurements in diastole, in accordance with the guidelines for adults from the American Society of Echocardiography. The Pettersen nomograms are based on inner edge to inner edge measurements in systole, in accordance with paediatric guidelines. Z-scores can easily be calculated using www.parameterz.blogspot.com or via www.marfan.org.

Conclusions

Level 4	Guidelines for adults and children recommend that ultrasound examination of the aortic root in patients suspect for Marfan syndrome measure in the parasternal long-axis view. Diameters are measured at the level of the aortic annulus, aortic root, ST junction and at the ascending aorta, one centimeter above the ST junction. D Lang et al., 2005; Lopez et al., 2010
Level 3	Literature cites various cut-off values for determining aortic root dilatation in adults with normal stature, with an aortic root diameter greater than 40 mm usually considered as abnormal. Depending on age and BSA, smaller diameters can already be considered abnormal. C Biaggi et al., 2009; Pellicia etl, 2010; Radonic et al., 2011; Kinoshita et al., 2000
Level 4	Most of the normal values for echocardiography in adults quoted in the literature are based on leading edge to leading edge measurements in diastole. D Lang et al., 2005; Roman et al., 1989
Level 4	Ultrasonography in children generally utilises Z scores for the expression of measured dimensions. Published nomograms specific for children are based on different methods of measurement. D Lopez et al., 2010; Gautier et al., 2010; Pettersen, 2008

Level 4	Specific nomograms have been developed for ultrasound measurements of aortic root dimensions in tall children and adolescents. D Rozendaal et al., 1998

Considerations

Aortic root Z-scores have a central role in the Ghent II criteria. In addition to the determination of the absolute diameter, it can sometimes also be useful to express the measured aortic root diameter as a standardised Z-score using one of the published nomograms, under the condition that the method of measurement corresponds to that of the nomogram. However, the Z-score is unreliable in patients with a large body surface area (>2 m^2), such as is often the case in Marfan syndrome (Radonic et al., 2011).
In the opinion of the working group, an aortic root diameter of >40 mm is always dilated, although this figure may still fall within the norm on the extrapolated data in the Roman nomograms. In individuals with a normal posture, the aortic root is usually smaller than 38 mm. When in doubt as to a dilatation, the form of the aortic root (pear shape) and the relation of the aortic root to the immediate area, left atrium and ascending aorta may help to confirm the diagnosis of aortic root dilatation.

The three sinuses of Valsalva can be visualised in the parasternal long -axis view. In cases of aortic root dilatation, a typical cloverleaf shape is seen, which is sometimes accompanied by an asymmetric dilatation of the sinus of Valsalva. This can mean that the diameter in the long-axisview may not be the maximal diameter.

Imaging the aorta with cardiovascular MRI (CMR) and/or CT
The entire aorta can be visualised using magnetic resonance techniques. This is especially important for adult Marfan patients because aortic dilatation can also occur beyond the aortic root in the distal aorta, especially following aortic root replacement and with increasing age. In adult Marfan patients, the entire aorta should therefore be visualised with MRI as soon as the diagnosis is made. The frequency of follow-up MRI depends on the conditions at baseline. In young children, the entire aorta can usually be clearly visualised using ultrasound, in addition to the fact that widening of the distal aorta occurs only rarely in young children. MRI is performed in this age group as indicated.
General MRI guidelines indicate that the aortic root should be measured on triggered

MR images in the short axis, from leading edge to leading edge. Magnetic resonance angiography (MRA) is not reliable when measuring the aortic root diameter because only the aortic lumen is depicted.
MRI is preferred for routine assessment of (young) adults. The advantage of MRI is that any cross-sectional plane can be chosen, allowing the aortic diameter to be measured in the longitudinal plane. This can be of importance in aortas with considerable tortuosity. In addition, radiation exposure and the administration of contrast agents is avoided.
For the frequency of surveillance, please refer to section 10.

Postoperative assessment
Because the chosen projection surfaces of consecutive MRI tests are not always exactly the same, an increase in the diameter of the aorta can sometimes be better assessed with CT scans. The CT scan is preferred for postoperative assessment in some clinics for this reason (for example, after a type A dissection or for following the growth of the descending aorta in the case of a persistent type B dissection). The possible development of pseudo-aneurysms after surgery can be determined at an early stage using CT. MRI is preferred where a patient shows a contrast allergy.

Recommendations

Perform ultrasound aortic measurements at 4 levels in the 2D parasternal long-axis perpendicular to the direction of blood flow, with the aim of visualising the maximum diameter of the aortic root. Also evaluate the asymmetry of the root in the short axis. Carefully asses the largest diameter at follow-up.

For adults, in the context of standardisation, follow the guidelines of the American Society of Echocardiography, with the ultrasonographic measurements in diastolic stop-frame, from leading edge to leading edge.

In an adult, an aortic root diameter of more than 40 mm should generally be regarded as dilated. In the assessment of aortic root diameter, always take into account the age, sex and BSA of the patient, and depending on these factors, smaller diameters may already be considered abnormal. The use of a Z-score may be helpful in these cases.

In children, express the values obtained by ultrasound in Z-scores, using published nomograms or digital formulas. Document the measurement technique and the matching nomograms used.

Image the entire aorta in adult Marfan patients with MRI once the diagnosis is established.
The frequency of follow-up MRIs depends on the conditions at baseline.

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Section 5 Differential Diagnosis

Introduction

The diagnosis of Marfan syndrome is based on internationally agreed criteria. The latest criteria were published in July 2010 (Loeys et al., 2010; Dietz et al., 1993).
A diagnosis of Marfan syndrome is based on a combination of cardiovascular, ophthalmic, and systemic (including skeletal) characteristics, together with molecular analysis.
These 'revised Ghent criteria' (Ghent II criteria) are still under discussion and no consensus has yet been reached.

Since no individual symptom is specific for Marfan syndrome, other diagnoses should always be considered. This also applies for individuals with Marfan syndrome, in whom no pathogenic *FBN1* mutation has been found. The Ghent II criteria incorporate the criteria for Ectopia Lentis syndrome (ELS), Myopia-Mitral valve prolapse-borderline and non-progressive Aortic root dilatation-Skeletal Findings-Striae (MASS) and Mitral Valve Prolapse Syndrome (MVPs) (see Table 5.1). In addition, in cases of ectopia lentis with aortic root dilatation or aortic root dilatation with a high systemic score, indications for other syndromes must be absent in order to establish a diagnosis of Marfan syndrome.
This distinction is also clinically important because the different diagnoses all have differing inheritance, prognosis and needs for guidance. Knowledge of differential diagnostic considerations is therefore essential.

Summary of the literature

Describing the medical conditions that must be considered in a differential diagnosis required an alternative literature search, because the scientific justification of this aspect is less relevant.
Table 5.1 has been prepared on the basis of two core articles, Loeys et al. (2010) and Dietz et al. (1993), and the first column includes the characteristics of Marfan syndrome. In addition, the specific characteristics of the other syndromes are described (and core articles were sought on these syndromes). Genereviews.org was frequently consulted (also available via Pubmed).
The table attempts to provide a summary of the alternative diagnoses for the main features of Marfan syndrome, ectopia lentis and aortic root dilatation. Since most referrals

to a Marfan clinic – including the question of presence or absence of Marfan syndrome - are based on the existence of an aortic root dilatation or dissection, ectopia lentis and distinctive habitus (usually tall stature), marfanoïde habitus is also included as an entry in the table, with its specific differential diagnosis. The characteristics appropriate to Marfan syndrome are in regular letters. Characteristics in in italics are appropriate to (one of the) differential diagnoses. The presence of a characteristic in italics indicates that the diagnosis of Marfan syndrome needs to be reconsidered.

The characteristics of each syndrome are classified as possibly overlapping with Marfan syndrome, or specific for the syndrome.

Ectopia Lentis syndrome (ELS) (Ades et al., 2004; Aragon-Martin et al., 2010)
Autosomal dominant and recessive inheritance (dominant: sometimes *FBN1*, which is not associated with aortic root dilatation / recessive: *LTBP2* and *ADAMTSL4* mutations).
Overlapping features: lens luxation, skeletal features.
Specific characteristics: none, the lens luxation is usually bilateral and congenital, without ectopia pupillae. N.B. Whether there is a long term risk of aortic root dilatation in the event of a *FBN1* mutation is unclear.

Myopia-Mitral valve prolapse-borderline and non-progressive Aortic root dilatation-Skeletal findings-Striae (MASS) (Loeys et al., 2010)
Autosomal dominant inheritance.
Overlapping features: Myopia, **M**itral valve prolapse, borderline and non-progressive **A**ortic root dilatation, **S**kin and **S**keletal features.
Specific characteristics: none.

Mitralis valve prolapse syndrome (MVPS) (Loeys et al., 2010)
Autosomal dominant inheritance.
Overlapping features: mitral valve prolapse, (subtle) skeletal features.
Specific characteristics: none.
Shprintzen Goldberg syndrome (SGS) (Greally, 2010)
Inheritance uncertain (a few *FBN1* or *TGFBR2* mutations described).
Overlapping features: phenotype: dolichostenomelia, arachnodactyly, pectus, scoliosis, aortic root dilatation (rarely), high palate.
Specific characteristics: Craniosynostosis, mental retardation, Chiari malformation, hypertelorism, proptosis, rib anomalies, clubfoot.

Table 5.1. Differential diagnostic considerations - Marfan syndrome

	Marfan	ELS	MASS	MVPS	SGS	LDS	AOS	CCA	FTAAD	WMS	EDS-CT	EDS HT	EDS-VT	EDS -KST	HHC	Stickler	FraX	Lujan-Fryns	AT	Cutis Laxa
Aortic dilatation and/or dissection											&	&								
Ectopia lentis																				
Marfanoid habitus^ (tall stature, long legs)																				
Mitral valve prolapse																				
Skeletal features#	all			pe, sc, ar	pe, sc, ar	pe, sc, ar, pl	pe, Sc, ar, pl	sc, ar						sc	pe, sc					pe, ar
Myopia																				
Facial features*	all				d											m		m		m, r
Skin abnormalities®																				
Contractures																				
Hypermobility																				
Craniosynostosis																				
Developmental delay																				
Chiari malformation																				
Rib abnormalities																				
Clubfoot																				
Hypertelorism																				
Bifid uvula/ cleft palate																				
Blue sclerae																				
Arterial tortuosity																				
Organ rupture																				
Thrombosis																				
Hearing problems																				
Skelet dysplasia/arthritis																				

ELS : Ectopia Lentis Syndrome
MASS : Myopia, Mitral valve prolapse, borderline Aortic root dilatation, Striae, Skeletal features
MVPS : Mitral Valve Prolapse syndrome
SGS : Sprintzen-Goldberg syndrome
AOS : Aneurysms-Osteoarthritis syndrome
LDS : Loeys-Dietz syndrome
CCA : Congenital Contractural Arachnodactyly
FTAAD : Familial Thoracic Aorta Aneurysms and Dissections
WMS : Weill-Marchesani syndrome
EDS-CT : Ehlers-Danlos Syndrome, classic type
EDS-HT : Ehlers-Danlos Syndrome, hypermobile type
EDS-VT : Ehlers-Danlos Syndrome, vascular type
EDS-KST : Ehlers-Danlos Syndrome, kyphoscoliosis type
FraX : Fragile-X syndrome
HHC : Hyperhomocysteinuria
AT : Arterial Tortuositas

^ Also consider MEN2B, Cohen, and certain genes involved in X-linked mental retardation (SMS, ZDHHC9)
* facial features: d=dolichocephaly, e=enophthalmos, pf=downslant palpebral fissures, m=malar hypoplasia, r=retrognathia
# Skeletal features: wt=wrist/thumb sign, pe=pectus, pl=pes planus, sc=scoliosis/kyphosis, ar=arachnodactyly
& McDonnell et al. (2006) described a mildly dilated aortic root in the classic and hypermobility type EDS patients; however, the cohort was small, aortic diameters were not normalised to BSA, and Marfan syndrome cannot be excluded in patients with aortic root dilatation. Furthermore, this publication seems to be the only one in which an aortic root dilatation is described.

Dark grey: frequent/pronounced.
Light grey: less frequent/less pronounced.

Loeys-Dietz syndrome (LDS) (Loeys & Dietz, 2008)

Type 1

Autosomal dominant inheritance (mutations in *TGFBR1*, *TGFBR2*).

Overlapping features: long face, downward slanting palpebral fissures, high palate, malar hypoplasia, micrognatie, retrognathism, pectus, scoliosis, arachnodactyly, hypermobility, dural ectasia, aortic root dilatation or dissection.

Specific characteristics: hypertelorism, broad or bifid uvula, cleft palate, learning disabilities, hydrocephalus, chiari malformation, blue sclerae, exotropia, craniosynostosis, cervical spine instability, club foot, soft pasty skin, translucent skin, easy bruising, generalised arterial tortuosity and aneurysms and/or dissection.

Type 2 (formerly: Marfan syndrome type 2)

Autosomal dominant inheritance (mutations *TGFBR1*, *TGFBR2*).

Absent LDS type 1 characteristics: arterial tortuosity and almost all other characteristics except aneurysms and/or dissection of aorta and other arteries.

Features overlapping with Marfan syndrome: sometimes marfanoid habitus and arachnodactyly.

Absent Marfa features: ectopia lentis.

Aneurysms-Osteoarhritis syndrome (van de Laar et al. 2011)

Autosomal dominant inheritance (mutations *SMAD3*)

Features overlapping with Marfan syndrome: aortic dilatation or dissection, mitral valve prolapse, hypermobility, marfanoid habitus, pectus deformities, scoliosis

Specific characteristics: hypertelorism, cleft palate/uvula, explicit osteoarthritis

Congenital Contractures and Arachnodactyly (Godfrey, 2012)

Autosomal dominant inheritance (heterozygous mutations in *FBN2*).

Overlapping features: long, thin fingers and toes, kyphosis, scoliosis, aortic root dilatation (occasionally).

Specific characteristics: ear abnormalities ("crumpled ears", "folded upper helix"), contractures of knees and ankles, flexion contracture at the proximal interphalangeal joints of fingers and toes, hip contractures, adducted thumbs, clubfeet, muscle hypoplasia.

(Familial) thoracic aortic aneurysms and dissections ([F] TAAD) (Milewicz & Regalado 2012)

Autosomal dominant inheritance (mutations in *MYH11*, *ACTA2*, *TGFBR1*/2, *Smad3*, *MyLK* and as yet undiscovered genes). (F)TAAD associated with mutations in *TBFBR1/2* without features of LDS is sometimes called LDS type 2 or Marfan syndrome type 2.

Overlapping features: aortic dilatation (but usually of the ascending aorta and not of the aortic root).

Specific characteristics: none.

Weill-Marchesani syndrooem (WMS) (Tsilou & MacDonald, 2007)

Autosomal dominant and recessive inheritance (*ADAMTS10* mutations).

Overlapping features: lens luxation, myopia, contractures.

Specific characteristics: small stature, microspherophakia, brachydactyly.

Ehlers-Danlos syndrome (EDS) classical type, type 1 (Malfait et al., 2011)

Autosomal dominant inheritance (mutations in *COL5A1, COL5A2*).

Overlapping features: hypermobility (more pronounced in EDS).

Specific characteristics: hyperelastic skin, abnormal wound healing, soft pasty skin.

EDS hypermobile type, type III (Levy, 2010)

Autosomal dominant inheritance.

Overlapping features: hypermobility.

Specific characteristics: None; there is overlap with familial and physiological hypermobility.

EDS vascular type, type IV (Pepin & Byers 2011)

Autosomal dominant inheritance (*COL3A1* mutations).

Overlapping features: hypermobility (limited to distal finger joints), Ao aneurysm/dissection.

Specific characteristics: thin skin (dark under the eyes), easy bruising, wide dystrophic scars; Facial: prominent eyes, sharp facial features, organ ruptures, artery dilatation/dissection, especially abdominal aorta (generalised).

Absent Marfan characteristics: Marfanoid habitus.

EDS kyphoscoliotic type, Type VI (Yeowell et al., 2008)

Autosomal recessive inheritance (mutations in *PLOD1*, *CHST14*).

Overlapping features: kyphoscoliosis, hypermobility, hypotonia, sometimes eye problems (sclerae weakness with globe rupture, myopia), MVP.

Specific characteristics: rupture of medium-sized arteries.

Homocysteinuria (HHC) (Picker & Levy, 2011)

Autosomal recessive inheritance (mutations in *CBS*).

Overlapping features: myopia, ectopia lentis, skeletal abnormalities, physique, pectus excavatum/carinatum, scoliosis, mitral valve prolapse, high palate, inguinal hernia.

Specific characteristics: mental retardation, intravascular thrombosis, thromboembolism.

Stickler syndrome (Robin et al., 2011)
Autosomal recessive inheritance (mutations in *COL2A1*, *COL11A1*, *COL11A2*).
Overlapping features: myopia, retinal detachment, mid-face hypoplasia.
Specific characteristics: hearing loss, cleft palate, spondyloepiphyseal dysplasia, premature arthritis, congenital abnormality of vitreous humor.

Fragile X syndrome (Saul & Tarleton, 2012)
Sex-linked inheritance (*FMR1* mutations).
Overlapping features: long face, hypermobility.
Specific characteristics: mild/moderate mental retardation, large head, prominent forehead and chin, large ears, large testes, behavioural problems.

Lujan-Fryns syndrome (van Buggenhout & Fryns, 2006)
Sex-linked inheritance, especially reflected in men.
Overlapping features: long narrow face, maxillary hypoplasia, small mandible, tall stature, marfanoid habitus.
Specific characteristics: prominent forehead, mental retardation, behavioural problems.

Arterial Tortuosity (Canadas et al., 2010; Callewaert et al., 2008)
Autosomal dominant inheritance (mutations *GLUT10*).
Overlapping features: long face, dilated arteries and aorta, hypermobility.
Specific characteristics: tortuosity of aorta and large arteries.

Cutis Laxa (Loeys B, 2011 ; Callewaert et al., 2012)
Autosomal recessive inheritance (mutations *FBNL4/EFEMP2*), autosomal dominant inheritance (mutaties *ELN*)
Overlapping features: aorta aneurysms, hypermoblity, retrognatia, arachnodactyly, pectus deformity, malar hypoplasia, high arched palate.
Specific characteristics: skin phenotype, arterial tortuosity, lung emphysema.

Recommendation

Consider other diagnoses, if a feature not matching Marfan syndrome is present (as in Table 5.1)

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Section 6 Treatment of Skeletal Abnormalities

Introduction

Abnormalities of the musculoskeletal system are often present in patients with Marfan syndrome. The most common problems are:

- Scoliosis;
- Spondylolisthesis;
- Pectus excavatum/pectus carinatum;
- Instability (multidirectional) of the shoulder;
- Instability carpometacarpal (CMC) joint 1;
- Protrusio Acetabuli;
- Patellar instability;
- Pes planovalgus;
- Hammer-/claw toes.

The question is whether the treatment of skeletal abnormalities in Marfan patients is different from that in patients without Marfan syndrome.

Summary of the literature

Scoliosis

Scoliosis is a common orthopaedic problem in patients with Marfan syndrome. A scoliosis is present in approximately 62% of patients, where a curve greater than 40° suggests a high probability of progression following skeletal maturation (Sponseller et al., 1995). The curves are noticeably more rigid in comparison with idiopathic scoliosis, which may mean that the probability of success of brace treatment is lower.

A brace is the only non-invasive method available to counter the progression of scoliosis. Because the use of a brace has a significant impact on the life of a patient, it is important to be able to reach a judgement on the effect of the treatment.

In idiopathic scoliosis, brace treatment is known to be more favourable than the natural course in patients with a curve of between 20 and 45 degrees whose skeleton is still immature (Risserstadium less than or equal to 2). In 60-80% of cases the curve increases by less than 5 degrees during the treatment, which often allows an operation to be avoided (Emans et al., 1986; Nachemson & Peterson, 1995; Rowe et al., 1997).

Only 2 retrospective studies describe the treatment of scoliosis in Marfan patients. Sponseller et al. (2000) have show that the effect of a Boston brace is limited. Of the 24 patients (demonstrated Marfan syndrome, curve of 20-45°, Risser 0-2 at the beginning of brace treatment), 22 could persist with the brace program, the remaining two being unable to tolerate wearing the brace. The patients were followed for at least two years (until skeletal maturation or surgery), and during follow-up 4 of the 24 patients (17%) were found to have a curve increase of less than 5 degrees and a curve of less than 45 degrees. Fifteen patients underwent a surgical intervention and the remaining 5 were indicated for surgery but an operation had not yet been carried out.
Birch & Herring (1987) treated eight Marfan patients (diagnosis based on clinical signs, no DNA test) with a Boston or Milwaukee brace and saw a stabilisation of the curve in 1 patient. She was already three months postmenarcheal, however. Six were surgically treated following curve progression, and one patient refused surgery (Birch & Herring, 1987).

Spondylolisthesis

There is evidence that the incidence of spondylolisthesis occurrence and the severity of the slip are slightly higher than in patients without Marfan syndrome (Sponseller et al., 1995). There is no evidence that treatment differs from that of patients without Marfan syndrome.

Pectus excavatum

There is no evidence in the literature that there are grounds to treat Marfan patients with pectus excavatum differently from non-Marfan patients, nor regarding the indication for surgery, nor regarding the timing of any surgical correction.

Other abnormalities

No literature was found to indicate that treatment should depart from the standard treatment applicable to the abnormalities mentioned in the introduction in patients without Marfan syndrome.

Conclusions

Level 3	There are indications that, in Marfan patients with a still immature skeleton (Risser 2 or less) and scoliosis curves of between 20 and 45 degrees, wearing a brace can avoid the need for surgery in a small proportion of patients. *C* Sponseller et al., 2000; Birch & Herring, 1987
Level 3	There are indications that the percentage of patients in whom a brace can prevent surgery is lower in Marfan than in non-Marfan patients. *C* Sponseller et al. 2000; Birch & Herring, 1987
Level 4	There is insufficient available literature to justify treating other skeletal abnormalities in Marfan syndrome differently to those in patients without Marfan syndrome. *D* Opinion of the working group

Considerations

Studies on the effects of wearing a brace do not clarify the issue of whether, without the brace, the scoliosis would have progressed to the extent to make surgery unavoidable. In other words, whether the brace affects the natural course of the disease. Adequate consideration of the advantages and disadvantages of both wearing a brace and of surgery should therefore always include the patient and possibly also the parents.

In cases of pectus excavatum, an indication for surgery is based on the psychological problems of a patient with this deformity. These problems usually start around adolescence, and do not differ between Marfan patients and non-Marfan patients.

There is no evidence in the literature for grounds to treat Marfan patients with pectus excavatum differently to non-Marfan patients, neither regarding the indication for surgery, nor regarding the timing of a possible surgical correction (Scherer et al., 1988; Jaroszewski et al., 2011).

Although improvements in cardiac and pulmonary function are mentioned in the literature, this phenomenon long remained difficult to objectify (Morshuis et al., 1994). This effect

was attributed to an improved confidence and thus greater participation in gymnastics, sports, etc. post-correction. More recently, there are some indications for a measurable improvement in exercise capacity following surgical correction of severe pectus excavatum (Malek et al., 2006). There also seems to be no difference between patients with Marfan syndrome and patients with other diseases on this specific issue.
There are also no differences between Marfan syndrome and non-Marfan patients with regard to the use of surgical techniques.

Publications on combined correction of the chest wall deformity and cardiac surgery (e.g. aortic root replacement) are limited to case reports.

It is important to realise that cardiac surgery is no longer possible following insertion of a Nuss bar. Prior to insertion of a Nuss bar, evaluation by a (child) cardiologist is recommended to evaluate the risk of cardiac surgery during the period the Nuss bar is in situ.

The working group is of the opinion that the musculoskeletal abnormalities found in Marfan patients should be treated in the same manner as in patients without Marfan syndrome. The treatment must be performed by a surgeon with specific experience in the area in question.

Recommendations

In general, treat abnormalities of the musculoskeletal system in patients with Marfan syndrome in the same manner as for patients without Marfan syndrome.

In Marfan patients with scoliosis of between 20 and 45 degrees and a still immature skeleton, the (low) expected success rate of a brace should be weighed against the discomfort and the advantages and disadvantages of an operation.

Consult a (child) cardiologist before insertion of a Nuss bar.

Problems with the musculoskeletal system in patients with Marfan syndrome should be treated by a specialist with experience in the field of the specific musculoskeletal problem. It is not necessary that this occur within a Marfan team.

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Section 7 Drug Treatment

Introduction

Current drug treatment in Marfan syndrome is focused on slowing aortic root dilatation through a reduction in the haemodynamic stress in the aorta, mainly by means of beta-blockers. However, there is increasing evidence that the renin-angiotensin system (RAAS) plays a role in the development of aneurysms. Blockade of the RAAS system not only lowers blood pressure, but also intervenes in the molecular mechanisms underlying the development of aneurysms, such as the TGFβ cascade and the so-called extracellular-signal-regulated kinases (ERKs).

Summary of the literature

The first open-label, randomised study of the effect of drug treatment in Marfan patients was published in 1994 (Shores et al., 1994), in which 70 Marfan patients aged between 12 and 50 years were treated with the beta blocker, propranolol. After 4 years of follow-up, the treatment group had a 73% lower rate of aortic root dilatation and a lower mortality. Mechanical effects of beta-blockade were considered to be the cause of the beneficial effect of beta blockers. Lowering of blood pressure and a decreased contractility of the left ventricle lead to a reduction in haemodynamic stress in the aorta. Although the utility of beta blockers was never conclusively proven in a prospective double-blind, randomised study, the use of beta-blockade is currently the standard treatment in patients with Marfan syndrome and is recommended in all guidelines (Baumgartner et al., 2010; Hiratzka, 2010; Silversides et al., 2010; Regitz-Zagrosek et al., 2011).

The cystic medial degeneration in the aortic wall seen in aneurysms is accompanied by apoptosis and loss of smooth muscle cells, degeneration of elastic fibres and build-up of proteoglycan, a major constituent of the extracellular matrix. Angiotensin II contributes to this process via angiotensin II type 1 or 2 (AT1- en/of AT2-) receptors. In recent years, a number of studies have been published on the effect of various antihypertensive drugs, and two small studies have compared the effect of ACE inhibitors and beta-blockers. ACE inhibitors were more effective in reducing arterial stiffness, improving aortic distensibility and delaying the growth of the aorta, compared to beta blockers (Ahimastos, 2007; Yetman, 2005).

In 2006, the AT1 receptor blocker Losartan was shown to have a beneficial effect on the structure of the aortic wall and on aortic growth in mice with a *FBN1* mutation (Habashi

& Loeys, 2006). In a small retrospective cohort study in 18 children with a severe form of Marfan syndrome and rapid aortic growth, an AT1 blocker was added to existing medication and the growth of the aorta was followed for 12-47 months. A significant slowing of aortic growth was seen following treatment with Losartan, after adjusting for age and BSA (Brooke et al., 2008).

The prophylactic use of beta blockers in children with Marfan syndrome is still a subject of discussion. Most published studies persist in enrolling only a small number of children, thus preventing a conclusive appraisal of the effect of beta-blockers in children with Marfan syndrome. In a retrospective study by Selamet Tierney (2007) of 63 children with Marfan syndrome, up to 18 years old, the beneficial effect of beta-blockers on the speed of aortic root dilatation could not be demonstrated after 6 years of follow-up (Selamet Tierney, 2007). The retrospective design, with a possible selection bias, and the small numbers make it impossible to draw definitive conclusions. In contrast, a similar retrospective study by Ladouceur et al. (2007) of 115 children younger than 12 years, including 77 treated with beta blockade, could show a decrease in the rate of aortic root dilatation of 0.16 mm/year in the treated group compared to the non-treated group (Ladouceur et al., 2007). The authors concluded that it is advisable to start beta blockade at the moment of diagnosis, but because the treated group had greater aortic root dilatation than the non-treated group, a selection bias seems plausible.

Conclusions

Level 3	There are indications for an inhibitory effect of beta blockers on the growth of the aortic diameter in Marfan patients over the age of 12. B Shores et al., 1994 D Baumgartner et al., 2010; Hiratzka, 2010; Silversides et al., 2010; Regitz-Zagrosek et al., 2011
Level 2	Results regarding the effect of beta-blockers on aortic growth in children are contradictory. B Selamet Tierney, 2007; Ladouceur et al., 2007
Level 3	There are indications that Losartan slows aortic root growth in children with Marfan syndrome. C Brooke et al., 2008

Considerations

Although the study by Brooke suggests that Losartan may have a beneficial effect on aorta growth, this beneficial effect should be confirmed through prospective randomised trials before definitive conclusions are drawn. Several trials, with a number of variations in the design, are currently underway worldwide. Until evidence regarding the effects of Losartan on the course of Marfan syndrome becomes available, a conservative approach to the administation of Losartan is appropriate.
Other potentially beneficial drugs, such as doxycycline (Chung, 2008) and pravastatin, are currently being investigated in mouse models.

Beta-blockers may have side-effects such as bronchospasm, fatigue, depression, insomnia and behavioural problems. These side-effects are especially unacceptable in children considering that the efficacy of these drugs on the inhibition of aortic growth is not conclusively proven.
Because a recommendation regarding the prophylactic use of beta-blockers in children with Marfan syndrome is lacking in current international guidelines, it seems sensible to carefully weigh the advantages and disadvantages of the use of beta-blockers for individual patients, and not to routinely prescribe these drugs. It is common practice in the Netherlands to prescribe beta blockers for children with evident aortic root dilatation.
The use of Losartan in children with Marfan syndrome is uncommon in the Netherlands. Furthermore, Losartan is not registered in the Netherlands as a hypotensive agent in children <6 years. Because the effect of Losartan on the course of Marfan syndrome is unproven, caution should be exercised when considering prescribing Losartan to children.

Recommendations

Prophylactic use of beta blockers should be continued in adult patients with Marfan syndrome, for the time being.

Do not routinely prescribe beta blockers to children with Marfan syndrome, but carefully weigh the advantages and disadvantages on an individual basis. It is customary to prescribe beta-blockers in children with evident aortic root dilatation.

Be conservative in the prescription of Losartan to children at present.

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Section 8 Timing of Aortic Surgery in Adults

Introduction

An aortic dissection or symptomatic aortic aneurysm is a potentially life-threatening condition. The risk of rupture is strongly dependent on the diameter, and an aneurysm with a diameter of >60 mm has a 25 times higher chance of rupture than when the diameter of the aneurysm is between 40 and 49 mm (Davies et al., 2002). A decision to operate is always a trade-off between the risk of rupture and the surgical risk. Elective surgery of the descending aorta carries a high risk of morbidity and a somewhat lesser risk of mortality (10.9%) (Davies et al., 2002), but it is still much lower than under emergency conditions. In contrast, elective surgery of the ascending aorta (and arch) has low mortality (2.5%) and morbidity, especially in centers with extensive experience in this area (Davies et al., 2002). This surgical risk is accepted in the light of a greatly improved life expectancy after surgery.

The diameter of the aorta is measured in various ways (see also text on diagnostic imaging). The surgical literature generally describes a CT examination that uses the internal diameter (inside wall to inside wall), while the cardiological literature often describes ultrasound, with the so-called 'leading edge to leading edge' method (front wall to front wall; thus including measurement of a single wall thickness). Unless otherwise expressly stated, the diameters of the aorta given below refer to the internal diameters.

Summary of the literature

Most articles focus on small groups of patients and do not focus specifically on the Marfan population. Randomised studies are generally lacking, and prospective studies are rare and usually contain small numbers of patients. The recommendations of the working group are largely based on U.S. (Hiratzka 2010) and European (Baumgartner et al., 2010) guidelines, which include the most relevant literature.

Asymptomatic aortic root and/or ascending aorta dilatation:
The most recently published American guidelines for the diagnosis and treatment of patients with thoracic aortic pathology propose referral of Marfan patients for elective surgical replacement of the aortic root and/or ascending aorta when the internal diameter is >45 mm (corresponding with an external diameter of ≥50 mm) (Hiratzka et al., 2010; Pearson et al., 2008).

A surgical correction is also considered indicated when the aortic root and/or ascending aorta has an internal diameter of <45 mm, in combination with:

- A rapid increase in the diameter (defined as >5 mm per year), and/or;
- A family history of aortic dissections and/or;
- A serious aortic regurgitation;
- Desire for pregnancy.

The European guideline (Baumgartner et al., 2010) also cites an upper limit of 50 mm (leading edge to leading edge) as an absolute indication and 45 mm when combined with the above mentioned risk factors.
The cited values are approximately 5 mm lower than in the non-Marfan patients.

Asymptomatic dilatation of the aortic arch, descending aorta or thoraco-abdominal aorta:
In the case of these patients, a diameter of 55 mm or larger is an indication for surgical intervention. In the case of rapid growth (>5 mm per year), a family history of dissections or a wish to become pregnant, an indication for surgical correction is usually given for a diameter of 50 mm or even for 45 mm (Hiratzka et al., 2010). This was supported by a recent study, specifically of Marfan patients, which showed a significant increase in the risk of rupture or dissection with a diameter 50 mm or more (Jondeau et al., 2012).

Symptomatic aortic dilatation:
As is the case with non-Marfan syndrome patients, a surgical correction with optimal medical treatment is always indicated for symptomatic aortic dilatation (Hiratzka et al., 2010), especially if a slow response to intravenous antihypertensive treatment is seen. If dilatation of the aorta is not clearly established, a plausible connection between the symptoms and aortic pathology should be confirmed.

Acute dissection type A:
All patients with an acute type A dissection (Stanford classification type A, DeBakey classification type I and II) have an absolute indication for emergency surgery (Hiratzka, 2010), whether or not he or she has Marfan syndrome.
N.B. An intramural haematoma should be treated as an acute dissection (Hiratzka et al., 2010).

Acute dissection type B:
The literature on the management of patients with acute type B dissection (Stanford classification type B; DeBakey classification types I and III) was inconclusive for some years. Experience with the placement of an endoprosthesis during the acute phase has remained

limited, due to an apparent association with high morbidity and mortality. Partly based on these experiences, and in accordance with current opinion, the generally recommended policies are:
A conservative policy, together with optimal drug treatment (including intravenous antihypertensive treatment), is recommended for uncomplicated dissections (i.e. without ischemia of the end organs, without leakage from the false lumen and without uncontrolled hypertension or persistent pain). The morbidity and mortality of surgical correction (open or endovascular) is high in the acute phase of the dissection, and is therefore only justified in the case of complications (Hiratzka et al., 2010).

Chronic dissection:
Indications for a patient with a chronic dissection are as applied for dilatation of the aorta (q.v.) (Hiratzka et al., 2010).

Considerations

The increased risk of rupture or dissection at larger aortic diameters and associated risks (including death), versus the risks of elective surgery, mean that the above recommendations from international guidelines are taken as standard despite the low level of evidence.

Individuals with a smaller body surface area (BSA) usually have a smaller aortic diameter. In general, the aortic root diameter in women is 5 mm smaller than in men (in part due to a smaller BSA). Recommendations should be applied using common sense. This means that the indication for surgery may be somewhat more aggressive in patients with a smaller BSA and more conservative in patients with an extremely high BSA.

Some remarks concerning surgical procedures:
In experienced centers, a valve-sparing procedure for the surgical correction of aortic root aneurysm or a type A dissection is usually possible, and this procedure should always be given preference. Referral to a center with experience in valve-sparing surgery is therefore recommended in cases of elective surgery. Due to an ongoing risk of annular dilatation in patients with Marfan syndrome, this procedure should not employ the remodelling technique (as Yacoub) but a modified reimplantation technique (as David) (Hiratzka et al., 2010; Kallenbach et al., 2007) .
The use of an endoprosthesis for aneurysms of the descending aorta is not yet recommended in patients with Marfan syndrome (Nordon et al., 2009). This is related to

an expectation of further aortic dilatation and the possibility of a type I or type V endoleak, an indication for a secondary procedure.

If a patient is scheduled to undergo cardiac surgery for another reason, for example, mitral regurgitation, a replacement may be considered even in cases with a less strongly dilated ascending aorta. An additional argument is the fact that damage to the aorta (aortic cannula, aortic root cannula and aortic clamp) could be a trigger for a dissection in cases with poor tissue quality.

Recommendations

Indications for elective surgical replacement of the aortic root and/or ascending aorta in asymptomatic Marfan patients occur at diameters of >45 mm on CT (internal diameter) or > 50 mm on ultrasound or MRI (leading edge to leading edge). Indications for surgery (some mm's) are earlier in case of:
- a rapid increase in the diameter (>5 mm per year), and/or;
- a family history of aortic dissection and/or;
- a major aortic regurgitation;
- desire for pregnancy.

Proceed with replacement if a patient has to undergo heart surgery for any other reason, even in cases with a lesser dilatation of the aortic root and/or ascending aorta.

Consider a diameter of 55 mm or larger as an indication for surgical intervention in asymptomatic Marfan patients with dilatation of the aortic arch, descending or thoracoabdominal aorta. Indicate surgical correction at a diameter of 50 mm or even 45 mm in case of rapid growth (> 5 mm per year), family history of dissections or a wish to become pregnant.

A surgical correction with optimal medication is indicated for all patients with symptomatic aortic dilatation, especially if a slow response to intravenous antihypertensive treatment is seen.

An acute type A dissection is an absolute indication for emergency surgery. Uncomplicated acute type B dissections are handled conservatively using optimal drug treatment. Surgical correction during the acute phase of a dissection carries a high risk and it is therefore only justified in the case of complications.

Apply indications for a patient with a chronic dissection as for dilatation of the aorta.

For surveillance following cardiac surgery, see section 10.

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Section 9 Timing of Aortic Surgery in Children

Introduction

While in adults the discussion on the optimal moment for aortic surgery centers around the question of at what absolute aortic root diameter is the probability of dissection or rupture so high that preventive aortic root replacement is justified, in the case of a growing child criteria are sought that can be related to physical proportions (weight, height, body surface area).
Aortic dissection is extremely rare under the age of 10 (Gillinov et al., 1997) or 12 (Zanotti et al., 2008). This means that preventive aortic root replacement is not indicated in young children. Because dissection is rare in children younger than 12 years, regardless of the degree of aortic root dilatation, Zanotti et al. (2008) are of the opinion that the use of z-scores as an indicator for surgery is unnecessary. This view is also expressed in a recent article (Everitt et al., 2009). (This approach contrasts with that for children with Loeys-Dietz syndrome, in which a z-score of greater than or equal to 3.5 is used as an indication for surgery.)

Summary of the literature

The natural course of aortic root dilatation in a growing child with Marfan syndrome has been described in several publications. Westaby (1999) describe how the aortic root already begins to dilate in early childhood, with a maximum increase in diameter between the ages of 6 and 14 years, followed by a decline in the pace of dilatation. In women, dilatation averaged 0.38 mm/year, and in males 0.42 mm/year (Meyboom et al., 2005). Karnebeek et al. (2001) followed 52 children and adolescents with Marfan syndrome for an average of 7.9 years. In the study group, 83% developed aortic root dilatation and 25% also showed aortic regurgitation. Eight patients underwent aortic root replacement, including two for acute dissection.

The majority of publications (Gillinov et al., 1997; Carrel et al., 2003) are from cardiac surgery groups and describe the results of aortic root surgery in relatively small numbers of children with Marfan syndrome (21 and 13 patients, respectively), without comparing outcomes with those of unoperated patients. The group of 45 Marfan patients described by Cattaneo et al. (2004) partly overlaps with the population described by Gillinov (Cattaneo

et al., 2004). The most recent study, by Everitt and colleagues, described a group of 204 children younger than 18 years, of whom 30 underwent aortic root surgery during follow-up (Everitt et al., 2009).
In the above publications, the indication for aortic root surgery was already determined in advance and primarily defined on the basis of experience (expert opinion). Indications actually differ little between the different surgical groups, effectively creating a 'common practice' (Zanotti et al., 2008).
The indication for preventive aortic root surgery in children can therefore be formulated as follows:

1) The presence of a 'giant aneurysm', which fulfills the criteria for intervention for adults, or;
2) A rapid increase in aortic root diameter (8-10 mm/year) and/or;
3) Rapidly progressive aortic regurgitation.

Based on database data from 410 patients (adults and children with and without Marfan syndrome) with aneurysms of the thoracic aorta, Davies (2006) introduced an "aortic-size index" (measured absolute aneurysm diameter in cm, divided by body surface area expressed in m^2) and defined three levels of risk for complications (rupture, dissection, death). An aortic diameter of less than 2.75 cm/mm^2 BSA equalled low risk, a diameter from 2.75 to 4.25 cm/mm^2 BSA represented a moderate risk, and an indexed aortic diameter greater than 4.25 cm/mm^2 BSA, a high risk of complications. Only 23 patients had proven Marfan syndrome and they were significantly younger, but the number of children included is not mentioned. This index does not seem to apply to young children with Marfan syndrome, in whom aortic root diameters greater than 2.75 cm/mm^2 BSA are normal.

Conclusions

Level 4	Preventive aortic root replacement is indicated in a child with Marfan syndrome if a 'giant aneurysm' is present that fulfills the criteria for intervention in adults or in the event of a rapid increase in the aortic root diameter (8-10 mm/jaar) or rapidly progressive aortic regurgitation. D Gillinov et , 1997; Carrel et al., 2003; Zanotti et al., 2008; Everitt et al., 2009

Considerations

There is only level 4 evidence for establishing the indication for aortic root replacement in a child with Marfan syndrome. This assessment is uniformly applied by several child heart surgery groups and therefore carries more weight.
Based on literature, it is not possible to indicate a z-score above which an indication for surgery in children becomes absolute.
In severely affected children with Marfan syndrome, mitral regurgitation is often prominent and this can be a reason for surgical intervention, even at a young age. Simultaneous aortic root surgery may then be considered. The risk of a second cardiac intervention is greatest when the first operation occurs at a young age and when significant mitral regurgitation is present (Gillinov et al., 1997; Everitt et al., 2009).

Valve-sparing aortic root replacement techniques require a functional and unaffected or little affected aortic valve. This may be a reason to chose aortic surgery, before aortic regurgitation arises due to a further increase in aortic root diameter.
The choice of surgical technique is determined by multiple factors. To what extent anticipated growth should role a play in this choice is unclear. In a study by Cattaneo et al. (2004), 26 children underwent a Bentall operation and during follow-up none of the survivors developed outflow obstruction as a result of the aortic prosthetic valve being too small.
Operated children with Marfan syndrome have a higher incidence of reoperation than adult patients (Gillinov et al., 1997). More than half will have to undergo reoperation within 10 years.

Recommendations

Perform preventive aortic surgery in Marfan syndrome children with a widely dilated aortic root. Criteria to be used, in addition to the Z-score, are an increase in aortic root diameter of greater than 8-10 mm/year and/or the emergence of aortic regurgitation. As evidence is lacking, the decision should be made by the responsible treatment team.

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Section 10 Clinical Follow-up

Introduction

There is no scientific literature on the need for and the frequency of medical follow-up in children or adults with Marfan syndrome.

Children

Throughout their childhood, children with Marfan syndrome are seen periodically by a variety of medical specialists, all preferably attached to and within the framework of a multidisciplinary clinic specifically designed for these children.

Frequency of assessment (see Table 10-1): these are usually determined by paediatric cardiology factors:
In case of a stable non-dilated aortic root: every 2 years.
In case of a dilated aortic root (Z < +3): every year.
In case of a dilated aortic root (Z > +3): every six months.
When determining beta blocker medication: every 3-6 months.

A paediatrician, paediatric endocrinologist or coordinating doctor sees the child at each check-up, at which a complete general physical examination takes place. In the case of extreme height increase and once a height of 150 cm has been reached, a height prediction is made by the paediatrician or paediatric endocrinologist based on bone age.
If more frequent follow-up by a paediatrician is needed than determined by the paediatric cardiologist, part of the care could be situated in a regional hospital after consultation wit the local paediatrician.

Paediatric cardiologist: sees the child at every check-up. Echocardiography at each check-up. ECG and aortic MRI, if indicated.

Paediatric orthopaedic surgeon: sees the child by indication, based on musculoskeletal complaints or at the instigation of the paediatrician/coordinator.

Geneticist: sees the child at the time of diagnosis, for the results of DNA diagnostics and then by indication.

Table 10.1 Follow-up scheme for children with Marfan syndrome

Specialist	Type of follow-up	Frequency
paediatric cardiologist	echocardiography, ECG	Stable non-dilated aortic root every 2 years; dilated aortic root but Z <+3 every year; dilated aortic root Z >+3 every 6 months
coordinating doctor[1]	physical examination; height prediction (at 150 cm or in case of extreme height increase); signalling of psychosocial problems; coordination of care	every assessment based on the cardiologic factors[2]
paediatric ophthalmologist	refraction test, slit lamp examination in maximum mydriasis	no important ophthalmologic abnormalities every 2 years; in case of ophthalmologic abnormalities, depending on the findings
paediatric orthopaedic surgeon	physical examination, X-ray	if indicated
clinical geneticist	at diagnosis, for results DNA-analysis	if indicated

[1] this can be a paediatrician or clinical geneticist
[2] if more frequent follow-up by a paediatrician is needed, part of the care could be situated in a regional hospital after consultation wit the local paediatrician

Paediatric ophthalmologist: sees the child at first examination, then if no important ophthalmological abnormalities, every two years. In the case of significant abnormalities (usually lens (sub)luxation), frequency of inspection dependent on eye abnormalities. If the initial examination takes place during the first year of life, then repeat within one year.

Surveillance after cardiac surgery: in children, ultrasound examination is generally sufficient.

Adults

Life-long and regular clinical assessment is carried out in a Marfan center, with the involvement of specialists with extensive expertise in this area. Annual check-ups by a cardiologist are usually sufficient. In patients who have undergone surgery of the thoracic aorta, it is important that the cardiologist and cardio-thoracic surgeon agree on which of them will continue to monitor the patient on specific issues related to the aorta. Referral to other specialties occurs as indicated, and specific examinations will be dictated by symptoms.

Ophthalmologist:
There are indications that primary open-angle glaucoma is more common in patients with Marfan syndrome (Izquierdo et al, 1992; Nahum & Spierer 2008). Therefore, periodic examination of eye pressure and the aspect of the optic nerve should be considered. In most cases, an ophthalmologist local to the patient may become involved.

Echocardiography:
In stable patients, an annual outpatient check-up involving echocardiography of the aortic root is recommended. Valve regurgitation and ventricular function can also be effectively monitored using echocardiography.

MRI:
Baseline MRI imaging of the entire aorta is performed at first check-up and repeated at least every 5 years as long as the dimensions beyond the aortic root are normal. In cases of aneurysm formation in the distal aorta, a repeat MRI is indicated at least annually. CT scintigraphy can replace MRI if there is a contraindication for MRI, such as claustrophobia or a pacemaker, and CT can sometimes be more suitable than MRI for the early detection of complications following aortic surgery. See Considerations in the section Imaging.
A patient should be assessed annually by CT (or MRI) in the event of a chronic aortic dissection, in order to allow timely detection of aneurysm formation (Hiratzka et al., 2010). The elasticity of the aorta can also be measured with MRI, and elasticity of the thoracic descending aorta may have predictive value for the occurrence of progressive local growth (Nollen et al., 2004).

If there is an indication for elective surgery, the probability of coronary artery disease can be assessed using a CT scan, as catheter interventions entail a certain risk in patients with a dilated and weakened aortic wall. If the CT scan shows indications for coronary artery disease, the catheterisation will, however, still be required.

For optimal comparison, repeated measurements of the aortic diameter should be performed using the same technique.

Follow-up after aortic surgery:
In adults, a baseline CT/MRI should be made prior to discharge and repeated after 6 months, 1 , 2 and 3 years. In case of stable diameters, a frequency of once every 2 years is sufficient, but surveillance should be increased as soon as growth is observed.

Recommendations

Children
Follow-up of children with Marfan syndrome should take place within a multidisciplinary Marfan clinic team.

If more frequent paediatric follow-up is needed than the cardiologic follow-up, part of the paediatric care can be performed by a regional paedriatrician after consultation.

Ophthalmologic follow-up should take place at least every two years.

Ultrasound follow-up of the aorta after cardiac surgery is generally sufficient.

Adults
Adult Marfan patients should receive life-long check-ups, provided by a cardiologist attached to a Marfan clinic or alternately by a cardiologist attached to a Marfan clinic and a regional cardiologist.

Assessments by an ophthalmologist or orthopaedic surgeon take place as indicated.

Consider periodic follow-up for eye pressure and the optic nerve.

Folowing aortic surgery, perform a baseline CT/MRI before discharge. Repeat after 6 months, 1, 2, 3 years and then every 2 years. Intensify surveillance as soon as growth is observed.

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Section 11 Family Studies

Introduction

Due to the dominant mode of inheritance, family studies are important in Marfan syndrome, and the intra-familial variability of the disorder means that it is not always clear which family members may or may not be affected. Family studies in Marfan syndrome are no different to those in other inherited disorders, especially if the mutation in the FBN1 gene is known. The question is how studies will be conducted, in which family members, and where the research will take place.

Summary of the literature

Using the original 'Ghent criteria' (De Paepe A. et al, 1996), 91% of individuals conforming to a diagnosis of Marfan syndrome showed an *FBN1* mutation, which confirmed the diagnosis at the genetic level (Loeys et al, 2004). In a recent publication by Sheikhzadeh, an *FBN1* mutation was detected in 80% of the patients who met the original, the new, or both Ghent criteria (Sheikhzadeh et al, 2011). There is no specific literature that focuses on methodologies for family studies in Marfan syndrome.

Conclusions

Level 3	A mutation in the FBN1 gene can be demonstrated in most patients with Marfan syndrome. C Loeys et al, 2004; Sheikhzadeh et al, 2011

Considerations

When a pathogenic mutation in the *FBN1* gene is detected in an index patient, use is made of the 'cascade-screening' customary in clinical genetics. This involves the examination of (vertical) first-degree relatives in first instance, followed by wider screening depending on the position of the family members that tested positive. Brothers and sisters of the affected person will be examined if a parent has Marfan

syndrome or if anamnestic evidence points to Marfan syndrome in the siblings. Prior to blood withdrawal, the implications of possible DNA test results should be explained and discussed with family members by a clinical geneticist or genetic counsellor, especially where relatives are apparently healthy (presymptomatic DNA diagnostics). Depending on the time required for a DNA diagnostic test, in the case of a new index patient, and where appropriate, one can consider echocardiography in first-degree relatives of the index patient if there is strong suspicion of Marfan syndrome in a family member or high levels of anxiety.

If no pathogenic mutation of *FBN1* is found in the index patient, a diagnostic examination is conducted as described in the section Diagnostics. This includes physical, cardiological and ophthalmological examination in all cases.

In practice, it is strongly recommended that the relatives of a Marfan syndrome patient be referred to a specialised Dutch Marfan clinic for diagnostics (where a clinical geneticist is involved).

Recommendations

Perform DNA analysis in the relatives of Marfan patients where possible, and otherwise by clinical diagnosis. Apply so called 'cascade screening': vertical (at risk) first-degree relatives are the first to be screened.

DNA diagnostics in relatives must take place in a clinical genetic centre, and clinical diagnosis - where appropriate - in a multidisciplinary Marfan clinic.

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Section 12 Pregnancy and Delivery

Introduction

Literature and hard data related to pregnancy, childbirth and Marfan syndrome are relatively scarce. The questions central to this section are: "What are the risks of pregnancy and childbirth for mother and child?", and "What are the recommendations for the guidance of pregnancy and childbirth?".
Large randomised trials are lacking, as most literature consists of case reports. This leaves considerable room for expert opinion in the treatment recommendations for pregnant women with Marfan syndrome. Progress has been made in treatment and there is now a better understanding of the genetics and natural history. In this regard, studies of the effects of beta-blockers during pregnancy are promising but have yet to be completed.
Multidisciplinary teamwork and a clear treatment plan, are important in pregnant women with Marfan syndrome.
The section is divided into two subsections. The first subsection concerns the risks of pregnancy and childbirth for mother and child. The second subsection concerns the supervision of pregnancy and childbirth. At the end a checklist is included.

1. Risks of pregnancy and delivery for mother and child

Summary of the literature
Most literature consisted of case reports, with only a few of reviews and studies in patient groups.
The main complications for pregnant women with Marfan syndrome are cardiovascular events, aortic dissections in particular. The physiological changes during pregnancy (increased cardiac output (30-50%) and increased blood volume) constitute an extra burden on the heart and major blood vessels. Cardiac output increases by a further 20% during the contractions of labour, and an extra burden is also present immediately after delivery (Hytten & Paitin, 1963; Wallenburg, 1990; Silversides et al., 2010). In addition, the hormonal changes during pregnancy have a negative influence on the strength of the aortic wall (Manola-Estrella & Barker, 1967).

1.1 Aortic dissections

The incidence of aortic dissection in the general population is about 2.9-3.5/100,000

person-years (Ramanath et al, 2009). The risk in pregnant Marfan patients is estimated to be 1-5% (Pacini et al., 2009; Lind & Wallburg, 2001; Canadas, 2010). This seems to be related to aortic diameter, aortic diameter growth, family history and previous surgery on the aorta. (Preconceptional) counselling is therefore an important component of care for pregnant women with Marfan syndrome.

The likelihood of an aortic dissection during pregnancy

The average age at which a dissection occurs in Marfan patients is around 30-32 years (Murdoch et al., 1972; Silverman, 1995). A dissection can occur in any trimester in pregnant women, but the greatest risk is in the last trimester and in the postpartum period. From two cases series including 57 (Husebeye et al., 1958) and 51 patients (Konishi et al., 1980) and a literature review by Lind (2000), it appears that around 5% of dissections occur in the first trimester, 10% in the second trimester, 50% in the third trimester, 15% during delivery and 20% in the postpartum period. Only a relatively small number of dissections have been described in the literature (500-600).

Meijboom (2005) followed changes in aortic diameter for an average of 6.4 years in a group of 127 female Marfan patients. Within this group, changes in aortic root diameter could be followed in 31 (22 females) pregnancies, and were compared with 22 matched patients who did not become pregnant during the follow-up period. In the pregnant group, no significant change was found in the aortic root diameter prior to, during and following pregnancy. There were also no significant differences in diameters between women who were or were not pregnant. However, within the 'pregnant' group a significant difference was seen in increases in aortic root diameter in those women with an initial aortic root diameter ≥40 mm, compared to women with a diameter <40 mm. There was also a significant difference in the long-term increase in aortic root diameter of 'pregnant' women with an initial aortic root diameter ≥40 mm and matched controls. In the group of pregnant women with an aortic root diameter of 40-45 mm and no haemodynamic abnormalities (n = 9; 11 pregnancies), no aortic dissections or aorta-related complications were found.

An aortic root diameter <40 mm is considered a relatively safe limit for women with Marfan syndrome who wish to become pregnant.

The European guideline (Regitz-Zagrosek et al., 2011)) also adheres to a limit of 40 mm. Furthermore, the guideline states:

a. An aortic root diameter of >40 mm and an increase in diameter of the aortic root during pregnancy are risk factors for dissection in pregnant women with Marfan syndrome;

b. Pregnancy is not recommended at aortic root diameters greater than 45 mm;
c. During the counselling of women with Marfan syndrome with an aortic root diameter of 40-45 mm, risk factors such as aortic growth and family history of dissection should be taken into account.

The Canadian guideline (Silversides et al., 2010) extends the threshold above which a pregnancy is not recommended to 44 mm.
Almost all literature indicates that even when the aortic root diameter is <40 mm, there is no guarantee that a dissection will not occur.

Pregnancy is discouraged in patients who have experienced a type A or B dissection (Regitz-Zagrosek et al, 2011).

Possibilities for prevention of aortic dissection
Beta blockers could potentially reduce the risk of aortic dilatation (Regitz-Zagrosek et al, 2011). It is recommended that prophylactic treatment with beta blockers be either continued or initiated, regardless of aortic diameter or length of pregnancy (Regitz-Zagrosek et al, 2011; Goland et al., 2009; Canadas, 2010; Pacini et al., 2009). Beta-blockers have been linked to foetal growth retardation and neonatal bradycardia, hypoglycemia, hyperbilirubinemia and apnea but these complications are generally rare. The maternal clinical benefits far outweigh these risks (Magee & Dukey, 2003).
In practice, metoprolol is most widely prescribed; atenolol has been more frequently linked to foetal growth retardation (Hogstedt et al., 1985).
The detection and treatment of high blood pressure is important. Beta blockers are not indicated for the treatment of gestational hypertension, with the exception of labetolol, which has postsynaptic α_1- en non-selective β-sympatholytic properties (NVOG 2005). Other agents which may be used for the treatment of gestational hypertension/pre-eclampsia include nifedipine, nicardipine and aldomet.
ACE-inhibitors and AT1-receptor blockers are contraindicated in pregnancy due to teratogenic effects (Cooper et al., 2006; Alwan et al., 2005).

In cases of planned pregnancy, both the ESC guideline 2011 and the Canadian guideline advise preconceptional preventive surgery at aortic root diameters above 45 mm (leading edge to leading edge).

Treatment of aortic dissection during pregnancy

Most acute situations involve a type A dissection (ascending aorta), and immediate surgery is required.

The length of gestation is of importance when considering whether a pregnancy should be terminated in cases of a type A dissection. At a gestational age of more than 30 weeks, a caesarean section is usually carried out and immediately followed by surgery of the aorta. The period between 24-30 weeks is still under debate. An emergency caesarean followed by surgery is common practice but in cases of extreme prematurity, one can elect to continue the pregnancy and to perform the operation under foetal monitoring, and with adjustments in anaesthesia and hypothermia. The risk of severe neonatal morbidity is about 3-6% and foetal mortality is high (Chambers, 1994). When a dissection occurs at a gestational age of less than 24 weeks, the same dilemmas have to be faced.

Type B dissections (no involvement of ascending aorta) are generally treated by conservative drug treatment with frequent monitoring by MRI. Surgery may be indicated in symptomatic dilatation of the descending aorta. If a foetus is viable, the European guideline recommends a caesarean section first, immediately followed by treatment of the aorta (Regitz-Zagrosek et al, 2011).

1.2 Other complications of pregnancy and delivery

The rate of spontaneous abortion in women with Marfan syndrome may be slightly increased (15-20%; normal 10-15%) (Meijboom et al., 2006; Lind, 2000; Lind et al., 2001; Rossiter et al., 1995) .

Premature birth and dysmaturity are also somewhat more frequent (5-30% and 10-15%, respectively, normally about 8% and 10%) (Lind et al., 2001; Rossiter et al., 1995; Lipscomb et al., 1997).

Little information is available on the incidence of postpartum bleeding, manual placental removal and total rupture in Marfan syndrome compared with the normal population, but it does not seem to greatly deviate from that of the normal population (Lind, 2000; Lind et al., 2001).

Published data specifically focused on pregnant women with Marfan syndrome and scoliosis is lacking. The percentage of caesarean sections in non-Marfan patients with scoliosis is no higher than in the normal population(Betz et al., 1987; Orvomaa et al., 1997).

Neonatal outcome is correlated with maternal health. If no cardiac or vascular surgery/ complications occur during pregnancy, neonatal outcome is comparable to that of a normal population (Regitz-Zagrosek et al, 2011).

Conclusions

Level 2	Aortic dissection is a major complication of pregnancy in women with Marfan syndrome, and occurs in 1-5% of cases. B Lind et al., 2001; Pacini et al., 2009 D Canadas, 2010
Level 3	Most aortic dissections occur in the 3rd trimester, during delivery and postpartum. C Husebeye et al., 1958; Konishi et al., 1980
Level 3	The probability of an aortic dissection during pregnancy is small, where the aortic diameter is stable and less than 40-45 mm. B Meijboom et al., 2005
Level 4	A type A dissection is an indication for emergency surgery. A type B dissection is usually treated conservatively. Depending on the findings and symptoms, a type B dissection may be treated surgically or by placing a stent. D Regitz-Zagrosek et al, 2011
Level 4	A type A dissection is an indication for emergency surgery. A type B dissection is usually treated conservatively. Depending on the findings and symptoms, a type B dissection may be treated surgically or by placing a stent. D European Society of Cardiology 2011

Level 4	The risk of spontaneous abortion or preterm birth in women with Marfan syndrome may be slightly elevated. C Meijboom et al., 2006; Lind et al., 2001; Rossiter et al., 1995; Lipscomb et al., 1997

Considerations

The probability of an aortic dissection in pregnancy is lower with elective aortic surgery in the anamnesis than with a history of surgery for acute aortic dissection. Although no studies or literature are known, it is generally believed that the risk of dissection in pregnancy following elective aorta surgery is acceptable. In cases with a history of surgery for acute aortic dissection, the aorta is known to be poor and to carry an increased risk of a new dissection. In this case, a pregnancy is discouraged.

Recommendations

Do not discourage women with Marfan syndrome and an aortic root diameter of less than 40 mm from becoming pregnant.

When making decisions on pregnancy, in cases of aortic root diameters of 40-45 mm, individual risk factors should be taken into account.

Encourage patients with an aortic root >45 mm to undergo elective aortic root replacement before becoming pregnant.

Advise against pregnancy in patients who have experienced an aortic dissection.

Diagnose high blood pressure actively and treat it.

Continue or begin prophylactic treatment with beta blockers during pregnancy.

2. Guidance during pregnancy and delivery

Summary of the literature

2.1 Pregnancy

There is little to no scientific literature on pregnancy guidance in Marfan patients. The recommendations therefore predominantly reflect the opinion of the working group.

2.2 Anticoagulants during pregnancy

Anticoagulants are indicated for a proportion of female Marfan patients, due to valve defects or following cardiovascular surgery.

There is no evidence that the use of anticoagulants in a pregnant patient with Marfan syndrome entails specific increased risks, but oral anticoagulants do cause an increased risk of miscarriage and haemorrhagic complications in pregnant women (Schaefer, 2006).

Patients taking anticoagulants should receive counselling appropriate to their situation. Congenital anomalies have been described following the use of coumarin derivatives, but not following the use of heparin and low molecular weight heparin (LMWH) derivatives (these substances do not cross the placenta). If oral anticoagulants (Marcoumar/ Sintromitis) were already in use before pregnancy, it is preferable to use Heparin/LMWH in the first and at the end of the 3^{e} trimester, and to prescribe coumarin derivatives in the intervening period. In general, a daily dose of <2 mg Sintromitis dfoes not result in teratogenic effects and can therefore be considered for use throughout pregnancy. The use of LMWH throughout pregnancy has been linked to a slightly increased risk of thrombosis in patients with non-biological prosthetic valves (Yinon, 2009). Concentrations of LMWH may fluctuate during pregnancy, potentially resulting in a fall of anti-Xa-activity below the therapeutic value (Friedreich, 2010; Barbour, 2004).

Prolonged use of unfractioned heparin can result in complications such as osteoporosis and “heparin-induced thrombocytopenia”. The use of ascal is acceptable, if indicated (NICE 2010).

2.2 Delivery

No scientific studies were found in which the risk of vaginal birth was compared with caesarean sections in women with Marfan syndrome. Reviews and guidelines do provide recommendations, but they are mainly based on expert opinion.

The European Society of Cardiology (Regitz-Zagrosek et al, 2011) provides recommendations and advice on parturition, based on the WHO risk classification. See Tables 12.1, 12.2 and 12.3.

Table 12.1 Modified WHO classification of maternal cardiovascular risk: principles

Risk class	Pregnancy-related risk with this condition
I	No detectable increased risk of maternal mortality and no/mild increase in morbidity.
II	Small increased risk of maternal mortality or moderate increase in morbidity.
III	Significantly increased risk of maternal mortality or severe morbidity. Expert counselling is required. If pregnancy is decided upon, intensive specialist cardiac and obstetric monitoring is needed throughout pregnancy, childbirth and puerperium.
IV	Extremely high risk of maternal mortality or severe morbidity: pregnancy is contraindicated. If pregnancy occurs, termination should be discussed. If pregnancy continues, care as for class III.

Table 12.2: Risk classification of the various cardiovascular abnormalities that may occur in Marfan syndrome

Risk class	Condition
WHO II-III	Mild left ventricular dysfunction. Marfan syndrome without aortic dilatation.
WHO III	Mechanical valve. Marfan syndrome and aorta between 40-45 mm.
WHO IV	Marfan syndrome and aorta >45 mm.

Table 12.3 : Recommendations based on risk classification

Recommendation	Level of recommendation[a]	Level of proof[b]
Marfan and aorta >45 mm: surgery before pregnancy	I	C
Ascending aorta <40 mm: vaginal delivery is favoured	I	C
In cases of severe hypertension: vaginal delivery with epidural and possibly elective assisted delivery	IIa	C
Marfan and aorta between 40-45 mm: vaginal delivery with epidural anaesthesia and expedited second stage should be considered	IIa	C
Marfan and aorta between 40-45 mm: caesarean delivery may be considered	IIb	C
Aorta >45 mm and Marfan syndrome: caesarean delivery should be considered	I	C
Type B dissection or history of type B dissection: should be advised against pregnancy*	III*	C
If delivery starts while on oral anticoagulants, caesarean delivery is indicated	I	C

Two non-systematic reviews have suggested that women at low risk can undergo vaginal birth, provided there are no obstetric or other contraindications (Canadas, 2010(II), Goland et al., 2009).
Both Goland et al. (2009) and Canadas et al. (2010) recommend the liberal use of epidural anaesthesia and forceps or vacuum pump during vaginal delivery, in order to reduce effects on blood pressure and to accelerate the second stage of labour.
A position on the left side or half sitting places the least stress on the aorta during labour (Regitz-Zagrosek et al, 2011).

In cases with a high risk, a controlled setting is usually chosen and a primary caesarean section performed, together with intensive maternal monitoring and, if possible, fractionated administered epidural anaesthesia with haemodynamic monitoring (Canadas, 2010; Goland et al., 2009).

The ability to administer epidural or spinal anaesthesia can sometimes be limited by dural ectasia (Goland et al., 2009; Canadas, 2010), previous back surgery, scoliosis or prolonged bleeding (anticoagulants). Dural ectasia cause the spinal block to fail through the dilution of the local anaesthetic agent and are responsible for a higher chance of a “dural tap” at the epidural punction.

A caesarean section is recommended in patients who enter labour while using oral anticoagulants (OAC), to prevent intracranial haemorrhage in the foetus. The OAC can cross the placenta and affect coagulation in the foetus (Regitz-Zagrosek et al, 2011).

Conclusions

Level 3	As a consequence of fluctuating concentrations of LMWH, anti-Xa activity may fall below the therapeutic concentration and should be monitored. C Friedreich, 2010; Barbour, 2004

Level 4	Vaginal delivery is safe in patients at low risk (aortic diameter <40 mm and no haemodynamic abnormalities). According to the ESC guideline, both vaginal and caesarean delivery should be considered in cases with a moderately increased risk, depending on individual circumstances. High risk cases (aortic diameter >45 mm, history of acute aortic dissection, haemodynamic limitations) usually undergo a primary caesarean section performed with intensive maternal monitoring and, if possible, fractionated administered epidural anaesthesia with haemodynamic monitoring. D Canadas, 2010 II; Goland et al., 2009; Regitz-Zagrosek et al, 2011

Level 4	The advice in literature is for liberal use of epidural anaesthesia and forceps or vacuum pump during vaginal delivery, in order to reduce the cardiovascular burden during the second stage of labour. D Goland et al., 2009; Canadas, 2010

Considerations

A preconceptional consultation is important and should include: a gynaecologist, a clinical geneticist, a cardiologist and a paediatrician. During this consultation, the dangers, the strategy and plan of treatment can be determined in advance and discussed. An anaesthesiologist can be consulted preconceptionally, but should always be consulted before delivery.
If no preconceptional consultation has taken place, the above consultation should take place as early as possible in pregnancy and a written treatment plan should still be prepared. The antenatal check-ups can, in principle, take place at regular intervals as described in the NVOG protocols. On theoretical grounds, frequent monitoring from 28 weeks appears logical.

Besides the routine 1e trimester foetal ultrasound (period; 11-14 weeks scan), a so-called advanced ultrasound examination of the foetus around 20 weeks gestation may be considered, with a repetition at 30-34 weeks. Although the chance of a foetus being

affected by congenital abnormalities visible on ultrasound is very small, there are case reports and personal communications that report large aortic diameters antenatally. Moreover, it is undesirable to overlook a foetal abnormality in a pregnancy that is already hazardous.

Periodic monitoring of aortic dimensions is clearly required. In the literature, no clear indications are given for the frequency of monitoring, but a general scheme is: before pregnancy, at 20-24 weeks, 36 weeks, 2 days postpartum and 6 weeks postpartum. Depending on the history and clinical findings, more frequent monitoring may possibly be indicated. In high-risk patients, the abdominal aorta should also be properly examined.

The choice of the mode of delivery depends not only on the risk, but also on individual circumstances and patient preference. The patient should be well-informed about the risks of the various options.
The safety of the mother and the child should take priority when making any choice.
Parturition should take place at the secondary or tertiary level, depending on the cardiovascular status of the patient and treatment capabilities of the hospital.
Low-risk patients (aortic diameter <40 mm) can receive check-ups and undergo childbirth at the secondary level, as long as clear agreement is reached with a cardiosurgical centre on check-ups, treatment and measures around parturition, and that the circumstances requiring referral are clearly discussed. A treatment plan for possible emergencies is important. Patients with moderate or high risk must give birth in a tertiary center with capabilities in cardiovascular surgery and an experienced team should be present.

Depending on general health and the results of cardiological investigations, in a medium-risk situation (aortic diameter 40-45 mm or 1x elective aortic surgery or heart valve replacement with a stable haemodynamic result) it is possible to opt for a vaginal delivery with progressive epidural anaesthesia, basal maternal monitoring and possibly a primary assisted vaginal delivery (forceps/vacuum extraction).

If medication is used by a mother during pregnancy, a consultation with a paediatrician is indicated following childbirth.

Recommendations

Both a (multidisciplinary) preconceptional consultation and a multidisciplinary treatment plan are important in Marfan patients planning pregnancy.

Consider a so-called advanced ultrasound examination of the foetus, in addition to routine foetal ultrasound, at around 20 weeks and around 30-34 weeks of pregnancy.

Pregnant Marfan patients should receive check-ups at the secondary or tertiary level, and these should include periodic monitoring of aortic diameter (before pregnancy, at 20-24 weeks, 36 weeks, 2 days postpartum and 6 weeks postpartum). Depending on history and clinical findings, more frequent monitoring may possibly be indicated and monitoring of high-risk patients should also include the entire abdominal aorta.

Patients on anticoagulants should receive appropriate counselling. Check coagulation using the indicator of anti-Xa activity during therapeutic use of LMWH. If delivery occurs while oral anticoagulants are still being used, a caesarean section is indicated.

Advise a vaginal birth in a secondary centre for patients at low risk (aortic diameter < 40 mm and no haemodynamic abnormalities), provided that arrangements with a tertiary centre are made in case of problems or complications. Use epidural anaesthesia and forceps or vacuum pump liberally, in order to reduce effects on blood pressure and to accelerate the second stage of labour.

Consider a primary progressive epidural and primary assisted delivery (vacuum/forceps extraction) at parturition, with fractionated administered epidural anesthesia and haemodynamic monitoring in patients at moderate risk (aortic diameter 40-45 mm or 1x elective aortic surgery or heart valve replacement with a stable haemodynamic result). Consider a caesarean section depending on individual circumstances.

Perform elective caesarean section with intensive maternal monitoring and with fractionated administered epidural anaesthesia and haemodynamic monitoring, if possible, in high-risk cases (aortic diameter >45 mm or acute aortic dissection or haemodynamic limitations) in the presence of an experienced team.

Patients at moderate or high risk should give birth in a tertiary care centre with options for cardiovascular surgery

The choice of the mode of delivery should be made in consultation with the patient.

If medication is used during pregnancy, a consultation with a paediatrician is indicated following childbirth.

Checklist: pregnancy and Marfan syndrome

Preconceptional consultation:

- Cardiovascular evaluation;
- Adjustments to medication: anticoagulants, ACE inhibitors, beta-blockers;
- R/ folic acid;
- Consultation with cardiologist and possibly other specialists as needed;
- Pregnancy preferably at a younger age;
- Prenatal diagnosis, consultation with clinical geneticist;
- General physical examination;
- Explanations of chance of aortic dissection, prevention, treatment, complications;
- Possible adjustments to lifestyle;
- Pregnancy check-ups at secondary/tertiary level, depending on health.

Pregnancy:

- Cardiovascular evaluation;
- Explanations of chance of aortic dissection, prevention, treatment, complications;
- Monitoring of aorta (at 20-24 and 36 weeks);
- Possible lifestyle guidelines;
- Consultation with cardiologist, anaesthesiologist and possibly other specialistss as needed;
- Consultation at a cardiosurgical centre;
- Prenatal diagnosis;
- Ultrasound: 11-13 weeks scan, 20 weeks ultrasound/advanced ultrasound examination type 1, 30-34 weeks scan, growth monitoring;
- Prepare a birth plan;
- Consultation with a paediatrician;
- Medication (anticoagulants, beta blockers, etc.);
- General physical examination;
- From approximately 28 weeks, more frequent check-ups in order to monitor blood pressure.

Childbirth:

- If risk is low and aortic diameter <40 mm: secondary/tertiary level, vaginal delivery;
- If risk is moderately increased (decided at multidisciplinary consultation): vaginal delivery, tertiary level, maternal monitoring, epidural anaesthesia and primary

assisted vaginal delivery or caesarean section;
- If high risk: tertiary level, intensive maternal monitoring, epidural anaesthesia, a primary caesarean section if possible.

Postpartum:
- Contraception;
- Evaluation of neonate;
- Ultrasound of aorta (2 days and 6 weeks postpartum).

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Section 13 Prenatal and Preimplantation Diagnosis

Introduction

Many patients with Marfan syndrome reach reproductive age in good health, thanks to the effective treatment options. Patients often have questions concerning fertility, pregnancy-related complications and the consequences for the child. Since Marfan syndrome is an autosomal dominant disorder, the chance that a Marfan parent will have a child with Marfan syndrome is 50%. As a molecular cause is currently found in a large percentage of Marfan patients, opportunities are available for prenatal diagnosis (PND) or preimplantation genetic diagnosis (PGD).

Brief explanation of procedure prenatal/preimplantation genetic diagnosis
Prenatal diagnosis of an existing pregnancy allows foetal material to be examined for a specific condition by chorionic villus sampling or amniocentesis. Chorionic villus sampling takes place via either the abdomen or vagina, around the 11th week of pregnancy. Amniocentesis can be performed from the 16th week onwards. PND involves certain risks: in general, the risk of miscarriage as a result of a chorionic villus sampling/amniocentesis is approximately 0.3-0.4%.

Preimplantation genetic diagnosis involves an IVF procedure whereby genetic diagnosis takes place prior to transfer of the embryo, and only embryos lacking a mutation will be returned. If a couple chooses PGD, the laboratory must conduct preliminary genetic research of the parents themselves, any children and any relatives. The duration of the preliminary investigation can vary from several weeks to one year. Since the *FBN1* gene is not prone to hot spot mutations and each family carries a specific mutation, marker analysis is usually chosen (Spits et al., 2006; Kilpatrick et al., 1996).

A gynaecologist, a clinical geneticist and a social worker will discuss the course of these studies (preparation, sampling, results, psychosocial aspects) with the prospective parents.

Summary of the literature
A literature search was carried out with the aim of identifying whether PND and PGD carry specific risks in the context of Marfan syndrome. If the father has Marfan syndrome, PND/PGD is not expected to entail a higher risk than for any other indication for PND/PGD, as the expectant mother is healthy. However, in the event that the woman herself has

Marfan syndrome and wishes to become pregnant, there are theoretical additional risks to consider, including those of the tests (chorionic villus sampling/amniocentesis/follicular puncture), the hormonal stimulation in PGD, the multiple pregnancy risk with PGD and the use of anticoagulants.
The specific cardiac-related risks for a woman with Marfan syndrome during pregnancy are discussed in section 12.

The literature related to prenatal/preimplantation genetic diagnosis and Marfan syndrome is limited to individual case reports (Blaszczyk et al., 1998, Godfrey et al., 1993; Harton et al., 1996, Kilpatrick et al., 1996, Smith et al., 2010) and a few small series (Loeys et al., 2002; Toudjarska et al., 2001; Spits et al., 2006). These articles report that prenatal/preimplantation diagnosis is possible, but do not discuss possible risks.

Whether amniocentesis/chorionic villus sampling/follicular puncture in an expectant mother with Marfan syndrome carries a higher risk of abortion than in the general population has not been studied. No complications associated with these procedures were found in the literature.

Adverse effects in female Marfan patients due to ovarian stimulation during an IVF procedure have not been described in the literature.

No report of a multiple pregnancy entailing additional risks for a Marfan patient could be found in the literature. The PGD guideline for Marfan syndrome includes no comment on the number of embryos that should be transferred. In general, the cardiac condition of a patient is taken into account (even for indications other than Marfan syndrome), and in consultation with the clinical geneticist, cardiologist and gynaecologist, 1 or 2 embryos will be transferred.

If a biopsy is to be performed on a woman using anticoagulant medication, this should be discussed with the cardiologist. Complications resulting from the use of anticoagulants in a pregnant patient with Marfan syndrome have not been described.

Conclusions

Level 3	Prenatal diagnosis for pregnancies that carry an increased risk of Marfan syndrome is possible if the familial mutation is known (or if there is linkage). A choice can be made between chorionic villus sampling (10-12 weeks gestation) or amniocentesis (15 - 18 weeks gestation). C Loeys et al, 2002; Toudjarska et al, 2001; Spits et al, 2006
Level 3	Pre-implantation genetic diagnosis for pregnancies that carry an increased risk of Marfan syndrome is possible if the familial mutation is known. C Loeys et al, 2002; Toudjarska et al, 2001
Level	No information is available from literature on whether invasive diagnosis (chorionic villus sampling/amniocentesis/follicular puncture) entails greater risks in a couple with Marfan syndrome than in the general population. No literature
Level	It is not known whether IVF-related hormone stimulation leads to increased risks for Marfan patients. No literature

Considerations

There is no scientific literature to support the conclusion that PND or IVF for PGD is contraindicated for Marfan female patients. Conversely, it can also not be concluded that there is no additional risk. On the basis of small series and personal experience, PND and PGD does not seem to present a major risk to pregnant Marfan patients.

The clinical variability of Marfan syndrome leads to complex reproductive choices, since a molecular diagnosis does not predict the severity of symptoms.
Theoretically, a twin pregnancy is less desirable because of the additional cardiovascular

stress. This is particularly important in cases where ovulation induction or IVF (with or without pre-implantation diagnosis) is applied, with the related increased risk of multiple births.

Recommendations

Prenatal or preimplantation genetic diagnosis is possible when the familial mutation of a parent with Marfan syndrome is known.

Offer (or re-offer) genetic counselling to young adults (male or female) with Marfan syndrome. Recurrence risk, cardiac condition before and during pregnancy and reproductive options should be discussed during this preconception counselling.

Do not transfer more than a single embryo during IVF.

The risks of PND and PGD for IVF should be studied in larger series

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Section 14 Lifestyle Recommendations

Introduction

The international guidelines on Marfan syndrome (Maron et al, 2004; Pelliccia et al, 2005; Maron et al, 2005a) advise patients to change their lifestyle. These recommendations mainly concern sport; advice related to daily activities and career choice is more limited. The motivation for lifestyle advice is to reduce the haemodynamic stress on the aortic wall. The increases in heart rate, blood pressure and stroke volume during intense activity may promote the development of aortic aneurysms and increase the rate of aortic dilatation. Distensibility declines and wall stress increases during progressive aortic dilatation, resulting in an aortic wall that is even less capable of compensating for the effects of haemodynamic stress during exercise and is thus at greater risk of aortic dissection or rupture (Elefteriades, 2008). Besides physical exertion, strong emotions also seem to play a role in the occurrence of aortic dissection (Hatzaras et al, 2007).

Limiting normal sports activity in children and adolescents with Marfan syndrome can have a dramatic effect, and may well have a negative impact on the physical and psychosocial well-being of the child. Adult Marfan patients experience this obligatory adjustment of their activity pattern as negative for their quality of life (Peters et al, 2001).

Summary of the literature

A *scientific statement* from the American Heart Association in 2004 (Maron et al, 2004) included advice on physical activity and **recreational** sport for young people with genetic heart disease, including Marfan syndrome. These recommendations were based primarily on the experience and insights of the panellists (expert opinion). Quote: "*There is a paucity of precise published evidence. Indeed, the panel encountered few absolute and truly quantitive data...*". When formulating their advice, the panellists attempted to strike a balance between the positive effects of sports activity and the inherent risks for this particular patient group.

Avoiding participation in sports that involve rapid accelerations and decelerations (e.g. basketball, squash and tennis) and those that demand intensive isometric (= static) exertion is advisable for all individuals with Marfan syndrome.

School gym classes deserve particular attention. Activities during school gym and outdoor play are acceptable at all times for junior school children, but competitive sports tests in high school are not suitable. The above recommendations are not applicable to Marfan patients who have already had surgery, as more stringent recommendations generally apply to this group.
In 2005, the European Society of Cardiology (ESC) published recommendations for participation in **competitive** sports, also at the level of expert opinion (Pelliccia et al, 2005). Competitive sport is not recommended for Marfan patients, at any age. Low intensity, recreational sport is acceptable. Contact sports are not recommended because of the risk of damage to the aorta or eyes. Moderately intense, **recreational** sports are allowed in individuals with normal aortic root dimensions. Recommendations have also been formulated for individuals with clinically suspected Marfan syndrome, but without definite genetic confirmation.

Aortic root diameter is at the centre of the recommendations formulated in 2005 at the 36th Bethesda Conference (BC#36) of the American College of Cardiology (ACC). Participation in low and moderate intensity static and low dynamic competitive sports is allowed if the aortic root diameter is below 40 mm or below 2SD in children, in the absence of haemodynamically significant mitral regurgitation and a family history of aortic dissection or sudden death. It is advisable to measure aortic root diameter by echocardiography every six months.
Marfan patients with an aortic root diameter above 40 mm or above 2SD in children with previous aortic surgery, chronic aortic dissection, moderate to severe mitral regurgitation and/or a family history of dissection or sudden cardiac death, may only participate in low intensity competitive sports. Participation in contact sports is not recommended for anyone with aortic dilatation, and in particular, for patients taking anticoagulant medication (Maron and Zipes, 2005b).
There are several published lists that provide a classification of the intensity of exercise in sports. The scheme by Mitchell et al (2005) may be helpful but the advice regarding sports is largely dependent on the individual situation of the patient (Mitchell et al, 2005).

In 2008, Pelliccia et al. (2008) compared the advice of the ESC and that of the ACC: the differences centred on whether or not to allow participation in competitive sport at (still) normal aortic root dimensions.

Sports advice aimed at lowering the burden on the joints is at the level of "common sense" ("A*lthough there have been no trials to investigate the effectiveness of sports limitation to*

avoid joint damage, common sense suggest that activities likely to stress the joints should be avoided.") (Dean, 2007).
In connection with an increased risk of pneumothorax, scuba diving is discouraged.

Conclusions

Level 4	Most guidelines discourage participation in sports involving rapid accelerations and decelerations, sports requiring intensive static exertion and contact sports. Participation in recreational low-to-moderate intensity sports is permitted in most guidelines. Guidelines recommend that children participate as much as possible in normal school gym and outdoor play activities. Participation in intensive sports tests such as the Cooper Test or the Shuttle Run Test is not recommended. D Maron et al, 2004; Maron & Zipes, 2005b; Pelliccia et al, 2005; Pelliccia et al, 2008

Considerations

The international literature contains clear advice regarding the sports participation of patients with Marfan syndrome. On the one hand, these recommendations are based on theoretical considerations and the partly investigated effects of haemodynamic stress on the aortic wall, especially when dilatation and decreased distensibility is already present, and on the other, on retrospective observations concerning the circumstances under which patients developed aortic dissection or rupture. The advice of the ESC and ACC expert panels differ in the details regarding Marfan patients without aortic root dilatation. Advice should be tailored to fit individual needs and with as few restrictions as possible, especially with regards to children. The same sports exercise advice should be followed for non-sports related (daily) activities. In the opinion of the working group, there is no reason to dissuade Marfan patients from participation in normal daily activities (including sexual activity).
National Marfan organisations have an important supporting role to play in disseminating this lifestyle advice.

Recommendations

Advice regarding participation in sports should be tailored to fit individual needs, for example, taking into account the condition of the aorta and the use of anticoagulants.

Caution should be exercised with regard to competitive sports, major peaks of exertion and intensive static exertion.
Participation in recreational low-to-moderate intensity exercise can usually be allowed.

Children should participate as much as possible in normal school gym classes and outdoor play activities. Advice against participation in intensive sports tests such as the Cooper Test or Shuttle Run.

There is no reason to dissuade Marfan patients from participating in normal daily activities (including sexual activities).

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Section 15 Organisation of Care

Introduction

Marfan patient care is organised around Marfan clinics. These centres provide a team of medical specialists with expertise in the area of Marfan syndrome. This section aims to provide an illustration of the requirements that must be met by a Marfan clinic and recommendations for the organisation of diagnosis, monitoring and treatment.

Literature

There is no scientific literature to prove that the care for Marfan patients in a center of expertise is better than elsewhere. Nevertheless, the working group considers that a relatively rare multisystem disorder such as Marfan syndrome requires a multidisciplinary approach by specialists with expertise in the field of Marfan syndrome. A multidisciplinary approach is in line with recommendations in the literature: "*The diagnosis and management of Marfan syndrome requires a multidisciplinary team approach, in view of its multisystem effects and phenotypic variability*"(Dean, 2007). This is endorsed by the patientgroup "Contactgroup Marfan Netherlands".

Out-patient clinics

Each clinic has at least one coordinator. This can be a doctor, but may also be a genetic counsellor or specialist nurse. The care coordinator is responsible for the organisational aspects of the Marfan out-patient clinic and may play a role in providing the patient with information. A Marfan team consists of at least a cardiologist, paediatric cardiologist, cardio-thoracic surgeon, paediatrician, clinical geneticist and an ophthalmologist. A paediatric endocrinologist, orthopaedic surgeon, gynaecologist and one physiotherapist can be part of the team or make contributions on a consultative basis. It is recommended that each Marfan team has a link via the website of the relevant University Medical Centre that clearly shows to whom and how a patient referral should occur and provides details of the coordinator or contact person.

Procedure

Referral

A patient consults the (family) physician with features reminiscent of Marfan syndrome. See also section "Diagnostics". It is recommended that in cases with a strong suspicion of Marfan syndrome (e.g. dilatation or dissection of the aortic root without obvious cause, lens (sub)luxation or a first-degree relative with Marfan syndrome), the patient should be referred to a Marfan clinic. Patients with less specific features of Marfan syndrome are reviewed by the coordinator following referral, who then prepares a treatment plan. The referring physician should have a clear idea of how and to whom a patient should be referred.

The first appointment should take place within 4-8 weeks of referral. If this deadline is not feasible, it is recommended that the patient undergo an ultrasound examination of the heart in a local hospital to exclude serious pathology.

If serious pathology is suspected on the basis of the assessment of the referring physician, then the patient should obviously be referred to either an emergency department or directly to an appropriately qualified specialist.

Diagnosis

In a Marfan clinic, all diagnostic studies necessary for determining a diagnosis are performed in a single day (or part of). In practice, this means: (family)history and physical examination by the clinical geneticist, and examination by a (paediatric)cardiologist and ophthalmologist. Upon adequate suspicion, a blood sample is taken by the clinical geneticist for DNA testing. Although in most cases the results of the DNA tests can be slow (months or longer), at the end of this first visit it is usually possible to provide a statement of the probability that the referred patient has Marfan syndrome. Preferably, this preliminary result is discussed with the patient by the coordinating physician at the end of the day of diagnosis, and confirmed by a letter within 2-4 weeks, with copies to the relevant specialists, referring doctor and GP.

The Marfan team coordinator/clinical geneticist draws definitive conclusions following receipt of the result of the DNA test. The patient is notified in a final interview and the check-up schedule at the Marfan clinic modified, if necessary. This is followed by confirmation in writing to the patient, GP and specialists involved.

If, based on clinical investigations, insufficient evidence is found for a diagnosis of Marfan syndrome, then alternative diagnoses should be considered; see "Differential Diagnosis".

Clinical assessment

See the section "Clinical assessment". The frequency of clinical assessment is determined by the clinical findings. Clinical assessment of children preferably takes place on a single day, in a multidisciplinary setting, with specialists from the Marfan team. In practice, adults only see a cardiologist and, after cardiovascular surgery, a cardio-thoracic surgeon. Assessment by other specialists takes place only on indication. Clear local arrangements are needed for the transfer of a patient from a paediatric cardiologist to a cardiologist. It should be clear at what moment the cardiologist should call in a young person for the first check-up and there should be a clear, written handover. The transition from a paediatric cardiologist to a regular cardiologist is an appropriate moment to offer a young person an appointment with the genetic counsellor/coordinator. The genetic aspects can be discussed and the patient can be informed of the possibility of making an appointment with the clinical geneticist should there ever be a wish to start a family.

Treatment

See sections 6 to 8, in which special attention is devoted to drug treatment, aortic surgery and the treatment of musculoskeletal disorders. Treatment is the responsibility of the treating specialist.

Pregnancy and childbirth

See the section 12 "Pregnancy and Childbirth". The clinical geneticist or coordinator of the Marfan team must tell patients that they should contact the coordinator should they wish to start a family, allowing a multidisciplinary treatment plan to be prepared in the case that the prospective mother has Marfan syndrome. With an already pregnant patient, this treatment plan should be prepared at the earliest possible stage of the pregnancy. The factors included are also listed in the checklist "Pregnancy and Marfan syndrome." During pregnancy, the gynaecologist and the cardiologist are the primary responsible physicians.

Recommendations

Each Marfan team has a coordinator who may be contacted by Marfan patients and referring physicians. The coordinator's contact details are known to patients and referring physicians.

Effort should be made to reach a clinical diagnosis of Marfan syndrome within 3 months of referral by the general practitioner.

It must be clear to referring physicians how and to whom the referral should be made; a clear link on the website of the University Hospital is recommended.

Check-ups are dictated by the abnormalities found.

During the transition from paediatric cardiologist to regular cardiologist, an appointment should be made with the clinical geneticist.

Pregnancy in a Marfan patient requires multidisciplinary care, coordinated by a gynaecologist and cardiologist.

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Section 16 Patient Information

Introduction

In many cases, the first confrontation with a (possible) diagnosis of "Marfan syndrome" will provoke considerable uncertainty in a patient and/or in his or her immediate family. It is common that those familiar with the Internet almost immediately launch on a veritable treasure hunt using "Marfan" and "Marfan syndrome" as search terms. Fortunately, there is now so much useful and readily available information that this initial information hunger can be partly satisfied. However, translation of the general information found on the Internet to their personal situation still appears to be difficult for many patients, and brings with it uncertainty and an unclear picture of the status and future of life with Marfan syndrome.
The website of the Contactgroup Marfan Netherlands (www.marfansyndroom.nl) offers a wealth of accessible information on a wide range of subjects. The Contactgroup can be easily reached for all questions arising from the first confrontation with Marfan syndrome, using a variety of channels. When a patient requires "peer support", the treating physician may refer him/her to the Contactgroup.

Diagnosis

It is clear that provision of information by the medical team regarding the process leading up to a final diagnosis can be of great importance to a patient. In addition to examination of the patient, examination of relatives may also be essential. Any advice is usually insufficient to remove (all) uncertainty in the patient and relatives, but it is usually possible to provide a more acceptable perspective.
It is important that patient information clearly explains the agreements between the medical team and the patient and/or family. This concerns both agreements on the specific roles of medical team members and the family doctor, and on the coordination of care. It is also important that the patient is aware of the length of time required to make the diagnosis. What can be expected from the medical team during the course of the diagnostic process, in terms of treatment and counselling, should also be clear.

Monitoring and treatment

During the course of the monitoring and treatment of a patient with Marfan syndrome, a phased, timely and targeted provision of advice, in everyday language, to the patient and/or the immediate family must remain a central priority of practitioners in the medical team.

The transition from "diagnosis" to "monitoring and treatment"

Just as in the diagnosis phase, the patient and/or relatives should be informed of agreements with the medical team practitioners. This is especially true if the team is different to that during the diagnosis phase. This concerns agreements on the specific roles of medical team members and the family doctor, and on the coordination of care. The patient must receive clear information on who the care coordinator is and who to approach during a specific course of treatment. What can be expected of the medical team during further stages, in terms of treatment and counselling, should also be clear.

Regular check-ups

Due to cardiac problems, Marfan patients should receive regular check-ups (every six months to once every few years). It is desirable that patients and families, through targeted and timely information, remain aware of the need for regular assessment. Being able to rely on one coordinating individual in a research institute medical team or Marfan clinic is not only a clear desire of individual Marfan patients, but can also represent an important stimulus to medically appropriate monitoring and compliance. This contact person can also play a coordinating role in informing the patient of results of examinations.

Coordinating information on medical consequences

Marfan patients express a clear need for practical advice and information. Besides the obvious need for information on the implications of cardiac and eye diseases, there is certainly a need for information on the implications with respect to muscle problems, orthopaedic abnormalities, dural ectasia, lung defect(s) and impact on daily life. Practical advice on everyday life is especially important.

The above-mentioned coordinating contact individual in the medical team can play an essential role in providing information.

Advice on lifestyle

In connection with cardiac and lung problems in Marfan patients, issues such as hypertension and smoking are important and require attention. The treating specialist can play a role by, for example, giving advice on lifestyle, by referral for help with stopping

smoking and/or treatment for hypertension. Advice on lifestyle is also possible with regard to ophthalmic and orthopaedic problems.

Sports advice (also see section 14)
The treating specialist may refer a patient to a sports physician if there is a desire for (intensive) sports participation. The sports physician can advise the patient on which branch of sport is suitable, based on an individual situation. It is recommended that the patient always seek the advice of a cardiologist before he/she actively participates in sport.

Psychosocial aspects

General
It can not be stressed enough that caregivers should pay special attention, in the form of advice, to the psychosocial aspects of Marfan syndrome. Due to the complexity of the symptoms, those aspects are often difficult for the patient to distinguish. An additional aggravation of the situation is represented by the incurable nature of Marfan syndrome. Despite the psychosocial burden of disease, the experience of the Dutch patient organisation suggests that Marfan patients are often very positive about life.
Signals that may indicate excessive physical and/or psychological stress on the patient, his or her partner and/or family should be noted by the care coordinator. The care coordinator can help a patient by offering a listening ear and more practically, by referring the patient to a psychologist or social worker.
Incidentally, the family doctor also has an important to role play in identifying excessive psychosocial stress.

Young children, their parents and the family
The psychosocial care of young children with Marfan syndrome, their parents and the family receives considerable attention at out-patient clinics for Marfan children. In other situations, there is an urgent need to provide the parents with the necessary information. Regular feedback should be sought from the young Marfan patient and possibly from other children in the family.

Adolescence
Psychosocial problems, especially in (pre)adolescents, may result from the sometimes extreme height growth and the physical appearance, but may also be caused by the impact of treatments such as hormonal or surgical length reduction or cardiac surgery. Adolescents may become socially isolated, and adolescent rebellion can also lead to denial

of the importance of regular check-ups. The care coordinator should be aware that during contact with the patient in this particular life phase (approaching autonomy), special attention should be paid to providing information on and stressing the importance of check-ups and compliance, on providing lifestyle advice and confronting any psychosocial problems. Any problems that may arise in school (introduction of necessary adaptations to furniture and/or classroom aids, changes to gym lessons, necessary additional educational support) can be (partly) overcome by targeted advice by the treating physician.
The transition from adolescence to adulthood requires that the care coordinator be prepared to provide extra attention and advice.

The older Marfan patient (50+)
Information on the consequences of specific symptoms that the older patient with Marfan syndrome may face is desirable. This information should preferably be available at the regular (annual) check-ups, and should be provided by the care coordinator.

The partner of a Marfan patient
The partner of a Marfan patient can play an important role in the process of aftercare. It is desirable that a patient's partner is involved in the provision of any advice. This is particularly the case if it appears that the partner exhibits a (great) need for psychosocial support and advice.

Psychosocial support
A patient with Marfan syndrome will have adapt to circumstances, given the physical constraints that the disease entails. This can be expressed in the choice of further education, career or the distribution of a limited energy reserve over (daily) activities. Some patients also have problems with their appearance or may encounter misconceptions amongst those around them. Due to chronic fatigue and other symptoms caused by Marfan syndrome, the patient will have to regularly call on those around them.

The working environment
The working environment can reveal the limitations of Marfan patients (e.g. endurance, joint strength). To what extent the (working) environment should be informed of these limitations and their cause can be a serious dilemma for Marfan patients. In such situations, the specialist treating the patient can recommend consulting the family or company doctor for help and advice.

Insurance

The specialist(s) treating a patient can provide further information to the insurance company doctor when a patient experiences problems taking out insurance.

Recommendations

Providing Marfan patients with good advice during diagnosis, monitoring and treatment is essential to both the success of the medical care and well-being of patients. In the field of advice provision, the following recommendations can be made.

After diagnosis

- Ask how the patient and/or family experienced the medical team's approach in the phase prior to the diagnosis.
- Assess to what extent the patient and relatives have processed and accepted the diagnosis.
- Ascertain whether the need for further investigations in the family is understood by the patient and relatives and whether sufficient explanation was given.

During control and treatment:

- Introduce a single care coordinator to act as a contact for the patient and his/her parents.
- Make the patient aware of how responsibilities are divided amongst the treating specialists. In the event that a Marfan patient has a prosthetic valve or a valve abnormality, the patient should be made aware of the increased risk of endocarditis and that profylaxis for endocarditis is indicated. It is of great importance that paradontitis and caries be prevented. The dentist and/or dental hygienist play an important role in providing advice. The NHG Endocarditis Profylaxis Guideline clarifies other circumstances under which profylaxis for endocarditis is indicated.
- Provide advice on lifestyle and sports advice, if relevant.
- Pay special attention to psychosocial issues including:
  - Specific issues in relation to age;
  - Family and friends;
  - Employment and insurance.

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A general website with information about Marfan syndrome and links to external sites: www.marfansyndroom.nl

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Prevention Bacterial Endocarditis 2008 (RP54): http://www.webshop.hartstichting.nl/producten/producten.aspx?CatID=71&pID=3765

Appendix 1 Clinical Questions

Diagnostics

- What are the symptoms that indicate Marfan syndrome and which diagnostic test is appropriate?
- What is the policy in relation to a newborn with a 50% risk of Marfan syndrome?

Differential Diagnosis

- What alternative diagnoses should be considered and what are the criteria?

Family studies

- How should family studies be conducted, where, in which family member and at what moment?

Pregnancy and childbirth

- What are the risks of pregnancy and childbirth in a patient with Marfan syndrome?
- Type and frequency clinical assessment during pregnancy.
- When and how should a birth take place?

Prenatal diagnosis/preimplantation diagnosis

- What are the possibilities for prenatal diagnosis/preimplantation diagnosis, and are there specific risks?

Clinical assessment

- Frequency and nature of clinical assessment, as applicable to both adults and children.

Treatment

- What is the effect of drug treatment on the dilatation of the aortic root?
- What is optimal moment for preventive aortic surgery in adults/children?
- Can brace treatment, compared with the natural disease course, prevent surgical intervention?

Lifestyle

- Does participation in (top)sport and certain other activities lead to greater complications?

Patient information

- Which information is important for patients with Marfan syndrome?

Appendix 2 Characteristics of Marfan Syndrome

	Gent II criteria (Loeys et al, 2010)	Gent I criteria (De Paepe A. et al, 1996)
Facial features	+	+
dolichocephaly	+	+
enophthlamos	+	+
downward slanting palpebral fissures	+	+
malar hypoplasia	+	+
retrognathia	+	+
high and narrow palate	-	+
dental crowding	-	+
long face	-	-
Skeletal features		
arachnodactyly (positive thumb and wrist sign)	+	+
pectus carinatum	+	+
pectus excavatum	+	+
chest wall asymmetry	+	-
hindfoot deformity	+	+
pes planus	+	+
protrusio acetabuli	+	+
long arms and legs	+	+
reduced elbow extension	+	+
scoliosis	+	+
thoracolumbar kyphosis	+	+
joint hypermobility	-	+
umbilical sign	-	-
Cardiovascular features		
aortic root dilatation or dissection	+	+
mitral valve prolapse	+	+
tricuspid valve prolapse	-	-
pulmonary artery dilatation	-	-
aortic dilatation or dissection distally from the aortic root	-	-
Ophthalmologic features		
lens(sub)luxation	+	+
myopia	+	+
increased globe length	+	-
Hypoplasia of the iris or ciliary muscle	+	-
corneal flattening	+	-
cataract	-	-
retinal tears	-	-
Family history		
first degree family member with Marfan syndrome	+	+
Other features		
pneumothorax	+	+
apical blebs	-	+
emphysema	-	-
dural ectasia	+	+
striae	+	+
Recurrent inguinal herniae	-	+
recurrent umbilical herniae	-	+
recurrent incisional herniae	-	+
reduced body muscle and fat	-	-
tall stature	-	-

+ feature is part of the criteria
- feature is not part of the criteria

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Appendix 3 Summary of Focus Group Meeting

Five people participated in the Marfan guideline focus group, three of whom themselves have Marfan syndrome, two have a child with Marfan syndrome, and one Marfan patient also has a child with Marfan syndrome. Three patients provided a report of their experiences, based on a set of prepared questions. The age at which Marfan syndrome was discovered ranged from 3 to 60 years. Several patients lost immediate family members prior to diagnosis, probably due to the symptoms of Marfan syndrome. The rapidity of diagnosis varied widely, and seemed to particularly depend on the familiarity with Marfan syndrome of the attending physician. There is room for improvement regarding the familiarity of the medical profession with Marfan syndrome.

The following points were discussed:

Communication and Marfan syndrome – have all the implications been clearly explained?
All participants expressed dissatisfaction with this aspect. The consequences for cardiology were clearly explained, and eye defects were explained briefly. However, the other abnormalities such as joint/muscle complaints, dural ectasia, lung disorders and implications for daily life received little or no explanation. The bulk of their knowledge was derived from the patient group website and from conversations with others with Marfan syndrome.

Have you heard about the do's and don'ts of Marfan syndrome?
Some patients received no advice, others were told that they should avoid strenuous exercise and endurance sports. They all indicated that they have received some information regarding the theoretical risks, but feel that the translation into practical advice for everyday life is lacking. However, the individual circumstances of the patient should not be overlooked, and individual limitations will vary from person to person.

What is your experience of the organisation of care and the collaboration between disciplines?
The patients were satisfied with the Marfan clinics in the Netherlands, and also preferred to be treated there. They did feel the lack of one central individual. Specialists give conflicting advice on what to do or what not to do.

How was the transition from paediatrician to several specialists in adulthood experienced?
The transition from the safe environment of a children's clinic to the anonymous,

impersonal environment of the adult clinic is a huge step. In the children's clinic, you are called in as necessary, but as an adult you have to suddenly do everything yourself. They feel the lack of a transitional period in which children are guided towards independence as a patient, and they would like to see transition nurses integrated into the Marfan clinic.

What are the typical symptoms in an older Marfan patient?
The distinction between age-related and Marfan-related ailments is currently unclear to both doctors and patients. This is an issue that they would like to see researched.

What are the good points of care?
Everyone is very satisfied with the children's clinics.

What are the main areas for improvement?
(Parents of) patients would also like to receive a copy of the letter sent to the GP following a visit to a specialist.
The cooperation between the specialists would be improved by appointing a single coordinator, generating the impression that there is an overview of all problems.
Some patients see a different ophthalmologist and cardiologist each time. Why can't this just be the same each time?
Greater attention should be paid to complaints relating to body regions/organs other than the heart, so that the patient can be referred to another specialist, if necessary. Complaints are discussed but do not lead to sufficient action or are not taken seriously.
There is too little preparation prior to a consultation, even by interns. Perhaps doctors could prepare a summary of all important information from the medical files.
Just as in the children's clinic, adults also prefer that all investigations take place on the same day, as much as possible.

Are there any questions/points that have not been addressed?
The partners of Marfan patients play an important role in the aftercare, but now are not sufficiently involved. If the partner is present during a consultation, he or she can also help ensure that all necessary information is available, both during and after the consultation.

<u>Improvements at a glance:</u>

- Improved information on what Marfan syndrome entails; the do's and don'ts are not communicated consistently.
- One central coordinator, above all specialists, is highly desirable.
- Preferably - only treatment by doctors in a Marfan (outpatient) clinic.

- The transition from child to adult care is too abrupt. A transition nurse is necessary to smooth this transition.
- Patient's partners should also be involved in the aftercare.
- Improved syndrome recognition by 'regular' doctors; for example, via a magazine read by many doctors.

Appendix 4 On behalf of - Financial support - Acknowledgements

On behalf of

The guidelines were developed on behalf of the Dutch Society of Paediatrics, Dutch Ophthalmological Society, Netherlands Association for Cardio-Thoracic Surgery, Dutch Society of Obstetrics and Gynaecology, Dutch Orthopaedic Association, Dutch Society of Clinical Genetic Diagnostic Laboratories and Netherlands Society of Cardiology.
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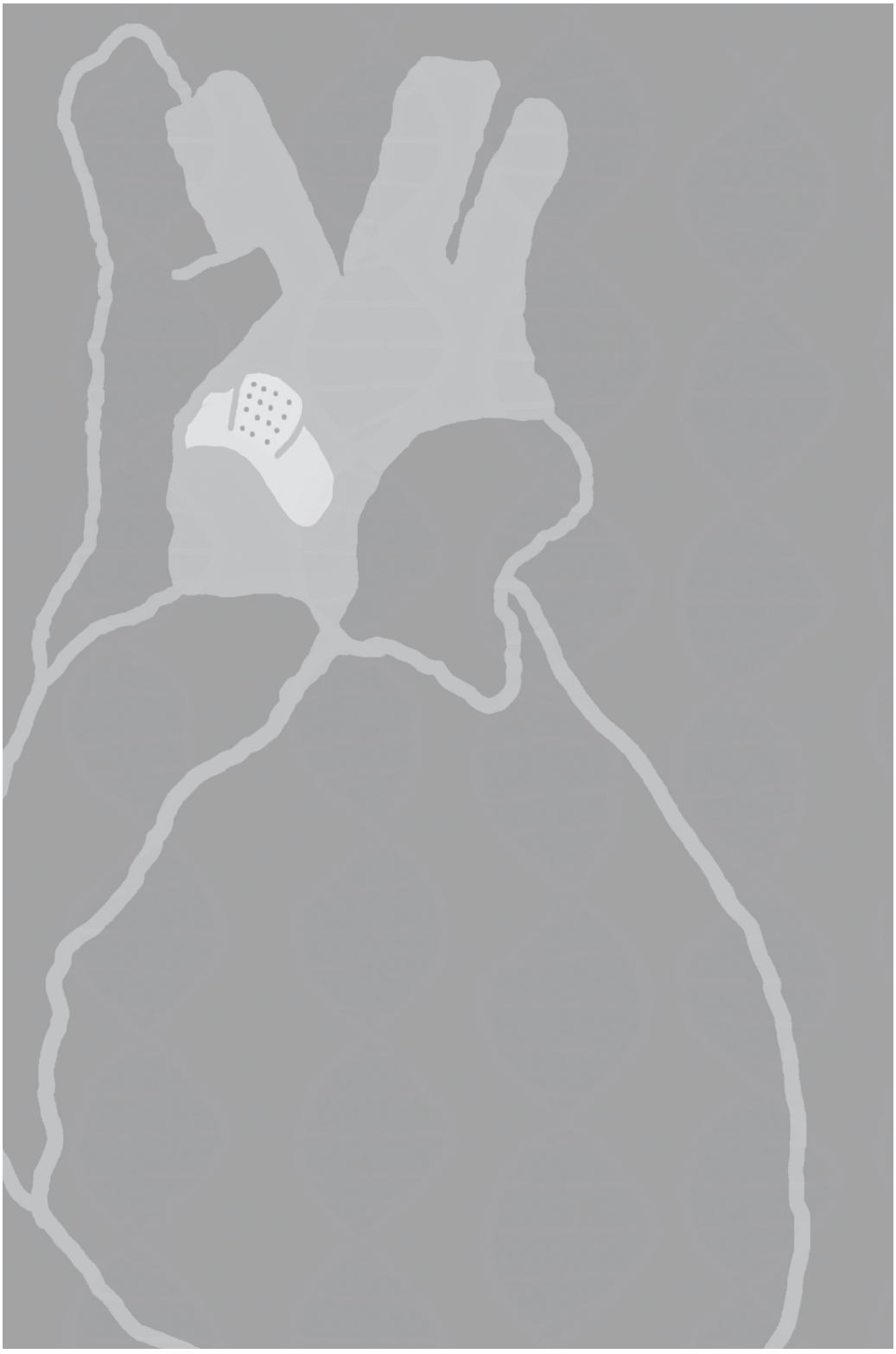

3

The revised Ghent nosology for the Marfan syndrome

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Abstract

The diagnosis of Marfan syndrome (MFS) relies on defined clinical criteria (Ghent nosology), outlined by international expert opinion to facilitate accurate recognition of this genetic aneurysm syndrome and to improve patient management and counselling. These Ghent criteria, comprising a set of major and minor manifestations in different body systems, have proven to work well since with improving molecular techniques, confirmation of the diagnosis is possible in over 95% of patients. However, concerns with the current nosology are that some of the diagnostic criteria have not been sufficiently validated, or are not applicable in children or necessitate expensive and specialised investigations. The recognition of variable clinical expression and recently extended differential diagnosis are further confounding accurate diagnostic decision making. Moreover, the diagnosis of MFS - whether or not established correctly- can be stigmatising, hamper career aspirations, restrict life-insurance opportunities and cause psychosocial burden.

An international expert panel has established a revised Ghent nosology, which puts more weight on the cardiovascular manifestations and in which aortic root aneurym and ectopia lentis are the cardinal clinical features. In absence of any family history, the presence of these two manifestations is sufficient for the unequivocal diagnosis of MFS. In absence of any of these two, the presence of a bonafide *FBN1* mutation or a combination of systemic manifestations is required. For the latter a new scoring system has been designed. In this revised nosology, *FBN1* testing, although not mandatory, has greater weight in the diagnostic assessment. Special considerations are given to the diagnosis of MFS in children and alternative diagnoses in adults.

We anticipate that these new guidelines may delay a definitive diagnosis of MFS but will decrease the risk of premature or misdiagnosis and facilitate worldwide discussion of risk and follow-up/management guidelines.

Introduction

Since Antoine-Bernard Marfan described the five-year-old Gabrielle with skeletal manifestations of the disease that now bears his name [1], important progress has been made in the delineation of the Marfan syndrome (MFS) and recognition of associated risks. The main features of this autosomal dominant disorder include disproportionate long bone overgrowth, ectopia lentis and aortic root aneurysm. In 1955, Victor McKusick first established a classification of connective tissue disorders, which resulted in the publication of his monograph 'Heritable Connective Tissue Disorders' [2,3]. In 1986, an international panel of experts defined a set of clinical criteria (Berlin nosology) for the diagnosis of Marfan syndrome (MFS) [4] with the aim of facilitating accurate communication about the condition between healthcare providers, researchers and patients. It was felt that this would improve proper patient management and effective patient counselling.

Following the identification of *FBN1* (encoding fibrillin-1) as the causal gene for MFS [5] it was recognised that the Berlin criteria falsely allowed a diagnosis of MFS in individuals with a positive family history of MFS, who had only nonspecific connective tissue findings themselves and who did not carry the mutation present in more typically affected family members. New diagnostic criteria were therefore put forth in 1996, referred to as the Ghent nosology [6]. These Ghent criteria were more stringent than the Berlin criteria, mitigating over-diagnosis of MFS and providing better guidelines to differentiate MFS from related, 'overlapping' conditions such as the MASS phenotype (myopia, mitral valve prolapse, borderline and non-progressive aortic root dilatation, skeletal findings and striae) and mitral valve prolapse syndrome (MVPS).

Since physicians associate the diagnosis of 'Marfan syndrome', above all else, with risk for aortic aneurysm/dissection, it can be detrimental to diagnose MFS in patients without tangible evidence of such risk. Avoidable consequences associated with misdiagnosis of MFS include: restriction of career aspirations or access to insurance benefits; additional financial burden associated with frequent medical care; anxiety or situational depression; unfounded marital or reproductive decisions; loss of health benefits or psychosocial stigmatisation associated with exercise restriction, a particularly important issue during childhood. The challenge is to balance such concerns with the paramount need to maintain good health through proper counselling and application of sound anticipatory medical practices. Toward this objective, it is also important to avoid the diagnosis of MFS when clinical or molecular observations could reveal alternative (and often more severe) diagnoses that mandate specialised counselling or management protocols.

The Ghent nosology employs a set of 'major' and 'minor' manifestations in numerous tissues including the skeletal, ocular, cardiovascular, and pulmonary systems and the dura,

skin and integument [6]. Major manifestations include ectopia lentis, aortic root dilatation/dissection, dural ectasia or a combination of ≥ 4 out of eight major skeletal criteria. The diagnosis of MFS in an index patient requires major involvement of at least two organ systems with minor involvement of a third organ system. In the presence of an mutation known to cause MFS or a first-degree relative who was unequivocally diagnosed based upon Ghent nosology, the presence of one major and one minor manifestation in different organ systems is sufficient to make the diagnosis.

Current status of the Ghent nosology

The Ghent criteria have found worldwide application in helping physicians to diagnose MFS appropriately. New molecular techniques allow the detection of *FBN1* mutations in up to 97% of Marfan patients who fulfill the Ghent criteria [7, 8]. This suggests that the current Ghent criteria have excellent specificity to identify patients with *FBN1* mutations. Consideration of sensitivity is highly complex due to varying definitions of the 'target' population and competing clinical priorities. For example, the current criteria have been criticised for taking insufficient account of the age-dependent nature of some clinical manifestations (making the diagnosis in children more difficult) [9] and for including some rather nonspecific physical manifestations or poorly validated diagnostic thresholds. Although the assignment of major and minor criteria within the Ghent nosology has contributed to its utility, several of those criteria are not intuitive when considered from the perspective of the differential diagnosis or patient management. Consideration of the diagnosis of familial ectopia lentis is particularly illustrative of the prevailing issues. This diagnostic category has been widely applied for individuals and families that show lens dislocation and skeletal features of MFS but do not show aortic enlargement or dissection. *FBN1* mutations are seen in familial ectopia lentis and are not easily distinguished from those causing MFS on the basis of character or location within the gene – suggesting either occult phenotype-genotype correlations or the influence of modifiers.

The Ghent nosology clearly attempted to accommodate the fact that some people with ectopia lentis, skeletal findings and even *FBN1* mutation have less cardiovascular risk (i.e. risk to the aortic root) than seen in classic MFS, by allowing the diagnosis of familial ectopia lentis in the absence of a second major Marfan manifestation. However, inadequate data were available to evaluate the critical issue of whether cardiovascular risk could be predicted by the presence of non-cardiac features, such as dural ectasia or major versus minor skeletal involvement. At the other extreme, is it justified not to diagnose MFS in someone with typical lens dislocation and aortic root enlargement simply because they lack minor skeletal or skin findings? To address some of these issues, an international panel (see acknowledgement) of experts in the diagnosis and management of MFS was

convened in Brussels, Belgium by the National Marfan Foundation (USA) and charged with considering modifications to the Ghent criteria. Other factors under consideration included the specialised nature, availability and cost of diagnostic tests for selected manifestations (eg, dural ectasia), the need to better define certain diagnostic categories better (eg, familial ectopia lentis, MASS phenotype[10] and MVPS), to define features that should trigger alternative diagnoses and a desire to complement diagnostic criteria with follow-up and management guidelines for various patient groups including children who do not yet fulfill the diagnostic criteria but may do so in the future.

Proposal for New Nosology

This proposal for a revised nosology (box 1) was based on critical review of clinical characteristics in large published patient cohorts [7, 8, 11, 12], and expert opinions of the panel members with extensive experience in applying the current criteria, the differential diagnosis of MFS and the strengths and limitations of molecular genetic testing. Several guiding principles were followed: maximal use of evidence-based decision making; attention to practical (patient centric) implications; a focus on features and criteria that distinguish MFS from other disorders and definition of purposeful thresholds for diagnosis. As a result, five major changes in the diagnostic guidelines are proposed.

First, more weight is given to two cardinal features of MFS, aortic root aneurysm/dissection and ectopia lentis. In the absence of findings that are not expected in MFS, the combination of ectopia lentis and aortic root enlargement/dissection should be sufficient to make the diagnosis. All other cardiovascular and ocular manifestations of MFS and findings in other organ systems, such as the skeleton, dura, skin and lungs, contribute to a 'systemic score' (box 2) that guides diagnosis when aortic disease is present but ectopia lentis is not.

Second, a more prominent role is assigned to molecular genetic testing of *FBN1* and other relevant genes (eg, *TGFBR1 and 2*), as well as other genes indicated in table 1. In practice, this does not make *FBN1* testing a formal requirement (which imposes financial burden in some countries, and does not yet have 100% sensitivity and specificity), but allows its appropriate use when available.

Third, some of the less specific manifestations of MFS were either removed or made less influential in the diagnostic evaluation of patients. This avoids the use of obligate thresholds that lack clear validation or general availability.

Fourth, the new criteria formalise the concept that additional diagnostic considerations and testing are required if a patient has sufficient findings to satisfy the criteria for MFS but also shows unexpected findings, particularly if they segregate with disease in the family or if they are suggestive of a specific alternative diagnosis. Particular emphasis

is placed on Sphrintzen-Goldberg syndrome (SGS), Loeys-Dietz syndrome (LDS) and the vascular form of Ehlers-Danlos syndrome (vEDS). SGS and LDS have substantial overlap with MFS, including the potential for similar involvement of the skeleton, aortic root, skin and dura (table 1). Occasionally, vEDS can show overlap in the vascular system, dura, skin and skeleton. It is essential to consider discriminating features (table 1) because each of these conditions has a unique risk profile and management protocol.

Finally, this nosology should help to allay concerns regarding delayed or ambiguous diagnoses by providing context specific recommendations for patient counselling and follow-up.

Box 1 Revised Ghent criteria for Diagnosis of Marfan syndrome and Related conditions

In the absence of family history:
(1) Ao (Z≥2) AND EL = MFS*
(2) Ao (Z≥2) AND *FBN1* = MFS
(3) Ao (Z≥2) AND Syst (≥7pts) = MFS*
(4) EL AND FBN1 with known Ao = MFS

EL with or without Syst AND with an *FBN1* not known with Ao or no *FBN1* = ELS
Ao (Z< 2) AND Syst (≥5 with at least one skeletal feature)without EL = MASS
MVP AND Ao (Z<2) AND Syst (<5) without EL = MVPS

In the presence of family history:
(5) EL AND FH of MFS (as defined above) = MFS
(6) Syst (≥7 pts) AND FH of MFS (as defined above) = MFS*
(7) Ao (Z≥2 above 20 yrs old, ≥3 below 20 yrs) + FH of MFS (as defined above) = MFS*

* Caveat: without discriminating features of SGS, LDS or vEDS (as defined in table 1) AND after *TGFBR1/2*, collagen biochemistry, *COL3A1* testing if indicated. Other conditions/genes will emerge with time
Ao, aortic diameter at the sinuses of valsalva above indicated Z-score or aortic root dissection; EL, ectopia lentis; ELS, ectopia lentis syndrome; *FBN1*, fibrillin-1 mutation (as defined in box 3); *FBN1* not known with Ao, *FBN1* mutation that has not previously been associated aortic root aneurysm/dissection; *FBN1* with known Ao, *FBN1* mutation that has been identified in an individual with aortic aneurysm; MASS,
myopia, mitral valve prolapse, borderline (Z<2) aortic root dilatation, striae, skeletal findings phenotype; MFS, Marfan syndrome; MVPS = mitral valve prolapse syndrome; Syst, systemic score (see box 2); Z, Z-score.

Box 2 Scoring of systemic features

- Wrist AND thumb sign – 3 (Wrist OR thumb sign – 1)
- Pectus carinatum deformity – 2 (pectus excavatum or chest asymmetry – 1)
- Hindfoot deformity – 2 (plain pes planus – 1)
- Pneumothorax – 2
- Dural ectasia – 2
- Protrusio acetabuli – 2
- Reduced US/LS AND increased arm/height AND no severe scoliosis – 1
- Scoliosis or thoracolumbar kyphosis – 1
- Reduced elbow extension – 1
- Facial features (3/5) – 1 (dolichocephaly, enophtalmos, downslanting palpebral fissures, malar hypoplasia, retrognathia)
- Skin striae – 1
- Myopia > 3 diopters – 1
- Mitral valve prolapse (all types) – 1

Maximum total: 20 points; score ≥7 indicates systemic involvement; US/LS, upper segment/lower segment ratio.

In the revised nosology, new diagnostic criteria have been defined for a sporadic patient and for an index patient with a positive family history (box 1). In the absence of a conclusive family history of MFS, the diagnosis can be established in four distinct scenarios:

1. The presence of aortic root dilatation (Z-score ≥2 when standardised to age and body size) or dissection [13] and ectopia lentis allows the unequivocal diagnosis of MFS, irrespective of the presence or absence of systemic features except where these are indicative of SGS, LDS or vEDS (table 1).
2. The presence of aortic root dilatation (Z≥2) or dissection and the identification of a bona fide *FBN1* mutation (box 3) are sufficient to establish the diagnosis even when ectopia lentis is absent. An overview of criteria that enhance confidence in the pathogenetic potential for MFS of particular *FBN1* mutations is provided in box 3. These include missense mutations that substitute or create cysteine residues, alter one of the conserved residues important for calcium binding in epidermal growth factor-like (EGF) domains, create a premature termination codon (nonsense mutations), delete or insert coding sequence, or disrupt the consensus sequence for pre-mRNA splicing. Evidence for pathogenicity of other types of missense mutations would include its absence in at least 400 ethnically-matched control chromosomes and co-segregation with disease in the family, or de novo occurrence in a sporadic case (with confirmation of paternity).

Definitive evidence of linkage to a predisposing *FBN1* haplotype can substitute for an *FBN1* mutation for diagnostic purposes but this linkage analysis requires at least six informative meioses in the patient's family to confirm the MFS-associated *FBN1* allele. The absence of a mutation in the *FBN1* gene despite complete screening is possible in MFS.

Box 3 Criteria for causal *FBN1* mutation

- Mutation previously shown to segregate in Marfan family
- De novo (with proven paternity and absence of disease in parents) mutation (one of the five following categories)
- Nonsense mutation
- Inframe and out of frame deletion/insertion
- Splice site mutations affecting canonical splice sequence or shown to alter splicing on mRNA/cDNA level
- Missense affecting/creating cysteine residues
- Missense affecting conserved residues of the EGF consensus sequence ((D/N)X(D/N)(E/Q)Xm(D/N)Xn(Y/F) with m and n representing variable number of residues; D aspartic acid, N asparagine, E glutamic acid, Q glutamine, Y tyrosine, F phenylalanine)
- Other missense mutations: segregation in family if possible + absence in 400 ethnically matched control chromosomes, if no family history absence in 400 ethnically matched control chromosomes
- Linkage of haplotype for n≥6 meioses to the *FBN1* locus

3. Where aortic root dilatation (Z≥2) or dissection is present but ectopia lentis is absent and the *FBN1* status is either unknown or negative, a MFS diagnosis is confirmed by the presence of sufficient systemic findings (≥7 points, according to a new scoring system) (box 2) confirms the diagnosis. However, features suggestive of SGS, LDS or vEDS must be excluded and appropriate alternative genetic testing *(TGFBR1/2*, collagen biochemistry, *COL3A1*, and other relevant genetic testing when indicated and available upon the discovery of other genes) should be performed.
4. In the presence of ectopia lentis but absence of aortic root dilatation/dissection, the identification of an *FBN1* mutation previously associated with aortic disease is required before making the diagnosis of MFS. If the *FBN1* mutation is not unequivocally associated with cardiovascular disease in either a related or unrelated proband, the patient should be classified as 'ectopia lentis syndrome' (see differential diagnosis).

In an individual with a positive family history of MFS (where a family member has been independently diagnosed using the above criteria), the diagnosis can be established in the presence of ectopia lentis, or a systemic score ≥7 points or aortic root dilatation with Z≥2 in adults (≥ 20 years old) or Z≥3 in individuals <20 years old.

Special consideration should be given to young individuals (<20 years old). In sporadic cases, these children may not fit in one of the four proposed scenarios. If insufficient systemic features (<7) and/or borderline aortic root measurements (Z<3) are present (without *FBN1* mutation), we suggest to use the term 'non-specific connective tissue disorder' until follow-up echocardiographic evaluation shows aortic root dilation (Z≥3). If an *FBN1* mutation is identified in sporadic or familial cases but aortic root measurements are still below Z=3, we propose to use the term 'potential MFS' until the aorta reaches threshold. Neonatal MFS is not considered as a separate category, but rather represents the severe end of the MFS spectrum.

In adults (>20 yrs), we define three main categories of alternative diagnoses: ectopia lentis syndrome (ELS), MASS phenotype (myopia, mitral valve prolapse, aorta, skin and skeletal findings), mitral valve prolapse syndrome (MVPS) (see differential diagnosis).

Finally, we recognise that some patients will remain difficult to classify due to overlap of phenotypes from different entities, the evolving nature of these connective tissue diseases, absence of mutation after screening of the appropriate genes or divergence between the phenotype and the genotype. However, these patients should be uncommon and will hopefully benefit from better definition of still unrecognised phenotypes in the future.

Organ system specific considerations

Cardiovascular Criteria

A key diagnostic criterion in the new nosology is aortic root aneurysm or dissection. Aortic root aneurysm is defined as enlargement of the aortic root at the level of the sinuses of Valsalva. Aortic root measurements should be done parallel to the plane of the aortic valve and perpendicular to the axis of blood flow. The largest correctly measured root diameter obtained from at least three transthoracic images should be corrected for age and body size and interpreted as a Z-score. There are varying practices regarding whether root measurements should be done in systole or diastole and whether the thickness of one aortic wall should be included (ie, the leading edge to leading edge method). The method employed must match that used to generate the normative data for Z-scores to be valid. For echocardiographic measurements made from inner wall to inner wall during systole in individuals ≤25 years, a convenient Z-score calculator can be found at www.marfan.org. For echocardiographic measurements made from leading edge to leading edge in diastole in all age groups, reference graphs and Z-score equations are available [13] . If transthoracic

echocardiographic evaluations do not allow precise visualisation of the proximal aorta, transoesophageal echocardiography or CT or MRI imaging should be applied, with special attention to using double-oblique images to obtain correct diameter measurements and use of the same nomograms [14].

Mitral valve prolapse is also a common finding in MFS and is included as a feature in the systemic score. Mitral valve prolapse should be defined by echocardiography as protrusion of one or both of the mitral valve leaflets across the plane of the mitral annulus during systole. This is best detected in parasternal long axis or apical long axis three-chamber or two-chamber views. There are no special criteria for diagnosing MVP in MFS and standard practices should be applied [15].

Pulmonary artery (PA) dilation (eg, main PA diameter >23 mm in adults) [16] is often seen in MFS, but it is not specific to this diagnosis. In addition, complications of pulmonary artery disease occur rarely. PA dilation was not therefore included in the systemic score because further research is needed regarding thresholds and the diagnostic utility of this finding.

Patients with MFS can develop aortic enlargement or dissection at segments distant from the aortic root. The frequency of this finding (particularly at the proximal descending thoracic aorta and in the abdomen) appears to be increasing with the prolonged survival due to improved management of disease at the aortic root. While descending aortic aneurysm or dissection in the absence of aortic root enlargement can occur in Marfan syndrome, [17, 18] this is rare and given the low specificity of this finding for MFS, this finding is not included in the diagnostic criteria. Intermittent imaging of the descending thoracic aorta is indicated in adult patients where there is a clinical suspicion of Marfan syndrome in the absence of aortic root enlargement. Widespread vascular disease is more common with other conditions in the differential diagnosis, such as vascular EDS and LDS. For example, systemic vascular imaging (head to pelvis) is recommended if there is a suspicion of LDS because of the high frequency of tortuosity, aneurysms and dissections throughout the vascular tree.

Ocular criteria

The most prominent ocular features of MFS are myopia and ectopia lentis. The diagnosis of ectopia lentis is based on slit-lamp examination after maximal dilatation of the pupil. Ectopia lentis reflects failure of supporting structures called ciliary zonules. Dislocation of the lens in MFS is most typically upward and temporal but is not specific as deviation in any direction may occur. If lens subluxation is deemed equivocal or minimal, manifesting only as a scalloped or ruffled lens margin at extremes of gaze, the eye exam should be repeated later before a definitive diagnosis of ectopia lentis can be made (such findings can occur outside the context of MFS, eg, in individuals with high myopia). Increased globe

length and corneal flattening are seen in MFS, but they have unclear specificity and are not routinely measured by ophthalmologists. Given that myopia is very common in MFS, is routinely monitored, and tends to show early onset, high severity and rapid progression, myopia of > 3 diopters contributes to the systemic score for diagnosis. However, since myopia is quite common finding in the general population we have only attributed one point to it in the systemic score.

Systemic criteria

Clinical manifestations of MFS in other organ systems were critically evaluated for their specificity and diagnostic utility based on expert opinion and the available literature. Several of the 'minor' criteria from the old Ghent nosology were eliminated, but the most selective systemic features were included in the 'systemic score'.

Three points are assigned to the combination of wrist and thumb signs. The thumb sign is positive when the entire distal phalanx of the adducted thumb extends beyond the ulnar border of the palm with or without the assistance of the patient or examiner to achieve maximal adduction. The wrist sign is positive when the tip of the thumb covers the entire fingernail of the fifth finger when wrapped around the contralateral wrist. If either of the two signs is absent, only one point is assigned.

Two points were assigned to each of five other specific systemic manifestations including anterior chest deformity, hindfoot deformity, spontaneous pneumothorax, dural ectasia and acetabular protrusion. Pectus carinatum is believed to be more specific for MFS than pectus excavatum and is assigned two points. Subjective qualifiers in the original Ghent criteria such as 'requiring surgery' have been eliminated but the examiner should be confident that a positive finding (pectus excavatum or chest wall asymmetry) extends beyond normal variation of chest contour in the general population before assigning one point. Hindfoot valgus [19] (two points) in combination with forefoot abduction and lowering of the midfoot (previously referred to as medial rotation of the medial malleolus) should be evaluated from anterior and posterior view. The examiner should distinguish this from the more common 'flat foot' (one point) without significant hindfoot valgus. As in the past, any spontaneously-occurring pneumothorax remains a diagnostic feature. For the detection of lumbosacral dural ectasia, no preferred method (CT or MRI) or uniformly accepted cut-offs have emerged from the literature [20-23] and local standards should apply. Dural ectasia is a sensitive but not a specific sign of MFS and, as such, is no longer considered on equal footing with lens dislocation or aortic root enlargement. It is commonly seen in Loeys-Dietz syndrome and has been described in mutation proven vEDS. Finally, an additional technical exam for detection of acetabular protrusion [24] can be helpful but is not mandatory: classical X-ray, CT or MRI can be used. On an X-ray anterior-posterior pelvis

angle, the medial protrusion of the acetabulum above 3 mm beyond the ilio-ischial (Kohler) line is diagnostic. Criteria on CT or MRI are not precisely defined but involve loss of the normal oval shape of the pelvic inlet at the level of the acetabulum.

One point is assigned to eight other manifestations, one cardiovascular (mitral valve prolapse), one ocular (myopia, ≥3 diopters) and six features from other organ systems. These are considered less specific features for MFS and can be observed in other connective tissue disorders or as normal variation in the general population [18].

The combined presence of reduced upper segment to lower segment (US/LS) ratio (for white adults <0.85; <0.78 in black adults; no data have been assessed in Asians) and increased arm span to height ratio (for adults >1.05) in the absence of significant scoliosis contributes one point to the systemic score. In Asians the incidence of an enlarged armspan to height ratio in Marfan patients was noted to be lower [25] and prior studies of Asian (and also Afro-Caribbean) populations demonstrated different distributions of arm span and height, so one should consider these ethnic differences when using cut-off values [26]. For the US/LS ratio in children, abnormal ratios are US/LS < 1 (for age 0-5 yrs), US/LS < 0.95 (for 6-7 yrs), US/LS < 0.9 (8-9 yrs old) and < 0.85 (above age 10 yrs).The lower segment is defined as the distance from the top of the symphysis pubis to the floor in the standing position and the upper segment is the height minus the lower segment. Importantly, neither of these ratios provides an accurate measurement of bone overgrowth in the presence of severe scoliosis or kyphosis. Scoliosis [27] can be diagnosed either clinically if, upon bending forward, a vertical difference of least 1.5 cm between the ribs of the left and right hemithorax is observed or if a Cobb's angle (angle between a line drawn along the superior end plate of the superior end vertebra and a second line drawn along the inferior end plate of the inferior end vertebra of the scoliosis measured on anterior-posterior view of the spine) of at least 20° is seen on radiographs. In the absence of scoliosis, one point can be contributed by the presence of an exaggerated thoracolumbar kyphosis. Elbow extension is considered reduced if the angle between the upper and lower arm measures 170° or less upon full extension. One point can be assigned based upon facial characteristics if the patient shows at least three of the five typical facial characteristics including dolichocephaly, downward slanting palpebral fissures, enophthalmos, retrognathia and malar hypoplasia. Striae atrophicae are considered significant as a diagnostic feature if they are not associated with marked weight changes (or pregnancy) and if they have an uncommon location such as the mid-back, lumbar region, the upper arm, axillary region or thigh.

The following criteria were removed from the current nosology because of lack of perceived specificity: joint hypermobility, highly arched palate, and recurrent or incisional herniae [18].

Differential diagnosis

Several conditions have been recognised which present overlapping clinical manifestations with MFS in the cardiovascular, ocular or skeletal systems. These include conditions with aortic aneurysms (LDS, bicuspid aortic valve, familial thoracic aortic aneurysm, vEDS, arterial tortuosity syndrome), ectopia lentis (ectopia lentis syndrome, Weil-Marchesani syndrome, homocystinuria, Stickler syndrome) or systemic manifestations of MFS (Shprintzen-Goldberg syndrome, congenital contractural arachnodactyly, LDS, MASS phenotype (myopia, mitral valve prolapse, aorta, skin and skeletal findings) and MVPS (mitral valve prolapse syndrome) (table 1).

Table 1. Features of differential diagnosis

Differential diagnosis	Gene	Discriminating features
Loeys-Dietz syndrome (LDS)	*TGFBR1/2*	Bifid uvula/cleft palate, arterial tortuosity, hypertelorism, diffuse aortic and arterial aneurysms, craniosynostosis, club foot, cervical spine instability, thin and velvety skin, easy bruising
Shprintzen-Goldberg syndrome (SGS)	*FBN1* and other	Craniosynostosis, mental retardation
Congenital arachnodactyly syndrome (CCA)	*FBN2*	Crumpled ears, scoliosis, contractures
Weill-Marchesani syndrome (WMS)	*FBN1 and ADAMTS10*	Microspherophakia, brachydactyly, joint stiffness
Ectopia lentis syndrome (ELS)	*FBN1* *LTBP2* *ADAMTSL4*	Lack of aortic root dilatation
Homocystinuria	*CBS*	Thrombosis, mental retardation
Familial thoracic aortic aneurysm syndrome (FTAA) FTAA with bicupid aortic valve (BAV) FTAA with patent ductus arteriosus (PDA)	*TGFBR1/2, ACTA2* *MYH11*	Lack of marfanoid skeletal features, levido reticularis, iris flocculi
Arterial tortuosity syndrome (ATS)	*SLC2A10*	Generalised arterial tortuosity, arterial stenosis, facial dysmorphism
Ehlers-Danlos syndromes (vascular, valvular, kyphoscoliotic type)	*COL3A1, COL1A2, PLOD1*	Middle sized artery aneurysm, valvular insufficiency, translucent skin, dystrophic scars, scoliosis, facial characteristics

Conditions with cardiovascular features of MFS

Historically the terms MASS phenotype and MVPS (mitral valve prolapse syndrome) have been used but several issues about the use of these terms have arisen. First, the definition of the MASS phenotype is not unequivocally applicable as it required at least two, but preferably three of the following manifestations: myopia, mitral valve prolapse, borderline aortic root enlargement, skin and minor skeletal features (insufficient to fulfill the major skeletal criterion of the original Ghent nosology) [6].

This definition indirectly also assumes a non-progressive nature of the aortic root dilatation but it is currently unknown to what proportion of patients this applies. Third, *FBN1* mutations have been found occasionally in MASS phenotype patients [18, 28] but the precise risk for the development of aortic aneurysm and progression for these patients is poorly studied. Analogous to the ectopia lentis syndrome, the spirit of the definition of MASS phenotype aims to avoid the diagnosis of MFS without documented risk for aortic root aneurysm development. The diagnosis of MASS is made in individuals with an aortic root size below Z=2, at least one skeletal feature and a systemic score ≥5. The presence of ectopia lentis precludes this diagnosis. If an *FBN1* mutation is identified in a MASS patient, this patient has the potential to evolve into MFS but it is currently unknown how often and which factors predict this transition over time.

Alternatively, when mitral valve prolapse is present in association with limited systemic features (score < 5), we suggest to use the term mitral valve prolapse syndrome (MVPS). MVPS is a common condition usually inherited in autosomal dominant mode [29] with several candidate gene loci [30] but with evidence for rare X-linked inheritance [31] which affects ~1.5% of the population. In addition to prolapse of the mitral leaflets, MVPS commonly includes pectus excavatum, scoliosis and mild arachnodactyly [32]. However, aortic enlargement and ectopia lentis preclude this diagnosis.

Loeys-Dietz syndrome (LDS) is an autosomal dominant aortic aneurysm syndrome characterised by the triad of hypertelorism, bifid uvula/cleft palate, and/or arterial tortuosity with ascending aortic aneurysm/dissection. It is caused by heterozygous mutations in the genes encoding the type 1 or 2 subunit of the transforming growth factor-β receptor (*TGFBR2* or *TGFBR2*) [33]. Other more variable clinical features that distinguish LDS from MFS include craniosynostosis, Chiari malformation, club foot deformity, congenital heart disease, cervical spine instability, easy bruising, dystrophic scarring, translucent skin and, most importantly, a high risk of aneurysm and dissection throughout the arterial tree. Patients with LDS are not typically inappropriately tall and do not exhibit disproportionally long extremities, although arachnodactyly is observed. Some patients with *TGFBR1/2* mutations lack overt craniofacial features despite an equal or greater severity of vascular or systemic findings. Importantly, the natural history of patients with LDS tends to be more aggressive than those with MFS or vEDS. In LDS, aortic dissections often occur at a younger age or at smaller aortic dimensions (<40 mm) compared to MFS, and the incidence of pregnancy-related complications is particularly high [34]. As with *FBN1* mutations, the phenotype associated with *TGFBR1/2* mutations can be variable, even within families, and can be associated with skeletal features of MFS leading to overlapping phenotypes in the old Ghent nosology [35-37]. In order to avoid persistent ambiguity even under the proposed criteria, molecular testing should be strongly considered because it influences the clinical

management [34]. It has been proposed that patients with *TGFBR1/2* mutations that lack outward discriminating features of LDS should be designated LDS2, highlighting the potential for more aggressive vascular disease than seen in Marfan syndrome (MIM 190181 and 190182).

With a population prevalence of up to 1% [38, 39], bicuspid aortic valve (BAV) is the most common congenital cardiac malformation. A subset of individuals with BAV present with ascending aortic aneurysm, however such patients usually lack ocular or other systemic findings that contribute strongly to MFS diagnosis. Skeletal findings such as pectus deformity and scoliosis can be observed in these families. Bicuspid aortic valve and aortic aneurysm can occur together in some family members but independently in others, indicating that they can be variably penetrant consequences of a common underlying genetic defect [40, 41]. Unlike MFS this condition commonly shows maximal or exclusive dilatation in the ascending aorta above the sinotubular junction [42]. Mutations have been identified in the *NOTCH1* and *KCNJ2* genes, but these account for only a small fraction of BAV patients, who may have prominent valve calcification or associated forms of congenital heart disease. Linkage analysis reveals genetic heterogeneity with putative loci on chromosomes 18q, 5q and 13q [43].

Familial thoracic aortic aneurysm syndrome dissection syndrome (FTAAD) is a clinically and genetically heterogeneous group of disorders where thoracic aortic disease predominates. The age of onset and rate of progression of aortic dilatation is highly variable and conditions that include variable or subtle systemic manifestations of a connective tissue disorder have been included in this designation. It is anticipated that future stratification of patients by genetic etiology will help to refine phenotypic descriptions and inform patient counselling and management. To date, there are five genes and two additional loci [44, 45] associated with FTAAD Mutations have been identified in *FBN1, TGFBR1/2, MYH11*, and *ACTA2*, the latter two encoding components in the smooth muscle cell contractile apparatus. Mutations in *MYH11* associate aortic root aneurysms with patent ductus arteriosus (PDA) [46]. Mutations in *ACTA2*, accounting for up to 16% of FTAAD, associate aortic aneurysm with other variable features including iris flocculi, livedo reticularis, cerebral aneurysm, bicuspid aortic valve and PDA [47]. In addition to thoracic aortic aneurysms and dissections, patients with *ACTA2* mutations can present with vascular disease in the cerebrovascular system (premature ischemic strokes, Moyamoya disease and cerebral aneurysms) or premature coronary artery disease [48]

The vascular type of EDS (previously EDS IV), is caused by mutations in *COL3A1*, the gene encoding type III collagen; it is characterized by vascular and tissue fragility. Cardinal features distinguishing vEDS from MFS include translucent skin, easy bruising, dystrophic scarring and a tendency for intestinal or uterine rupture. Typically, dissection or rupture occurs in

medium sized arteries in vEDS although aortic involvement is sometimes observed. There is no particular predisposition at the aortic root. About half of the aneurysms/dissections occur in thoracic or abdominal branch arteries; arteries of the head, neck and limbs are less frequently involved [49].

Three other rare types of Ehlers-Danlos syndrome have been associated with vascular problems. The kyphoscoliotic type (previously type VI EDS) is characterised by kyphoscoliosis, joint laxity, and muscle hypotonia. This autosomal recessive condition is caused by defects in the enzymatic activity of lysyl hydroxylase, encoded by the *PLOD1* gene. Aortic dilation/dissection and rupture of medium-sized arteries have been observed [50]. Patients with the so-called 'cardiac valvular subtype of EDS', which associates severe cardiac valvular problems and features of the classic type of EDS (atrophic scars, skin hyperelasticiy and joint hypermobility), were found to have a complete deficiency of the proα2-chain of type I collagen (*COL1A2*) [51]. Most recently, patients with arginine to cysteine substitutions in the proα1-chain of type I collagen (*COL1A1*) displayed classic EDS but evolved to a vascular EDS-like phenotype later in life, with increased risk for spontaneous arterial rupture, most prominently affecting the femoral and iliac arteries [52].

Arterial tortuosity syndrome (ATS) is a rare autosomal recessive connective tissue disorder, characterised by severe tortuosity, stenosis, and aneurysms of the aorta and medium-sized arteries [53]. Skeletal and skin involvement is common. The underlying genetic defect is homozygosity or compound heterozygosity for loss-of-function mutations in *SLC2A10*, the gene encoding the facilitative glucose transporter GLUT10 [54]. The condition is lethal in infancy in a subset of patients but some survive into adulthood and seem to do well [55].

Conditions with ectopia lentis

Patients with familial ectopia lentis typically have some skeletal features of MFS and an *FBN1* mutation. While lack of aortic disease is a defining feature of this condition, it may be difficult to distinguish from emerging MFS in the absence of other affected family members or at a young age. Even within extended pedigrees with familial ectopia lentis, later onset aortic aneurysm may be observed. In order to highlight the systemic nature of this condition better and to emphasise the need for assessment of features outside the ocular system, we propose the designation ectopia lentis syndrome (ELS). The presence of a personal or family history of aortic aneurysm or the identification of an *FBN1* mutation previously associated with aortic aneurysm would be sufficient to transition the diagnosis to MFS, independently of the number or distribution of systemic features. To ensure that adequate vigilance of other organ systems is maintained, the diagnosis of ELS cannot be formally invoked before the age of 20 years. The disorder is genetically heterogeneous,

with autosomal dominant inheritance caused by *FBN1* mutations [56] and recessive forms caused by *LTBP2* and *ADAMTSL4* mutations [57, 58]. Importantly, in ELS patients with *FBN1* mutations, cardiovascular follow-up by imaging should be maintained throughout life.

Ectopia lentis can be present as a component of other rare conditions. Ectopia lentis et pupillae is an autosomal recessive condition in which remnants of the pupillary membrane are present. However, it is not associated with cardiovascular or skeletal features of MFS.

In contrast to Weill-Marchesani syndrome (WMS), the lens dislocation is typically associated with microspherophakia (small, rounded and thickened crystalline lens) and a shallow anterior eye chamber. WMS patients are short with brachydactyly and joint stiffness. Both autosomal dominant and recessive forms of WMS have been described and are caused by *FBN1* mutations [59, 60] and mutations in the *ADAMTS10* gene [61], respectively. Homocystinuria is often easily differentiated from MFS by the presence of mental retardation and thrombosis, and can be excluded by urine amino acid analysis in the absence of pyridoxine supplementation. In homocystinuria, the lens usually dislocates downward due to complete loss of support by ciliary zonules. In Stickler syndrome, patients can present with a Marfanoid habitus. Typical ocular signs include retinal detachment, myopia and open-angle glaucoma. Early cataracts are common, but lens subluxation is not. Other potential discriminating features from MFS include cleft palate, hearing loss and epiphyseal changes of the bones.

Conditions with overlapping systemic features

Shprintzen-Goldberg syndrome (SGS) is a rare craniosynostosis syndrome characterised by some of the systemic features found in MFS (pectus abnormalities, scoliosis, arachnodactyly), craniofacial dysmorphism (exophtalmos, hypertelorism, downslanting palpebral fissures, maxillary and mandibular hypoplasia, high arched palate and low set ears) and developmental delay. So far, only two SGS patients have shown an *FBN1* mutation [62, 63]. Another patient reported by Kosaki et al. [63] as SGS was felt to have LDS based on arterial tortuosity and the presence of a bifid uvula [64]. Other important distinguishing features between SGS and either LDS or MFS are the high incidence of cognitive impairment and the low frequency of vascular disease in the former.

Congenital contractural arachnodactyly (CCA) is an autosomal dominant disorder characterised by a Marfan-like body habitus and arachnodactyly [65]. Most affected individuals have 'crumpled' ears that present as a folded upper helix and contractures of major joints (knees and ankles) at birth. The proximal interphalangeal joints of the fingers and toes have flexion contractures (camptodactyly). Kyphosis/scoliosis is present in about half of affected individuals. Mild enlargement of the sinuses of Valsalva has been reported, but there is no evidence that the aortic dilatation progresses to dissection or rupture [66].

CCA is caused by mutations in the *FBN2,* gene encoding the extracellular matrix microfibril fibrillin-2 [67].

Management

Management guidelines for MFS patients

Aortic root dilatation in MFS is usually progressive. Therefore absence of aortic root enlargement on initial clinical examination does not necessarily exclude the diagnosis, even in adulthood.

All individuals who meet the criteria for MFS should initially have at least yearly echocardiograms. More frequent imaging should be performed if the aortic diameter is approaching a surgical threshold (≥4.5cm in adults; less well defined in children) or shows rapid change (≥0.5 cm/year) or with concerns regarding heart or valve function. Individuals under age 20 with systemic findings suggestive of MFS but no cardiovascular involvement should have annual echocardiograms due to the potential for rapid evolution of the phenotype. Adults with repeatedly normal aortic root measurements can be seen at intervals of 2-3 years.

Although several alternative medical treatments have been proposed (angiotensin converting enzyme (ACE) inhibitors, calcium channel antagonists), the standard of care in most centers for the prevention of aortic complications in MFS remains β-blockade [68]. More data are required before ACE inhibitor therapy can be considered standard treatment [69]. β-blockade should be considered in all patients with MFS, including children and those with aortic root diameters of < 4 cm, unless contraindicated. The β-blocker should be titrated to maximum effect, aimed at a heart rate after submaximal exercise (eg, running up and down two flights of stairs) < 100 beats/ min in individuals over 5 years of age. Angiotensin receptor blockers (ARBs) have shown the ability to prevent aortic enlargement in a mouse model of Marfan syndrome [70], and encouraging results were observed in a pilot experience in children with severe MFS [71]. Several multicenter trials of losartan versus or on top of atenolol in MFS are currently underway [72]. If β-blockers are contraindicated or not tolerated, other classes of antihypertensive agents can be used, but there is not definitive evidence that they will afford protection in people with MFS.

Management of acute dissection of the ascending aorta (type A dissection) is emergency surgery. Consideration of prophylactic surgery is recommended when the diameter at the sinuses of Valsalva approaches 5.0 cm. Other factors that inform the timing of surgery include a family history of early dissection, the rate of aortic root growth, the severity of aortic valve regurgitation, associated mitral valve disease, ventricular dysfunction, pregnancy planning in women, and the desire for a valve sparing operation.

Type B dissection (originating in the thoracic descending aorta) accounts for about 10%

of all dissections in MFS. Possible indications for surgery include intractable pain, limb or organ ischemia, an aortic diameter exceeding 5.5 cm or a rapid increase in the aortic diameter. Open surgery is still preferred as experience with intravascular stenting in MFS is very limited, and the pressure endovascular stents need to apply against the wall of adjacent normal sized aortic segments to remain well seated may not be tolerated by weakened connective tissue, or the adjacent aorta may also be dilated. Regular imaging of the entire aorta is encouraged after root surgery and in adulthood.

Mitral valve repair or replacement is advised for severe mitral valve regurgitation with associated symptoms or progressive left ventricular dilatation or dysfunction. Repair should be considered, especially in patients undergoing aortic valve-sparing root replacement. If a mechanical aortic valve prosthesis is chosen, mitral valve replacement may be considered, although preservation of left ventricular function may be better with mitral valve repair. After isolated mitral valve repair, one should carefully monitor aortic root size as increased rates of enlargement have been observed.

Decisions regarding exercise restriction should always be made on an individual basis. Recommendations from the National Marfan Foundation (www.marfan.org) and guidelines from the American Heart Association/American College of Cardiology task forces [73] are useful templates. In general, patients with MFS should avoid contact sports, exercise to exhaustion and especially isometric activities involving a Valsalva manoeuver. Most patients can and should participate in aerobic activities performed in moderation.

Pregnancy in MFS women is associated with increased cardiovascular risk, with the majority of aortic complications (progressive dilatation and dissection) occurring in the third trimester or in the early postpartum period. The risk of aortic root complication is increased when the aortic root diameter is above 4.0 cm at the start of the pregnancy [74].

Annual ophthalmological evaluation for the detection of ectopia lentis, cataract, glaucoma and retinal detachment is essential. Early monitoring and aggressive refraction is required for children with MFS to prevent amblyopia. Indications for surgical lens extraction include lens opacity with poor visual function, anisometropia or refractive error amenable to optical correction, impending complete luxation and lens-induced glaucoma or uveitis.

Skeletal manifestations such as scoliosis and pectus deformity should be treated according to standard orthopedic management rules.

Management guidelines for related conditions

Regular follow-up including annual cardiovascular imaging and ophthalmological evaluation is advised in MASS, MVPS and ELS to monitor aortic size, and the degree of mitral regurgitation, over time. Counselling for patients with either ELS or MASS phenotype

should include the risk of a more severe presentation in their offspring, including aortic enlargement.

Careful cardiovascular and ophthalmological follow-up is of course strongly indicated in children with potential MFS or non-specific connective tissue disorders.

Conclusion

The diagnostic evaluation for MFS is unavoidably complex due to the highly variable presentation of affected individuals, the age-dependent nature of many of its manifestations, the absence of gold standards and its extensive differential diagnosis. While diagnostic criteria should emphasise simplicity of use and the desire for early diagnosis, accuracy receives highest priority in order to avoid the deleterious and often irreversible consequences of ungrounded or erroneous assignment. While the increased focus on vascular disease for the diagnosis of MFS in this proposal will likely prove controversial, it is responsive to the practical burden faced both by patients and physicians and does not represent a true departure from the spirit of prior diagnostic guidelines. Ongoing concerns about delayed diagnosis and/or the use of diagnostic categories that may prove provisional should be offset by additional discussion of ongoing risk and the definition of follow-up and management principles. A comparative analysis on different retrospective datasets has shown ~90% concordance between the old and revised Ghent nosology. The 10% discordance was generally beneficial by facilitating earlier diagnosis in young children with a convincing clinical phenotype and delayed diagnosis in individuals without clear cardiovascular risk. The current proposal will benefit from a prospective analysis, leading to further refinement. A web based diagnostic tool for the application of these criteria can be accessed at http://www.marfan.org.

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Competing interests None

chapter
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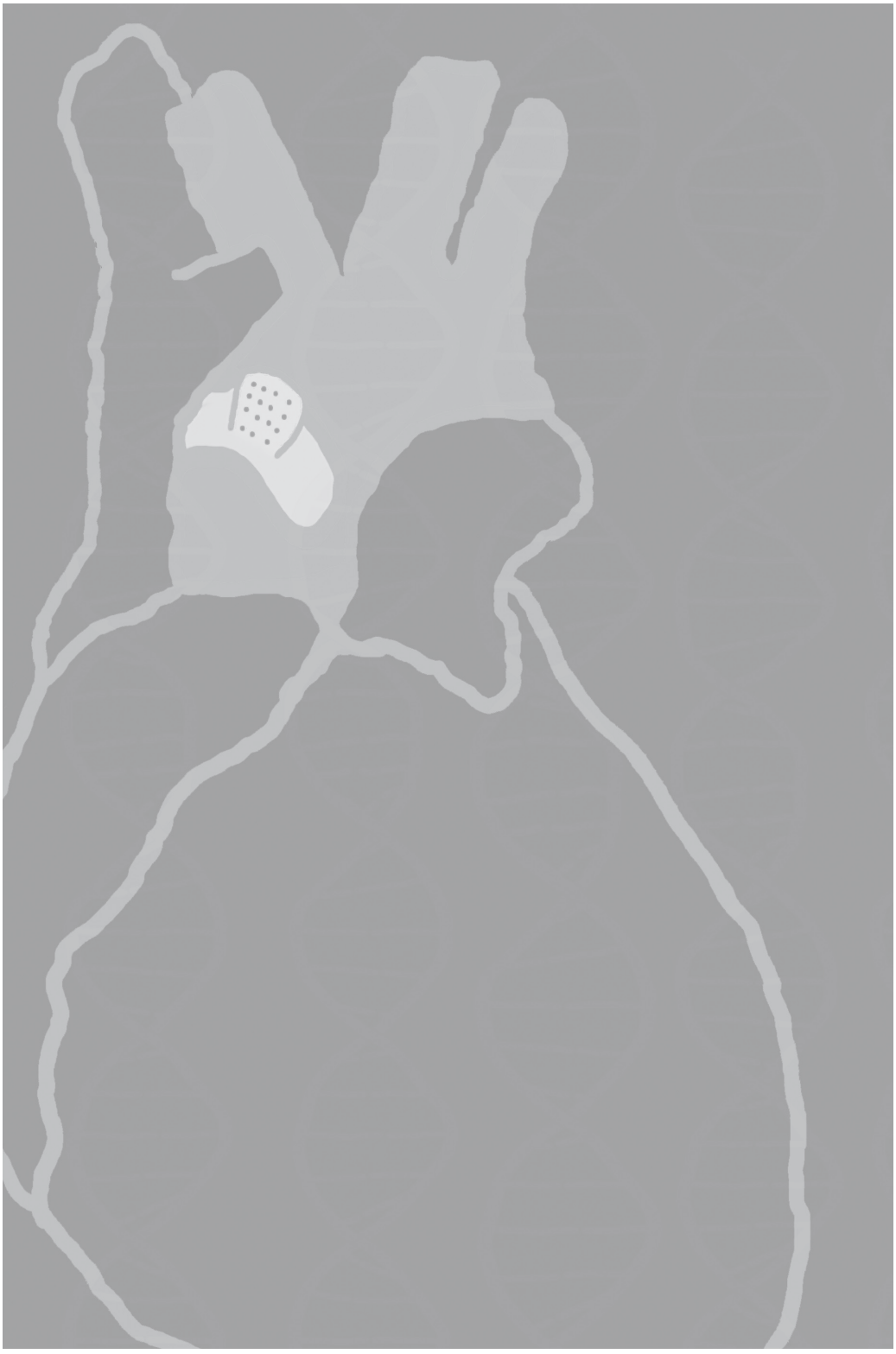

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The Clinical Spectrum of Missense Mutations of the First Aspartic Acid of cbEGF-like Domains in Fibrillin-1 Including a Recessive Family

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Abstract

Marfan syndrome (MFS) is a dominant disorder with a recognizable phenotype. In most patients with the classical phenotype mutations are found in the fibrillin-1 gene (*FBN1*) on chromosome 15q21. It is thought that most mutations act in a dominant negative way or through haploinsufficiency. In 9 index cases referred for MFS we detected heterozygous missense mutations in *FBN1* predicted to substitute the first aspartic acid of different calcium-binding Epidermal Growth Factor-like (cbEGF) fibrillin-1 domains. A similar mutation was found in homozygous state in 3 cases in a large consanguineous family. Heterozygous carriers of this mutation had no major skeletal, cardiovascular or ophthalmological features of MFS. In the literature 14 other heterozygous missense mutations are described leading to the substitution of the first aspartic acid of a cbEGF domain and resulting in a Marfan phenotype. Our data show that the phenotypic effect of aspartic acid substitutions in the first position of a cbEGF domain can range from asymptomatic to a severe neonatal phenotype. The recessive nature with reduced expression of *FBN1* in one of the families suggests a threshold model combined with a mild functional defect of this specific mutation.

KEY WORDS: Marfan syndrome, fibrillin-1, *FBN1* gene, autosomal recessive inheritance, pathogenesis

Introduction

Human fibrillin-1 is a large protein of approximately 350 kD and member of a family of extracellular cysteine-rich glycoproteins. Since 1991 mutations in the fibrillin-1 (*FBN1*) gene have been found to be responsible for Marfan syndrome (MFS; MIM# 134797) (Kainulainen et al., 1990; Dietz et al., 1991). Fibrillin-1 is characterized by a highly conserved modular domain organization. The most prominent domain is the Epidermal Growth Factor-like (EGF) domain present 46 times and containing six highly conserved cysteine residues stabilizing the structure by three disulfide bonds. Of these EGF domains, 43 have a consensus sequence for calcium binding (cb) in the N-terminal pocket of the domain which may mediate protein-protein interactions. The EGF domains are interrupted by seven transforming growth factor (TGF)-binding protein domains characterized by 8 cysteine residues involved in intra-domain disulfide bonds (Pereira et al., 1993; Robinson et al., 2006). In the last update of the Universal Marfan Database – *FBN1* (UMD-*FBN*; http://www.umd.be) (Faivre et al., 2007) 803 different mutations are reported. Most of the mutations are

missense mutations (56%) mainly substituting or creating a cysteine in a cbEGF domain. Other mutations are frameshift mutations, splice mutations and nonsense mutations. About 14% of mutations are recurring.

All cbEGF domains start with a highly conserved aspartic acid, which is crucial for binding of a positively charged Ca^{2+} ion (Whiteman et al., 2007). We have identified 10 index cases with a substitution of the first aspartic acid substitution of a cbEGF domain and reviewed a further 14 published cases. Most of them exhibit a complete MFS phenotype. Surprisingly, in one family the substitution only led to MFS in homozygous state in three family members, whereas 13 family members carrying the heterozygous mutation do not have Marfan syndrome after thorough clinical examination.

There is still a lot of debate how mutations in *FBN1* result in the MFS phenotype, but increasing evidence for different models is emerging. Possible explanations for the observed extreme variation in expression of the substitution of the first aspartic acid of a cbEGF domain are discussed. The observation of recessive inheritance of an expected dominant mutation also underscores the fact that mutations which are predicted to have a pathogenic effect, may not always lead to clinical symptoms.

Patients and methods

Patients

The patients were referred for DNA analysis of the fibrillin-1 gene to confirm the clinical diagnosis of MFS. Case 1, 2, 5, 7, 9 and 10 fulfilled the clinical Ghent criteria (De Paepe A. et al., 1996) for the diagnosis MFS. All index patients fulfilled the Ghent criteria when the finding of a pathogenic mutation in *FBN1* was included.

Case 9 belongs to a large Turkish pedigree (Figure 1, III-1). She was examined at the age of 22 years. At the age of 6 weeks she was operated on a right sided hernia inguinalis. She was diagnosed with bilateral subluxation of the lenses when she was 3 years old. From that time on she has been operated several times for retinal detachments and lens luxation. At the age of 14 years an aortic root replacement was performed for progressive aortic root dilatation and aortic valve regurgitation. A spontaneous pneumothorax occurred at the age of 16 years.

Clinical examination at the age of 18 years showed a marfanoid habitus, slight downslanting of palpebral fissures and a high and gothic palate. Despite long fingers, wrist and thumb signs were negative. She exhibited limited extension of her elbows, mild asymmetry of the chest, and bilateral flat feet. Her skin showed several striae on the chest, shoulders, hips and lower back. Her length was 179.5 cm (+3,7SD for Turkish descent) and an

arm span of 175 cm (within normal limits). The brother of case 9 (III-3) was a 13 year old boy with Marfan syndrome. He had mild skeletal manifestations of Marfan (pes planus), a mildly dilated aortic root, ectopia lentis and dural ectasia with an anterior sacral meningocele. Furthermore he suffered from recurrent episodes of intracranial hypertension treated by drainage of cerebrospinal fluid. He has been described in a case report (Hilhorst-Hofstee et al., 2008).

The third patient (II-15) died at the age of 22 years. His case history was obtained from the medical records. At the age of 2 years bilateral subluxation of lenses was diagnosed. He developed severe aortic and mitral valve regurgitation with an aneurysm of the aortic root. He had skeletal involvement and an anterior sacral meningocele. When he was 17 years of age an aortic root replacement was performed with reconstruction of the aortic and mitral valve. Due to progressive aortic regurgitation, a re-operation was performed a year later. He died at the age of 22 after a second episode of ventricular fibrillation.

All heterozygous family members had a thorough skeletal, cardiologic and ophthalmologic examination including anthropometric measurements, echocardiography and slit lamp evaluation (Table 1). Only the mother and father of case 9 had an MRI evaluation for dural ectasia. The obtained clinical data of all family members are summarized in Table 1. The father of case 9 (II-6) had no clinical signs of Marfan syndrome. The mother (II-7) was tall, with a height on +2.5 SD but with normal body proportions. She had no other skeletal, ocular or cardiovascular involvement, but had several striae on the lumbar region and around the knees. Furthermore she suffered from spontaneous pneumothorax at the age of 21 years. An MRI-scan showed a dural ectasia at S2 with otherwise a normal dural sac. The father and mother of II-15 did not exhibit any signs of Marfan syndrome. None of the nine other heterozygous family members had a major criterion in one of the organ systems. Some were found to have a non-specific or minor sign. Individual II-1 has an arm span to height ratio of 1.06 and a mild dilatation of the abdominal aorta. II-4 had recurrent inguinal hernias but this was during a period of performing heavy physical labor. II-8 had an arm span to height ratio of 1.07 and bilateral flat feet. II-9 had reduced extension of the elbows.

The clinical data of cases 1- 10 and published cases are summarized in Table 2 together with the molecular data.

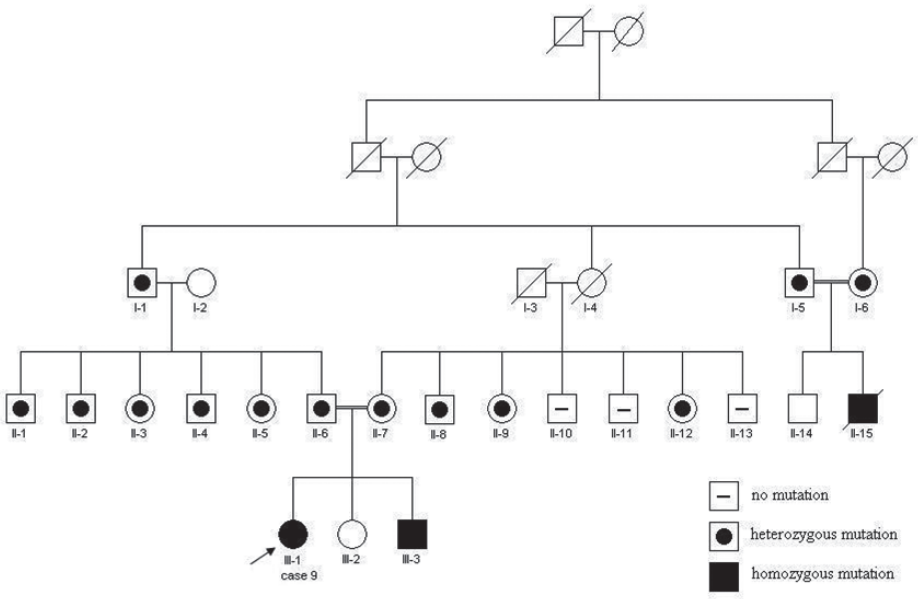

Figure 1. Pedigree of the family of case 9. Squares, male subjects; circles, female subjects. Affected subjects with a homozygous mutation (c.7454A>T) are represented by solid symbols. Presence or absence of the heterozygous mutation is represented by an open symbol with a black dot or a minus symbol respectively.

Table 1. Summary of the clinical features in the family of case 9

	III-1 Case 9	III-3	II-6	II-7	II-15	I-5	I-6	I-1	II-1	II-2	II-3	II-4	II-5	II-8	II-9	II-12
Age at examination (years)	22	10	42	43	9	57	56	61	44	41	37	35	33	55	48	37
Sex	F	M	M	F	M	M	F	M	M	M	F	M	F	M	F	F
height (cm)	180,9	150,5	167,1	174,4	135,0	164,0	157,5	166,0	167,5	174,5	167,0	188,5	161,0	181,0	164,7	161,0
height SDS (for Turkish descent)	3,7	1,6	-1,2	2,5	-0,2	-1,7	-0,6	-1,4	-1,1	0,1	1,1	2,5	0,8	1,2	0,7	0,2
arm span : height ratio	0,97	1,02	1,04	1,00	1,00	1,01	1,03	1,01	1,06	1,04	0,98	0,99	1,02	1,07	1,02	1,03
sitting height : height ratio	0,515	0,488	0,536	0,513	np	0,535	0,528	0,519	0,527	0,517	0,531	0,516	0,523	0,482	0,520	0,526
sitting height : height ratio SDS	-0,8	-2,0	1,6	-0,8	np	1,5	0,2	0,3	1,0	0,3	0,3	0,2	0,0	-1,8	-0,4	0,0
Skeletal system	**involv**	**involv**	**none**	**none**	**involv**	**none**	**none**	**none**	**none**	**none**	**none**	**none**	**none**	**involv**	**none**	**none**
major																
pectus carinatum	no	no	no	no	yes	no	no	no	no	no	no	no	no	no	no	no
pectus excavatum requiring surgery	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
sitting height : height ratio <2 SD or armspan : height ratio >1.05	no	yes	no	no	np	no	no	no	yes	no	no	no	no	yes	no	no
wrist and thumbsigns	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
scoliosis of >20° or spondylolisthesis	no	no	no	no	np	no	no	no	no	no	no	no	no	no	no	no
reduced extension at the elbow (<170°)	yes	no	no	yes	np	no	no	no	no	no	no	no	no	no	yes	no
pes planus	yes	yes	no	no	yes	no	no	no	no	no	no	yes	no	yes	no	no
protrusio acetabulae	np	np	np	np	np	np	np	np	np	np	np	np	np	np	np	np
minor																
pectus excavatum of moderate severity	no	no	no	no	yes	no	no	no	no	no	no	no	no	no	no	no
joint hypermobility	no	no	no	no	np	no	no	no	no	no	no	no	no	no	no	no
highly arched palate with crowding	yes	no	no	no	yes	no	no	no	no	no	no	no	no	no	no	no
facial appearance	yes	no	no	no	yes	no	no	no	no	no	no	no	no	no	no	no
Ocular system	**major**	**major**	**none**	**none**	**major**	**none**	**none**	**none**	**none**	**none**	**none**	**none**	**none**	**none**	**none**	**none**
major																
ectopia lentis	yes	yes	no	no	yes	no	no	no	no	no	no	no	no	no	no	no
minor																
abnormally flat cornea	np	np	np	np	np	no	no	no	no	no	no	no	no	no	no	no
increased axial length of globe	np	np	np	np	np	np	np	np	np	np	np	np	no	no	no	no
hypoplastic iris or ciliary muscle	np	np	no	no	yes	no	no	no	no	no	no	no	no	no	no	no

Table 1. Continue

	III-1 Case 9	III-3	II-6	II-7	II-15	I-5	I-6	I-1	II-1	II-2	II-3	II-4	II-5	II-8	II-9	II-12
Cardiovascular system	**major**	**major**	**none**	**none**	**major**	**none**	**none**	**none**	**involv**	**none**	**none**	**none**	**none**	**none**	**none**	**none**
major																
Z-score aortic root diameter	10,5[1]	4	0.3	-1.4	>2[2]	-0.9	-0.8	-1.8	1.4	-1.1	-0.8	-2.9	-2.5	0.4	0.5	0.8
dilatation ascending aorta	yes (arr)	yes	no	no	yes (arr)	no	no	no	no	no	no	no	no	no	no	no
dissection of ascending aorta	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no	no
minor																
mitral valve prolaps	yes	yes	no	no	yes	no	no	no	no	no	no	no	no	no	no	no
dilatation of main pulmonary artery	no	no	no	no	np	no	no	no	no	no	no	no	no	no	no	no
calcification of the mitral annulus < 40 years	no	no	no	no	np	no	no	np	no	no	no	no	no	no	no	no
dilatation or dissection of descending aorta < 50 years	no	no	np	no	np	no	no	no	yes	no	no	no	no	no	no	no
Pulmonary system	**involv**	**none**	**none**	**involv**	**none**	**none**	**none**	**none**	**none**	**none**	**none**	**none**	**none**	**none**	**none**	**none**
minor																
spontanous pneumothorax or apical blebs	yes	no	no	yes	no	no	no	no	no	no	no	no	no	no	no	no
Skin and integument	**involv**	**none**	**none**	**involv**	**np**	**none**	**none**	**none**	**none**	**none**	**involv**	**involv**	**none**	**none**	**none**	**none**
minor																
striae atrophicae	yes	no	no	yes	np	no	no	no	no	no	yes	no	no	no	no	no
recurrent or incisional herniae	no	no	no	no	np	no	no	no	no	no	no	yes[3]	no	no	no	no
Dura	**np**	**major**	**none**	**major**	**np**	**np**	**np**	**np**	**np**	**np**	**np**	**np**	**np**	**np**	**np**	**np**
major																
lumbosacral dural ectasia	np	yes	no	yes	np	np	np	np	np	np	np	np	np	np	np	np
Family or genetic history	**major[4]**	**major[4]**	**major[4]**	**major[4]**	**major[4]**	**major[4]**	**major[4]**	**major[4]**	**major[4]**	**major[4]**	**major[4]**	**major[4]**	**major[4]**	**major[4]**	**major[4]**	**major[4]**
major																
1st degree relative with Marfan syndrome	no	yes	yes	yes	no	yes	yes	no	no	no	no	no	no	no	no	no
pathogenic mutation in *FBN1*	hom	hom	het	het	hom	het	het	het	het	het	het	het	het	het	het	het

F female; M male; involve involvement; np not performed; arr aortic root replacement; hom homozygous c.7454A>T *FBN1* mutation; het heterozygous c.7454A>T *FBN1* mutation; Z-score related to body surface area and age according to Roman et al. (Roman et al., 1989); 1) aortic root measurement at the age of 14 years just before aortic root replacement; 2) no exact measurement available; 3) recurrent inguinal hernias during a period of heavy duty; 4) major criterion on the basis of a first degree affected family member or the presence of a pathogenic *FBN1* mutation.

Molecular studies

DNA was extracted from peripheral blood or paraffin embedded tissue, using standard techniques, analyzed by DHPLC and direct DNA sequencing as described previously (Matyas et al., 2002).

The reference sequence used to describe the mutations is the *FBN1* cDNA GenBank reference sequence: NM_000138.3. Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence, according to journal guidelines (www.hgvs.org/mutnomen).

Skin biopsies of case 1, 9 and her mother and case 10 were available. Fibroblasts were cultured and mRNA was isolated from confluent monolayers. For each individual two RNA isolations were performed: one cell culture of each was incubated with cycloheximide (0.25 mg/ml) for 4.5 hours prior to RNA isolation, to prevent possible nonsense mediated decay (NMD) of aberrantly spliced mRNA.

RNA was isolated using the RNA isolation minikit (Qiagen) according to the manufacturers instructions. Full length single stranded cDNA was prepared with oligo-dT-primer and Superscript™II RT reverse trancriptase (Invitrogen). To detect possible splice errors, the complete coding sequence of *FBN1* was analyzed by direct sequencing in 24 overlapping PCR fragments. The primers for overlapping fragments were positioned in different exons, to avoid allele dropout in case of exon skipping. For analysis of the mutation in exon 60, primers in exon 55 (forward) and 63 (reverse) were used.

Primer sequences are given in Supp. Table S1.

Results

Heterozygous mutations leading to a substitution of the first aspartic acid of a cbEGF domain were found in nine index cases and a homozygous mutation was found in 1 index case. These mutations and 14 comparable mutations described in the literature or in the UMD-*FBN1* (UMD-*FBN*; http://www.umd.be) are listed in Table 2. The phenotypes were classical MFS in 15 cases, neonatal MFS in two cases, thoracic aortic aneurysm in one case and lens luxation with striae in one case. In four cases described in the literature the phenotype is not clear.

The mutation was de novo in cases 2, 4, 5, and 8. In case 1, 3 and 6 parental DNA was not available. In case 1 five sibs were tested negative for the mutation. In case 6 the parents and seven sibs had no clinical symptoms of MFS. Case 7 has an affected sister with the same mutation. Their father died as a consequence of his third aortic dissection when he was 52 years of age. He was thought to have MFS. The mother of case 10 is probably affected but

was not molecularly tested. Of the published mutations three were de novo, three were familial and in eight inheritance was unknown (Table 2).

Using DHPLC for mutation scanning of all 65 exons of *FBN1*, initially no mutation was found in case 9. As the parents are consanguineous, a recessive mutation was suspected. Testing one of the parents, a heterozygous mutation was detected in exon 60: c.7454A>T, leading to the amino acid substitution p.Asp2485Val. This mutation was found in a homozygous state in the patients III-1, III-3 and II-15. The parents (II-6 and II-7) of patients III-1 and III-3 are first cousins. The parents of patient II-15 are distantly related and both are related to II-6 and II-7 (Figure 1). In the parents of patients III-1, III-3 and II-15 and 9 family members the mutation was heterozygous. The mutation was not detected in 1000 Caucasian and 60 Turkish control chromosomes.

Sequence analysis of cDNA, made from mRNA from cultured fibroblasts of case 1, case 9 and her mother, and case 10 showed no evidence of erroneous splicing of exon 60. Treatment with cycloheximide, to prevent possible nonsense mediated decay of erroneously spliced mRNA, gave the same results. Fibrobasts of the other patients were not available.

Table 2. Published and observed missense mutations leading to the substitution of the first aspartic acid of a cbEGF domain

Nucleotide change	Amino acid change	Exon	cbEGF domain	Diagnosis	Phenotype	Reference	Aberant mRNA splicing	Predicted aberant mRNA splicing[a]	Inheritance
c.1468G>T	p.Asp490Tyr	11	#3	Classical MFS	ard, el, sk	(Hayward and Brock, 1997)	np	yes	Unknown
c.2168A>C	p.Asp723Ala	18	#7	Severe classical MFS	ard, mvp, el, myop, sk	(Dietz et al., 1993)	np	no	De novo
c.2168A>T	p.Asp723Val	18	#7	Classical MFS	ard, mvp, el, sk, myop	(Katzke et al., 2002)	np	no	De novo
c.2728G>A	p.Asp910Asn	22	#10	Classical MFS	unknown	UMD	np	yes	Unknown
c.3209A>G	p.Asp1070Gly	26	#12	Neonatal MFS	unknown	UMD	np	no	Unknown
c.3338A>G	p.Asp1113Gly	27	#13	Phenotype unknown	unknown	(Liu et al., 1997)	np	no	Unknown
c.3463G>A	p.Asp1155Asn	27	#14	Thoracic aortic aneurysm	ard, mvp, diss	(Milewicz et al., 1996)	no	yes	De novo
c.3464A>G	p.Asp1155Gly	28	#14	Classical MFS	ard, el	**Case 1**	no	no	Parents not available, 5 sibs neg for mutation
c.3712G>A	p.Asp1238Asn	29	#16	Phenotype unknown	unknown	(Yuan et al., 1999)	np	no	Unknown
c.3713A>G	p.Asp1238Gly	30	#16	Classical MFS	ard, mvp, sk	(Tiecke et al., 2001)	np	no	Familial
c.3964G>A	p.Asp1322Asn	31	#18	Neonatal MFS	ard, mi, ti, myop, sk	**Case 2**	np	yes	De novo
c.4210G>T	p.Asp1404Tyr	33	#20	Classical MFS	ard, el, sk	(Hayward et al., 1997)	yes	yes	Familial
c.5422G>C	p.Asp1808His	43	#26	Lens luxation and striae	el, str	**Case 3**	np	no	Parents not available
c.5671G>A	p.Asp1891His	45	#28	Classical MFS	ard, sk	**Case 4**	np	no	De novo
c.5788G>C	p.Asp1930His	45	#29	Classical MFS	ard, el, sk	**Case 5**	np	yes	De novo
c.5788G>A	p.Asp1930Asn	46	#29	Phenotype unknown	unknown	(Liu et al., 1997)	np	yes	Unknown
c.5788G>A	p.Asp1930Asn	46	#29	Classical MFS	ard, el, sk, de, str	**Case 6**	np	yes	Parents not available
c.6037G>T	p.Asp2013 Tyr	48	#31	Classical MFS	ard, el, sk, de, str	**Case 7**	np	yes	Familial
c.6379G>T	p.Asp2127Tyr	51	#32	Classical MFS	ard, el	(Matsukawa et al., 2001)	np	yes	Familial
c.6381T>A	p.Asp2127Glu	52	#32	Classical MFS	ard, sk	(Kainulainen et al., 1994)	np	no	Familial
c.7331A>G	p.Asp2444Gly	59	#38	Classical MFS	ard, sk	**Case 8**	np	no	De novo
c.7454A>T	p.Asp2485Val	60	#39	Classical MFS in homozygous state	ard, el, sk, str, her, pn	**Case 9**	no	no	Familial
c.7819G>A	p.Asp2607Asn	62	#42	Classical MFS	ard, mvp, sk	**Case 10**	no	no	Mother suspect for MFS
c.7820A>G	p.Asp2607Gly	63	#42	Phenotype unknown	unkown	(Liu et al., 1997)	np	no	Unknown

UMD Universal Marfan Database – *FBN1* (UMD-*FBN*; http:/www.umd.be); cbEGF calcium binding Epidermal Growth Factor domain; bp basepair; np not performed; neg negative; pos positive; ard aortic root dilatation; diss aortic dissection; mvp mitral valve prolapse, mi mitral valve insufficiency; el ectopia lentis; pal high arched palate; ti tricuspid valve insufficiency; myop high myopia; ar arachnodactyly; hm hypermobility; contr contractures; str striae; her hernia; sk skeletal involvement; de dural ectasia; pn pneumothorax; unknown. The gray row represents the recessive mutation described in this article.

Mutation numbering refers to the *FBN1* cDNA GenBank reference sequence: NM_000138.3, with the A of the ATG translation initiation codon as nucleotide +1 (www.hgvs.org/mutnomen). [a]) Alamut mutation interpretation software version 1.5; Interactive Biosoftware, Rouen France.

Discussion

We identified a heterozygous substitution of the first aspartic acid of a cbEGF domain in *FBN1* in nine index patients and a homozygous substitution in one index patient with MFS. Reviewing the literature we found 12 reports of substitution of the first aspartic acid, and a further two unpublished cases are quoted in the UMD database (UMD-*FBN*; http://www.umd.be) (Collod-Beroud et al., 2003) as summarized in Table 2. All 10 index patients found in our center fulfilled the Ghent criteria when the finding of a pathogenic mutation was included. Of the 14 published cases, eight were reported to have a classical Marfan phenotype, one was a neonatal Marfan and one had a thoracic aortic aneurysm. Of four cases the phenotype was not reported. In all cases the acidic amino acid aspartic acid is replaced by a nonpolar or noncharged polar amino acid, apart from one mutation where aspartic acid is replaced by another acidic amino acid (p.Asp2127Glu) (Kainulainen et al., 1994). The codons of the first aspartic acids in the cbEGF domains always contain the last base of one exon and the first two bases of the next. Consequently, mutations of these codons may affect splicing. Aberrant splicing was excluded in cases 1, 9, and 10 and in one of the published cases (Milewicz et al., 1996). The mutation c.4210G>T was shown to destroy a donor splice site with abnormal splicing as a consequence (Hayward et al., 1997). Of the 19 cases in which no cDNA analysis was performed, prediction software predicted aberrant splicing in eight cases (Table 2). Exon skipping, as a result of these mutations, may have more severe effects than missense mutations, because the exons are all in frame and consequently, skipping will lead to a shorter protein that may exert a dominant negative effect (Robinson et al., 2002).

There are several reasons to argue that a substitution of the first aspartic acid of a cbEGF domain will lead to a MFS phenotype in the heterozygous state. Calcium binding of cbEGF domains is necessary for stabilization of the secondary structure, prevention of proteolytic degradation and for protein-protein interaction (Dietz et al., 1993; Handford et al., 1991; Cooke et al., 1987). The first aspartic acid of a cbEGF domain is highly conserved in evolution and in the human fibrillin-1 gene all cbEGF domains start with an aspartic acid, which is crucial for binding of a positively charged Ca^{2+} ion (Figure 2) (Whiteman et al., 2007). Furthermore mutations of the first amino acid of a cbEGF domain of coagulation factor IX in haemophilia B have been proven to reduce calcium binding even if the aspartic acid is replaced by the acidic amino acid glutamic acid (Handford et al., 1991; Winship and Dragon, 1991). The finding of 23 MFS or MFS-like cases with a heterozygous substitution of an aspartic acid in this position of the cbEGF domain underscores the crucial role of this amino acid. In this view the recessive nature of the mutation p.Asp2485Val in the family of case 9 came as a surprise. The family of case 9 (Figure 1) has been thoroughly investigated.

Patients III-1 (case 9), III-3 and II-15 have the classical type of Marfan syndrome according to the Ghent criteria (De Paepe A. et al., 1996). Based on the pedigree with three affected patients and healthy consanguineous parents recessive inheritance could be expected. This was confirmed by finding a homozygous missense mutation in all three affected patients. The four unaffected parents and nine other unaffected relatives were found to be carriers of the mutation. Unexpectedly in none of the investigated heterozygous carriers obvious signs of Marfan syndrome could be found. Only after thorough clinical examination one of them (II-7) was found to have a dural ectasia at S2, which as yet is considered a major symptom in the Ghent criteria. Together with the family history and some minor signs (pneumothorax, striae and reduced extension of the elbows) this classifies II-7 as having Marfan syndrome. However, compared to the homozygous cases, the cardinal Marfan features in the skeletal, cardiac and ophthalmological systems are absent.

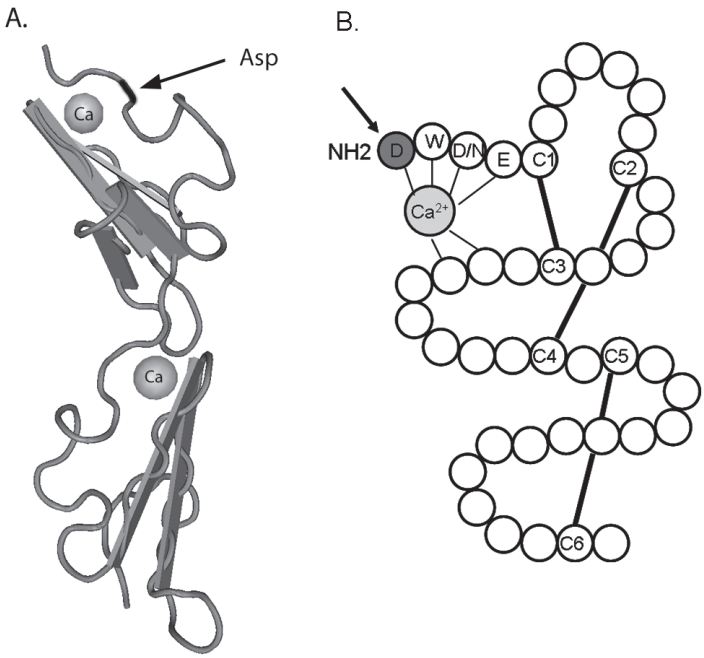

Figure 2. Class I cbEGF domain showing the position of the first Asp in relation to the calcium molecule. (A) 3D picture of a cbEGF domain of fibrillin-1. The arrows point to the first Asp. Picture derived from the NCBI database (http://www.ncbi.nlm.nih.gov/) (Downing et al., 1996). (B) cbEGF like domain (Handford et al., 1991). The arrow points to the first aspartic acid residue. Solid lines are the disulphide bridges between cysteine residues.

To our knowledge very little is known about recessive *FBN1* mutations. Only one family has been reported in which Marfan syndrome was found in two affected cousins homozygous for a *FBN1* mutation while the four normal parents were heterozygous carriers, indicating recessive inheritance of the syndrome (De Vries et al., 2007). The mutation is located in exon 11 and leads to an amino-acid substitution creating a cysteine. Like us the authors expected this mutation to have a dominant effect in the heterozygous state. Two of the parents were sibs and exhibited minor signs of Marfan syndrome (increased arm span to height ratio and a highly arched palate). No other family members have been investigated. Only one other probably recessively inherited form of Marfan syndrome has been described but could not be proven by molecular analysis as the gene was not yet known (Fried and Krakowsky, 1977).

Three families have been described in the literature in which both parents are affected with more severely affected children. The first is an Italian couple of first cousins, both affected with Marfan syndrome, who had 4 affected children. Two of the four affected children showed more severe manifestations than other affected family members, presumably due to homozygosity (Capotorti L. et al., 1959). In 1984, Chemke described a family with Marfan syndrome. Two sibs suffered from a severe phenotype reminiscent of neonatal Marfan syndrome. Their parents were cousins and had a much milder phenotype. Remarkable is that the probably homozygous sibs were the only patients in the family with ectopia lentis (Chemke et al., 1984). In 1988 a severely affected boy has been described (Schollin et al., 1988). Both parents were affected and were found to carry a missense mutation in *FBN1*. Compound heterozygosity was identified in the severely affected child (Karttunen et al., 1994).

In the recessive family described here the heterozygous mutation does not exert an important effect on the phenotype. Only in the homozygous state the abnormal fibrillin causes the classical clinical phenotype of Marfan syndrome. This observation together with the few other described families with bi-allelic inheritance, may support both alternative pathogenetic models of Marfan syndrome. A dominant negative effect of *FBN1* mutations has been the leading hypothesis for the pathogenesis of Marfan syndrome for a long time. However, several manifestations of Marfan syndrome like bone overgrowth, craniofacial features, lung disease, and muscle and fat hypoplasia could not be explained by a structurally abnormal protein. The observation that fibrillin interacts with a variety of proteins, including the latent TGFβ binding proteins (LTBP's) has lead to several investigations indicating that fibrillin-1 can interact with TGFβ signaling (Annes et al., 2003; Azhar et al., 2003; Isogai et al., 2003; Hubmacher et al., 2006; Kaartinen and Warburton, 2003; Loeys et al., 2006; Neptune et al., 2003; Ng et al., 2004; Ten Dijke and Arthur, 2007).

According to splice site prediction software (Alamut mutation interpretation software

version 1.5; Interactive Biosoftware, Rouen, France) the c.7454A>T mutation, found in the family described here, is not predicted to cause erroneous splicing. This was confirmed by cDNA studies, showing no evidence of splice error. The position of the mutation (exon 60) may explain the lack of expression as mutations in the more C-terminal end of the gene are expected to give a milder phenotype (Faivre et al., 2007; Robinson et al., 2002). However the mutation in case 10 is even more terminal and still leads to a classical MFS phenotype.

Hutchinson et al. (Hutchinson et al., 2003) hypothesized that variable expression of the normal *FBN1* allele could moderate the phenotypic effect of the mutant allele. A compensatory higher level of *FBN1* expression from the normal allele would explain a milder phenotype. As the normal alleles are inherited from 5 different parents in our recessive family, this mechanism is highly unlikely.

We hypothesize that the p.Asp2485Val mutation acts as a hypomorphic allele with a minimal dominant negative effect. Reduction of gene expression of both alleles could be the main determinant of the phenotype in homozygotes. The observation of only one major clinical sign in one of the heterozygotes (dural ectasia in II-7, Figure 1) and no major clinical signs in 12 other heterozygotes could be explained by sufficient gene expression with only a mild functional defect of the mutant allele product. This was also shown in a mouse model, however in this model the mutation had a severe dominant negative effect (Pereira et al., 1997). In this model with a deletion of 272 amino acids in the central part of fibrillin-1, a tenfold reduction in expression of the mutant allele was shown in heterozygous mice, resulting in a normal phenotype. Homozygous mutant mice however died shortly after birth due to severe vascular complications. The other mouse model of Pereira was a targeted *FBN1* mutation leading to 15% expression of a normal product with no abnormal phenotype in heterozygous mice, while mice homozygous for this mutation have severe abnormalities comparable with the neonatal MFS phenotype. These mouse models suggests that there is a threshold of expression of the normal allele below which the abnormal phenotype will develop (Pereira et al., 1999; Dietz and Mecham, 2000).

To understand the exact pathogenetic mechanism expression studies and studies on protein synthesis, secretion and matrix incorporation of the Asp2486Val mutation are necessary For comprehensive studies of this type, a mouse model should be created.

The finding of a homozygous substitution of A>T, as described here, has implications for mutation screening in MFS. Homozygous substitutions will not have an effect on denaturation of double stranded DNA, because the basepair remains the same. Consequently, heteroduplex based testing, such as DHPLC, working with the principle of differential denaturation of double stranded heteroduplex DNA, cannot detect this mutation in homozygous state. Formerly, based on the presumed dominant inheritance mode, only heterozygous mutations were expected. Now it is clear that recessive

inheritance is also possible and mutations may have been missed in similar cases, because heteroduplex based testing has been used until recently in many large diagnostic centers. Most laboratories nowadays use direct sequencing, which avoids this problem.

In conclusion we have shown that the first aspartic acid of a cbEGF domain in *FBN1* is important for the function of fibrillin-1, but may not always lead to a clinical effect in the heterozygous state. This underscores that missense mutations must be interpreted with care.

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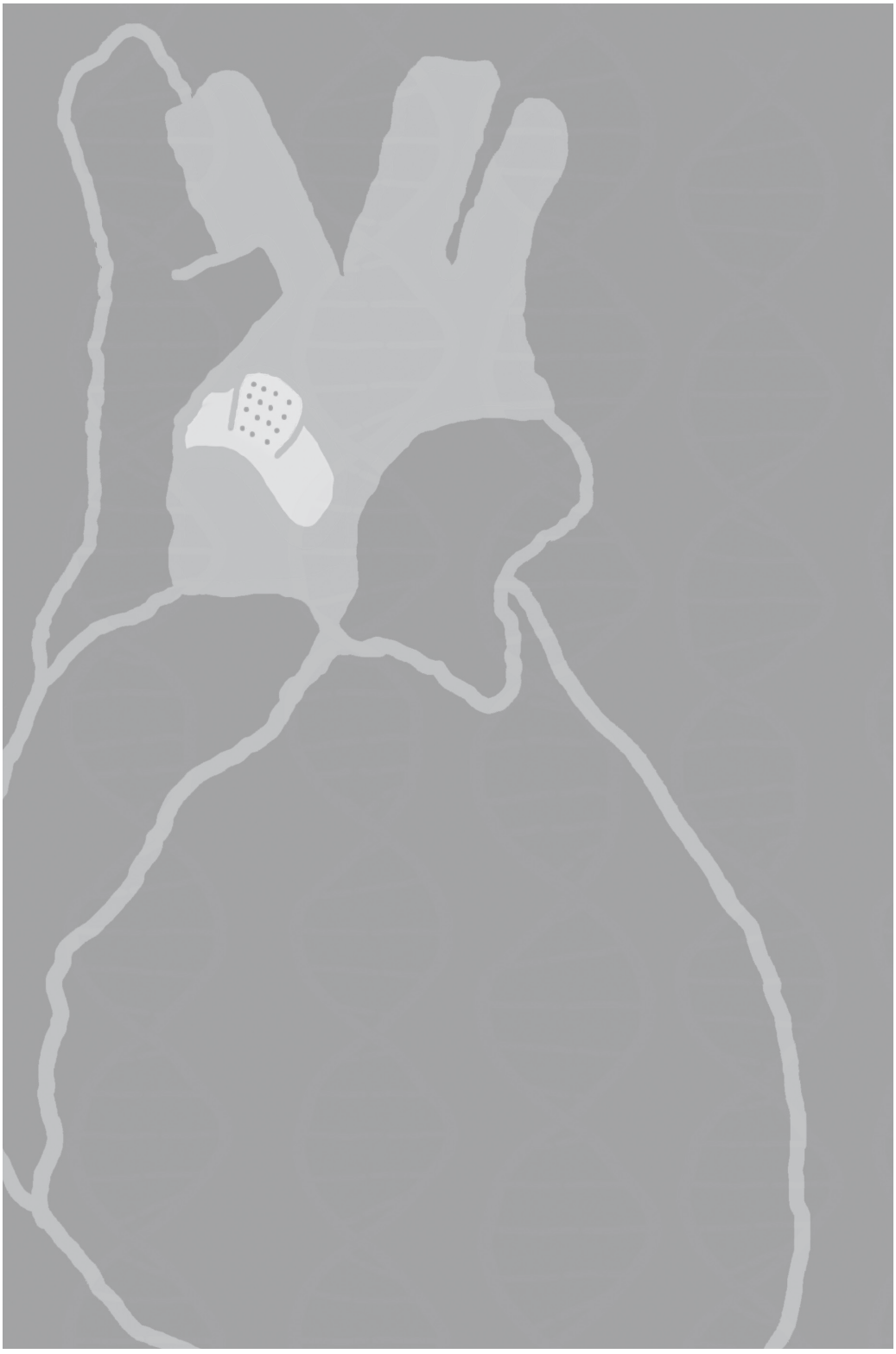
Supp. Table S1. Primers for cDNA sequencing of FBN1: F=forward; R=reverse; numbers refer to position in cDNA sequence.

fragment	primername	Sequence 5' > 3'	Length bp
1	FBN1F1	ATGCGTCGAGGGCGTCTGCT	384
	FBN1R384	GCTACCTCCATTCATACAGCGA	
2	FBN1F318	GATAGCTCCTTCCTGTGGCTCC	406
	FBN1R728	CCGTGCGGATATTTGGAATG	
3	FBN1F657	CCCCTGTGAGATGTGTCCTG	407
	FBN1R1064	TTGGTTATGGACTGTGGCAGC	
4	FBN1F1012	ACAGCTCTGACAAACGGGCG	384
	FBN1R1396	TGCAGCGTCCATTTTGACAG	
5	FBN1F1358	GCCAGTTGGTCCGCTATCTC	330
	FBN1R1688	ACATGAAAGCCCGCATTACAC	
6	FBN1F1609	AATGGCCGGATCTGCAATAA	398
	FBN1R2007	CTGGCCTCTCTTGTATCCACCA	
7	FBN1F1927	CTGGCTGTGGGTCTGGATGG	362
	FBN1R2289	GCAGTTTTTCCCAGTTGAATCC	
8	FBN1F2212	ATCTGTGAAAACCTTCGTGGGA	399
	FBN1R2611	AGGTGGCTCCATTGATGTTGA	
9	FBN1F2458	GTCTGCAAGAACAGCCCAGG	357
	FBN1R2815	TGGGACACTGACACTTGAATGA	
10	FBN1F2699	TACTCAAGAATTAAAGGAACA	525
	FBN1R3224	CGGCATTCGTCAATGTCTGTGC	
11	FBN1F3141	CATTGGCAGCTTTAAGTGCAGG	460
	FBN1R3621	ACCACCATTCATTATGCTGCA	
12	FBN1F3558	CCATTCAACTCCCGATAGGCT	335
	FBN1R3893	TTTTCACAGGTCCCACTTAGGC	
13	FBN1F3783	CAGGTGCTTGTGTTATGATG	392
	FBN1R4173	GCACAGACAGCGGTAAGA	
14	FBN1F4062	GATTGGAGATGGCATTAAGTGC	451
	FBN1R4513	TGTTGACACAGTTCCCACTGA	
15	FBN1F4425	CTACGAACTGGACAGAAGCGG	593
	FBN1R5018	ATACAGGTGTAGTTGCCAACGG	
16	FBN1F4910	ACTACCTGAATGAAGATACACG	626
	FBN1R5536	GACCTGTGGAGGTGAAGCGGTAG	
17	FBN1F5348	TCAACATGGTTGGCAGCTTCC	476
	FBN1R5824	AAAGATTCCCATTTCCACTTGC	
18	FBN1F5722	ACAATTGGTTCCTTCAACTG	356
	FBN1R6074	GCACAAATTTCTGGCTCTT	
19	FBN1F5973	CTTGGATGGGTCCTACAGATGC	579
	FBN1R6552	CACATTCTTGCAGGTTCCATT	
20	FBN1F6466	GGTTATACTCTAGCGGGAATG	450
	FBN1R6937	TCCCACGGGTGTTGAGGCAGCG	
21	FBN1F6842	AGCGGAGACCTGATGGAGAGG	645
	FBN1R7487	CAGTTGTGTTGCTTGGTTGCA	
22	FBN1F7429	CAAGAGGATGGAAGGAGCTGC	476
	FBN1R7905	GAACTGTTCATACTGGAAGCCG	
23	FBN1F7785	CTACCTCCAGCACTACCAGTGG	384
	FBN1R8169	GTAGCCATTGATCTTACACTCG	
24	FBN1F8024	CACCTGGTTACTTCCGCATAGG	672
	FBN1R8696	ATGATTCTGATTGGGGGAAAA	

chapter
4



5

The clinical spectrum of complete *FBN1* allele deletions

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Abstract

The most common mutations found in *FBN1* are missense mutations (56%) mainly substituting or creating a cysteine in a cbEGF domain. Other mutations are frameshift mutations, splice mutations and nonsense mutations. There are only a few reports of patients with marfanoid features and a molecularly proven complete deletion of a *FBN1* allele. We describe the clinical features of 10 patients with a complete *FBN1* gene deletion. Seven patients fulfilled the Ghent criteria for Marfan syndrome (MFS). The other three patients were examined at a young age and did not (yet) present the full clinical picture of MFS yet. Ectopia lentis was present in at least two patients. Aortic root dilatation was present in six of the 10 patients. In three patients, the aortic root diameter was on the 95th percentile and in one patient the diameter of the aortic root was normal, the cross-section however had a clover-leaf appearance. Two patients underwent aortic root surgery at a relatively young age (27 and 34 years old). Mitral valve prolapse was present in four of 10 patients and billowing of the mitral valve in one. All patients had facial and skeletal features of MFS. Two patients with a large deletion extending beyond the *FBN1* gene had an extended phenotype.

We conclude that complete loss of one *FBN1* allele does not predict a mild phenotype, and these findings support the hypothesis that true haploinsufficiency can lead to the classical phenotype of Marfan syndrome.

Keywords: Marfan syndrome, *FBN1*, fibrillin-1, deletion, haploinsufficiency

Introduction

Marfan syndrome (MFS) is a dominant disorder mainly caused by mutations in the fibrillin-1 gene (*FBN1*) on chromosome 15. The estimated prevalence is about 1 in 10.000 [1]. The disorder has a very variable intra- and interfamilial expression. Different tissues and organs can be affected, with main features in the cardiovascular, skeletal, and ocular systems. Revised international criteria for the diagnosis were published in 1996 to facilitate the clinical diagnosis [2,3].

FBN1 mutations are detected in the majority of patients fulfilling the clinical criteria, but also in incomplete phenotypes referred to as type 1 fibrillinopathies [4]. Mutations in other genes have been reported to cause Marfan syndrome related disorders, such as *TGFBR1* and *2* in Marfan syndrome type 2 [5,6] and Loeys-Dietz syndrome [7,8] and *MYH11* and *ACTA2* in familial thoracic aortic aneurysms and dissections [9,10].

To date over 600 mutations have been published in the Universal Marfan Database (UMD-*FBN1*; http:\www.umd.be), but only a minority are recurring mutations. Missense mutations substituting or creating a cysteine in one of the calcium binding EGF domains are most prevalent. Other mutations are frameshift, splice-site and nonsense mutations [11].

Deletions of single and multiple exons can be detected using appropriate methods like Multiplex Ligation-dependent Probe Amplification *(*MLPA), cDNA or Southern blot analyses. Most of these deletions are associated with a severe or classical Marfan phenotype[12-17] . Only four reports are known of a molecularly proven whole gene deletion of *FBN1*[18-21]. We describe 10 patients including a family with five patients with whole gene deletions and show that complete loss of one *FBN1* allele does not predict a mild phenotype. These findings support the hypothesis that true haploinsufficiency can lead to the classical phenotype of Marfan syndrome.

Patients and methods

Patients

We screened DNA samples of 300 patients with clinical features of MFS or a related phenotype by MLPA. All samples had been previously screened by DHPLC and no mutations in *FBN1* were found. In one patient, chromosome analysis and array CGH performed for mental retardation screening revealed a deletion including the *FBN1* gene. In all patients the size of the deletion was determined by SNP array analysis.

The clinical features of the patients are listed in Table 1. A more detailed description can be found in the Supplementary Material.

Table 1. Clinical features of patients carrying a complete *FBN1* allele deletion described in this study

Patient	Age	Gender	MR	Aortic root dilatation	Aortic root surgery (age)	Aorta dissection (age)	MVP	Eye (age)	Skeletal	Face	Skin	Pneumo thorax	DE	Family	Ghent	other
1	41	F	No	Yes	No	No	Yes	EL, lensextraction (36)	Arachnodactyly, hypermobility	Normal	Normal	No	Np	Pos	Yes	
2	39	F	No	Yes	Yes (34)	No	Yes	Myopia	Arachnodactyly, hypermobility	High palate	recurrent herniae	No	No	Pos	Yes	
3	16	M	No	Yes	No	No	No	Myopia	Pectus carinatum, flat feet, hypermobility	High palate	Normal	No	Np	Pos	Yes	
4	13	F	No	Yes	No	No	Yes	Dubious EL	Pectus carinatum, flat feet, hypermobility,	High palate	Normal	No	Np	Pos	Yes	
5	27	F	No	Yes	No	No	No	No	Flat feet, elbow contracture, scoliosis, pectus excavatum, hypermobility	High palate	Normal	No	Np	Pos	Yes	
6	21	F	No	No, but cloverleaf appearance	No	No	No	Mild EL right eye, left eye normal	Severe pectus excavatum, flat feet, scoliosis	High and narrow palate, marfanoid face	Normal	No	Yes	Neg	Yes	
7	34	M	No	Yes	Yes (27)	No	No	EL	Pectus excavatum, arachnodactyly, scoliosis, hypermobility	Long face, high palate	Stretch marks	Yes	Np	Neg	Yes	Congenital hip dislocation
8	5	F	Yes	On P95	No	No	Billowing	Translucent irides	Arachnodactyly, hypermobility, flat feet, long toes	Mild dysmorphisms	Normal	No	Np	Neg	No	Hypotonia,
9	13	F	Yes	On P95	No	No	Yes	Anisohypermetropia, amblyopia	Arachnodactyly, hypermobility, pectus excavatum, flat feet	Pale skin and hair, dysmorphisms	General hypo-pigmentation	No	No	Neg	No	Ataxia
10	8	M	No	On P95	No	No	No	No	Arachnodactyly, hypermobility, pectus carinatum, mild scoliosis	High palate	Normal	No	Np	Pos	No	

MR mental retardation; *MVP* mitral valve prolapse; *DE* dural ectasia; *Ghent* Ghent criteria; *EL* ectopia lentis; *np* not performed; *pos* positive; *neg* negative

Multiplex ligation-dependent probe amplification

MLPA analysis was performed as described elsewhere[22] with the SALSA MLPA kits P065 and P066 that contain probes for 53 of the 65 *FBN1* exons (including exons 1 and 65) and all the 7 *TGFBR2* exons and reference probes widely spread over the genome (MRC-Holland, see http://www.mlpa.com).

PCR products were analysed on a fluorescent capillary sequencer (ABI3130, Applied Biosystems, Torrence, CA, USA) using Genemarker software (Softgenetics Inc., State College, PA, USA).

Cytogenetic analysis (patient 8 and 9)

Conventional chromosome analysis was performed on phytohemagglutinin stimulated lymphocytes from peripheral blood cultures using GTG-banding according to standard protocols.

High density microarray analyses, SNP Arrays

The Affymetrix GeneChip Human Mapping 262K *NspI* array (Affymetrix, CA, USA) contains 262.000 25-mer oligonucleotides with an average spacing of approximately 12 kb. An amount of 250 ng DNA was processed according to the manufacturer's instruction (www.Affymetrix.com). SNP copy number was assessed using the software program Copy Number Analyzer for Genechip (CNAG) Version 2.0 (see http://www.genome.umin.jp)[23].

Fluorescence *in situ* hybridization (FISH) analysis

Fluorescence *in situ* hybridization (FISH) analysis was performed following the manufacturer's instructions using the BAC clone RP11-42K15 (Children Hospital Oakland Research Institute, Oakland, CA, USA).

Results

In nine patients, MLPA revealed reduced relative peak areas for all probes within the *FBN1* gene indicating a deletion of the entire *FBN1* allele.

Five patients (patients 1-5) are part of one family (Figure 1). The parents of patients 1, 2 and 5 have no clinical features of MFS. However in the mother, both MLPA and SNP array analysis showed lower intensity signals for the probes in the deleted area but higher signals than in the patients, suggesting a mosaic deletion.

Figure 2 shows the MLPA results in patient 2 and her mother. The other eight patients have MLPA results comparable to the results of patient 2. FISH analysis with a probe within the

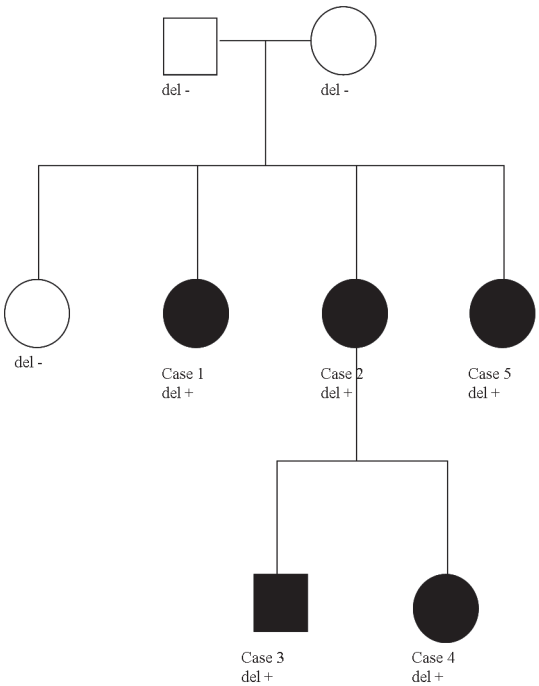

Figure 1 Pedigree of the family with five members carrying a complete deletion of a *FBN1* allele, del – no deletion present; del + deletion present

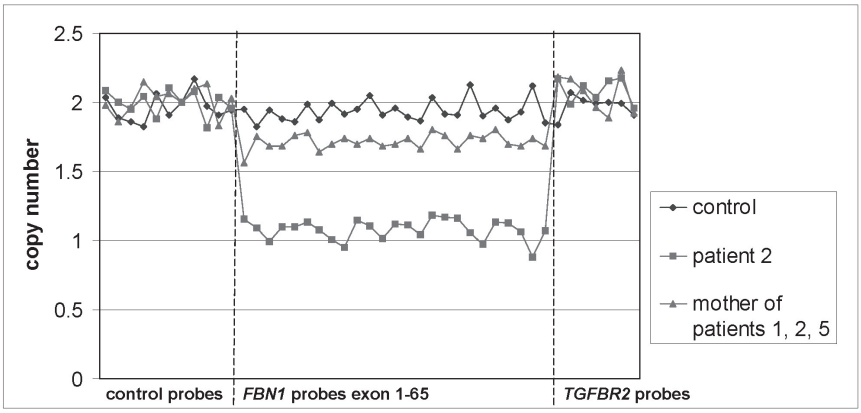

Figure 2 MLPA results of *FBN1* in mother and daughter (patient 2), compared to healthy control (MLPA kit P065, MRC Holland). The control probes are normalized to 2 copies. The probe signals for *FBN1* relative to control probes and to *TGFBR2* probes show a single copy for *FBN1* in patient 2. All other patients discussed in this paper show MLPA results comparable to patient 2. The healthy mother of patient 2 has reduced probe signals for all *FBN1* probes, indicative of somatic mosaicism for the deletion. The mean (±standard deviation) signals for all *FBN1* probes were: 1.93±0.08 (control), 1.08±0.08 (case 2) and 1.71±0.05 (mosaic mother; $p<10^{-15}$ compared to control, according to a two tailed t-test).

FBN1 gene confirmed the mosaic deletion in 21% of the totally 200 analyzed interphase nuclei. MLPA and FISH analysis in 200 interphase nuclei of the father showed a normal result (results not shown).

In three patients (patients 6, 8 and 9) the deletion occurred *de novo*. In one patient (patient 7), the parents were not tested for the deletion but appeared completely normal by clinical, ophthalmologic and cardiologic examination. The mother of patient 10 was not available for molecular testing.

In patient 8, the cytogenetic analysis revealed a *de novo* translocation between the long arms of chromosome 12 and chromosome 15. Additional array CGH analysis, with a resolution of 1 Mb, detected a 4,9 Mb interstitial deletion at the translocation breakpoint of the long arm of chromosome 15 between the bands q21.1 and q21.2 (results not shown). The *FBN1* gene is located in this region. At the translocation breakpoint of chromosome 12 no deletion was dectected by array CGH or SNP array analysis. Conventional karyotyping of case 9 was performed as part of the mental retardation screening showing a normal female karyotype. In the other patients no standard cytogenetic analysis was performed.

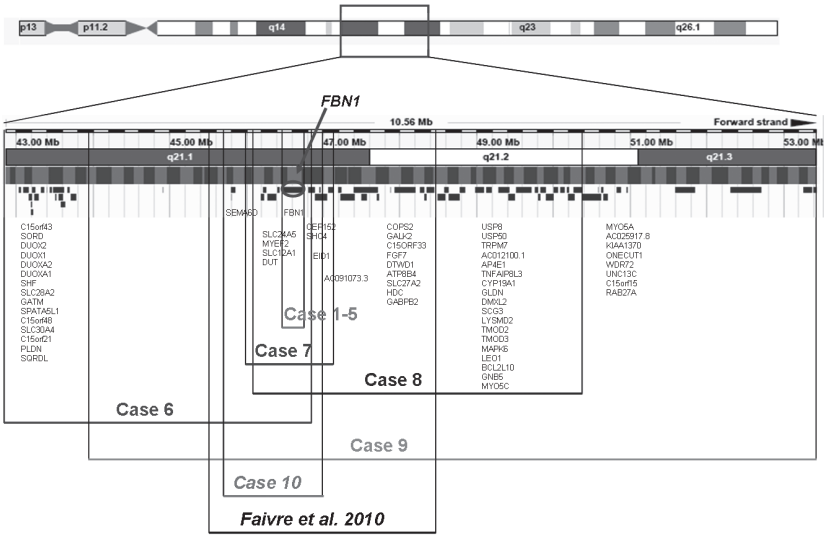

Figure 3 Position of the deletions on chromosome 15 (Ensemble release 53, March 2009) including the deletion described by Faivre et al.[19]. The bars underneath the chromosome depict the known protein coding genes according to Ensemble release 53, March 2009. The names of the genes are written below. The horizontal colored lines show the size of the different deletions and their overlap.

For all probands the size of the deletion was characterized by SNP array analysis. The results are depicted in Figure 3 and Table 2.

The clinical features of the patients are summarized in Table 1. Except for patients 8, 9 and 10, all patients fulfilled the Ghent criteria for Marfan syndrome. The young age of patients 8, 9 and 10 could explain why they do not yet present the full clinical picture of MFS. Ectopia lentis was present in patient 1 and 7. Patient 4 had questionable lens subluxation, and patient 6 had very mild lens subluxation of her right eye. Aortic root dilatation was present in six of the 10 patients. In patients 8, 9 and 10 the aortic root diameter was on the 95th percentile. In patient 6 the diameter of the aortic root was on the 50th percentile. the cross-section however had a cloverleaf appearance. Patient 2 and patient 7 underwent aortic root surgery at a relative young age (27 and 34 years old). Mitral valve prolapse was present in four of ten patients and billowing of the mitral valve in one. All patients had facial or skeletal features of MFS.

The two children with larger deletions (patients 8 and 9) had an extended phenotype with psychomotor retardation and additional features. Patient 8 was a 5-year old girl who presented at the age of 2.5 years with psychomotor retardation and hypotonia with severe motor delay. Patient 9 presented with psychomotor retardation with non-progressive ataxia. Apart from het marfanoid features she had a very pale skin and hair without other ectodermal manifestations. She had facial dysmorphisms consisting of a brachycephalic skull, long philtrum, broad nose and prognathism.

Further details about the clinical manifestations of the 10 described patients are found in the Supplementary Information.

Table 2. Size of the deletions and number of genes deleted. The location of the SNP's are derived from Ensemble release 53, March 2009

Patient	Starting SNP	Ending SNP	SNPs	Starting bp	Ending bp	Max. size (bp)	Genes
2	SNP_A-1876769	SNP_A-1938857	44	46.434.718	46.742.196	307478	1
6	SNP_A-2169073	SNP_A-2251237	429	42.867.884	46.950.476	4082592	23
7	SNP_A-2099749	SNP_A-1871856	107	46.098.636	47.145.902	1047366	9
8	SNP_A-2055581	SNP_A-1811386	366	46.116.834	50.383.848	4267009	36
9	SNP_A-2127245	SNP_A-2283948	2007	44.000.164	53.427.159	9426995	46
10	SNP_A-1826755	SNP_A-1823624	141	45.715.212	46.963.495	1248283	9

Abbreviations: bp, basepair; SNP single nucleotide polymorphism

Discussion

There are several reports of deletions of the long arm of chromosome 15 involving chromosome band q21.1. However, in most of these reports the deletion of *FBN1* or the presence of marfanoid features are not discussed[24-29]. In four reports the deletion of *FBN1* is confirmed by molecular techniques, with marfanoid features in three cases[18,19,21] and absence of marfanoid features in one case which could be due to the young age of this patient[20].

In this study we describe 10 patients with a deletion of an entire *FBN1* allele. To our knowledge this is the first series of complete *FBN1* allele deletions published so far. These patients and three previously described sporadic patients[18,19,21] have a Marfan phenotype due to pure haploinsufficiency. The phenotype of the patients in our series varies from mild features of MFS to the classical MFS phenotype. One family (patients 1-5) has a deletion encompassing only the *FBN1* gene, whereas patients 6, 7, 8, 9 and 10 have much larger deletions spanning 1 to 9.4 Mb, with 9 to 46 genes respectively (Figure 3 and Table 2). Patients 6, 7 and 10 have no other features than those which can be attributed to the deletion of *FBN1*. Patients 8 and 9 have psychomotor retardation and dysmorphic features. In addition, patient 9 has an extended phenotype with more severe neurological impairment and lack of skin and hair pigmentation. The deleted genes Myosin 5A (*MYO5A*, MIM 160777) and RAS associated protein (*RAB27A*, MIM 603868) could play a role in the phenotype of this girl. Mutations in *MYO5A* and *RAB27A* cause Griscelli syndrome type 1 and type 2 respectively. These rare autosomal recessive disorders are characterized by partial albinism, immunological problems and/or neurological impairment. Further studies of these genes on the normal allele are pending. The three previously published patients[18,19,21], also have a deletion extending beyond the *FBN1* gene. Faivre *et al*[19] describes a teenage girl with a deletion of 2.97 Mb with some skeletal features of MFS and mitral valve prolapse, but absence of aortic root dilatation and ectopia lentis. Apart from language disabilities she was not mentally retarded. The size of the deletion was characterized by array CGH and 13 genes were found to be deleted including *FBN1*. In Figure 3 the size and position of this deletion is compared with the deletions described in this study. The patients described by Adès *et al*[18] and Hutchinson *et al*[21] have psychomotor retardation with additional features, probably due to haploinsufficiency of other genes. The size of the published deletions is unknown but in the patient described by Hutchinson *et al* the *MFAP1* locus was deleted. That means that this deletion is extending more centromeric than our deletions. No further information is available about the breakpoints in these patients. Hutchinson *et al*[21] found that in the deletion patient the fibrillin-1 protein and mRNA levels were significantly higher than expected for a single *FBN1* allele. They suggest that the clinical variability in MFS could

be due to variable *FBN1* expression from the normal allele. They compared their results with three members of one family with a premature termination codon (PTC) mutation and showed that the variable expression in these individuals appeared to correlate with variability in *FBN1* expression of the normal allele and not with variable rates of nonsense-mediated decay (NMD).

Apart from the PTC mutations where the phenotype will be due to partial haploinsufficiency caused by NMD and a dominant negative effect of the fibrillin-1 molecules which escape NMD, few other mutations have been described leading to a haploinsufficiency state. Milewicz *et al*[30] described a patient with only skeletal features of MFS and a missense mutation in the *FBN1* gene. This mutation co-segregated with tall stature in the family. The mutation disrupted the normal processing of one-half of secreted profibrillin in fibrillin. Half the normal amount of fibrillin was shown to be deposited in structurally normal-appearing microfibrils. They hypothesized that this mutation mimics a null allele of *FBN1* and leads to a milder phenotype, analogous to the null allele of *COL1A1* which leads to the milder form of osteogenesis imperfecta [31,32]. Our results, however, show that haploinsufficiency of *FBN1* is sufficient for the development of the full clinical expression of MFS with some carriers exhibiting severe features. For instance, two of our patients (patients 2 and 7) needed aortic surgery at a relatively young age (age 27 and 34, respectively). Additional evidence supporting the haploinsufficiency model are the two patients with classical MFS described by Mátyás *et al* [16] with a deletion of the putative regulatory and promoter region of *FBN1*, resulting in complete loss of transcription of the corresponding allele. Both patients fulfilled the Ghent criteria with major manifestations in the skeletal and cardiovascular systems, but no ectopia lentis. The authors conclude that these two patients represent true haploinsufficiency.

Although no mouse model is known with a complete deletion of one *FBN1* allele, the mouse model of Pereira suggests that there is a threshold of expression of the normal allele below which the abnormal phenotype will develop [33,34]. Mice with a heterozygous targeted mutation leading to 15% expression of a normal product have no abnormal phenotype, while the mice with the same mutation on both alleles have severe abnormalities comparable with the neonatal MFS phenotype.

Judge *et al* [35] used yeast artificial chromosome-based transgenesis to overexpress a disease-associated mutant form of human fibillin-1 (C1663R) on a normal mouse background. These mice showed no abnormalities whereas a heterozygous comparable cysteine mutation in mice leads to the Marfan phenotype and histological changes as seen in heterozygous human. They showed that haploinsufficiency for the WT protein can be a significant factor in the pathogenesis of MFS when combined with an abnormal *FBN1* allele. In keeping with the hypothesis of the critical contribution of haploinsufficiency, introduction of a wild-

type transgene in the heterozygous mouse rescues the aortic phenotype.
How the lower production and deposition of fibrillin-1 will affect the TGFβ signaling pathway, and how it leads to the aortic and skeletal features is subject for debate. Recent evidence of the role of the TGFβ signaling pathway in the pathogenesis of MFS shows that fibrillin has a stabilizing effect on the latent TGFβ binding protein-1 (LTBP-1) in the extra cellular matrix (ECM) [34-37]. *LTBP1* plays a role in the release of TGFβ in the ECM. Mice with a Marfan phenotype and a centrally deleted *FBN1* allele showed marked dysregulation of TGFβ activation and enhanced signaling [38]. They hypothesize that deficiency of fibrillin-1 causes excessive amounts of active TGFβ to be liberated from the matrix. This might as well be the case in the patients with a deletion of the *FBN1* gene. Increased TGFβ signaling is also shown in aortic tissue of patients with Loeys-Dietz syndrome [8]. The exact mechanism how changes in TGFβ signaling lead to such a specific phenotype has still to be elucidated. Ectopia lentis was present in at least two of our patients. The published patients with molecularly proven complete *FBN1* allele deletions did not exhibit ectopia lentis[18-21]. None of the patients with a *TGFBR2* or *TGFBR1* mutation have ectopia lentis. We hypothesize that ectopia lentis in our patients is caused by the lower production of fibrillin-1 and not by perturbation of the TGFβ signaling. This is in keeping with the observation that mutations in *LTBP2* (latent transforming growth factor beta binding protein 2) cause recessive eye abnormalities including ectopia lentis[39,40]. LTBP2 is the only member of the LTBP family not to bind to latent forms of TGFβ, and is thought to have an important structural role in the ciliary body together with fibrillin-1[41].

In conclusion, our patients with a complete *FBN1* allele deletion show that haploinsufficiency has a major contribution to the pathogenesis of MFS and can lead tot the whole spectrum of MFS. We hypothesize that the skeletal and aortic phenotype are caused by aberrant TGFβ signaling and the ocular phenotype by the lower production of the fibrillin-1 microfibrils.

Conflict of interest
The authors declare no conflict of interest.

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Supplementary information

Family 1 (patients 1-5) (Figure 1)

Patient 1 was diagnosed with MFS at the age of 4 years, when bilateral ectopia lentis and mitral valve prolapse were detected. At the age of 36 years bilateral lensextraction was performed. At the age of 22 years she had a height of 175 cm (+0.7 SDS) with normal body porportions, arachnodactyly with bilateral positive wrist and thumb signs, general joint hypermobility, mild scoliosis and varicosis. She had a mild non-progressive aortic root dilatation.

Patient 2 is a younger sister of patient 1. She had progressive aortic root dilation, for which elective root replacement was performed at the age of 33 years. Prior to the aortic root surgery she delivered two children by caesarian section after uncomplicated pregnancies. She had high myopia, but no ectopia lentis. Physical examination revealed a normal height of 174.5 cm with normal body proportions. She had a long face, arachnodactyly with positive wrist and thumb signs, camptodactyly of the right fifth finger and mild scoliosis. As a child she was operated for recurrent inguinal hernias. She fulfilled the Ghent criteria for the diagnosis MFS when the mutation or a first affected family member is accounted for. Her first child (patient 3) was a boy with marfanoid build with a height of 179 cm (+1.4 SDS) at the age of 14, with an arm span/ length ratio of 1.04. He had a high arched palate with crowding of teeth, arachnodactyly, pectus carinatum, pes planus, and general joint hypermobility. No ectopia lentis was found. He had a dilated aortic root. His sister (patient 4) had a marfanoid build with a long and narrow face, high arched palate, pectus carinatum, mild scoliosis and long narrow feet with flat arches. At the age of 11 years she had a height of 185 cm with normal body proportions. Cardiologic examination showed aortic root dilatation with mitral valve prolapse. On ophthalmologic examination she had dubious ectopia lentis.

Patient 5, the sister of patient 1 and 2, had a marfanoid build with typical facial appearance, high arched palate, blue sclera, arachnodactyly, bilateral flexion contractures of the elbows, scoliosis of 32 degrees, general joint hypermobility, permanent clavicula dislocation, mild pectus excavatum and varicosis. At the age of 18 years her height was 174.6 cm (+0,8 SDS) with normal arm span and sitting height. Cardiovascular examinations showed a progressive aortic root dilatation. Apart from translucent irides she had a normal ophthalmologic examination.

The parents of patient 1, 2, and 5 had no clinical features of MFS but the mother was shown to be mosaic for the deletion.

All members of family 1 had normal intelligence.

Patient 6

Patient 6 was a high school student. She was referred for a severe pectus excavatum. Her pectus deformity was corrected by a nuss bar at the age of 14 years. At examination at the age of 17 years her height was 179.5 cm (+1.5 SDS) with normal body proportions. She had a long narrow face with downslanting palpebral fissures, a high arched palate with crowded teeth and an asymmetric nose. She had no arachnodactyly, but a severe pectus deformity, general joint hypermobility, and bilateral flat feet. She had stretch marks on the lower lumbar region (normal finding for females of this age). She had normal aortic root dimensions, but the root has a cloverleaf appearance on ultrasound examination. Ophthalmologic examination revealed a minimal lens subluxation on the right. An MRI scan of the lower spine revealed lumbosacral dural ectasia.

Patient 7

Patient 7 is a male patient presenting with hip dislocation at 3 months of age. He was treated with splints for a period of about 3 years. At the age of 8 years a severe pectus excavatum was corrected. At the age of 18 years he suffered from a pneumothorax, due to rupture of an apical bulla which had to be removed surgically. He had progressive aortic root dilation, and an elective aortic root replacement was performed at the age of 27 years. Furthermore, he had a dilatation of the pulmonary artery and the abdominal aorta. Ophthalmologic examination revealed bilateral superior lens dislocation. Physical examination showed an outspoken marfanoid habitus with a height of 201 cm (+2.4 SDS) with normal arm span and sitting height. He had a long face with high arched palate, dental crowding, general joint hypermobility, arachnodactyly with positive wrist and thumb signs, pectus excavatum and numerous striae.

Clinical examination of his parents revealed no clinical, ophthalmologic or cardiologic abnormalities.

Patient 8

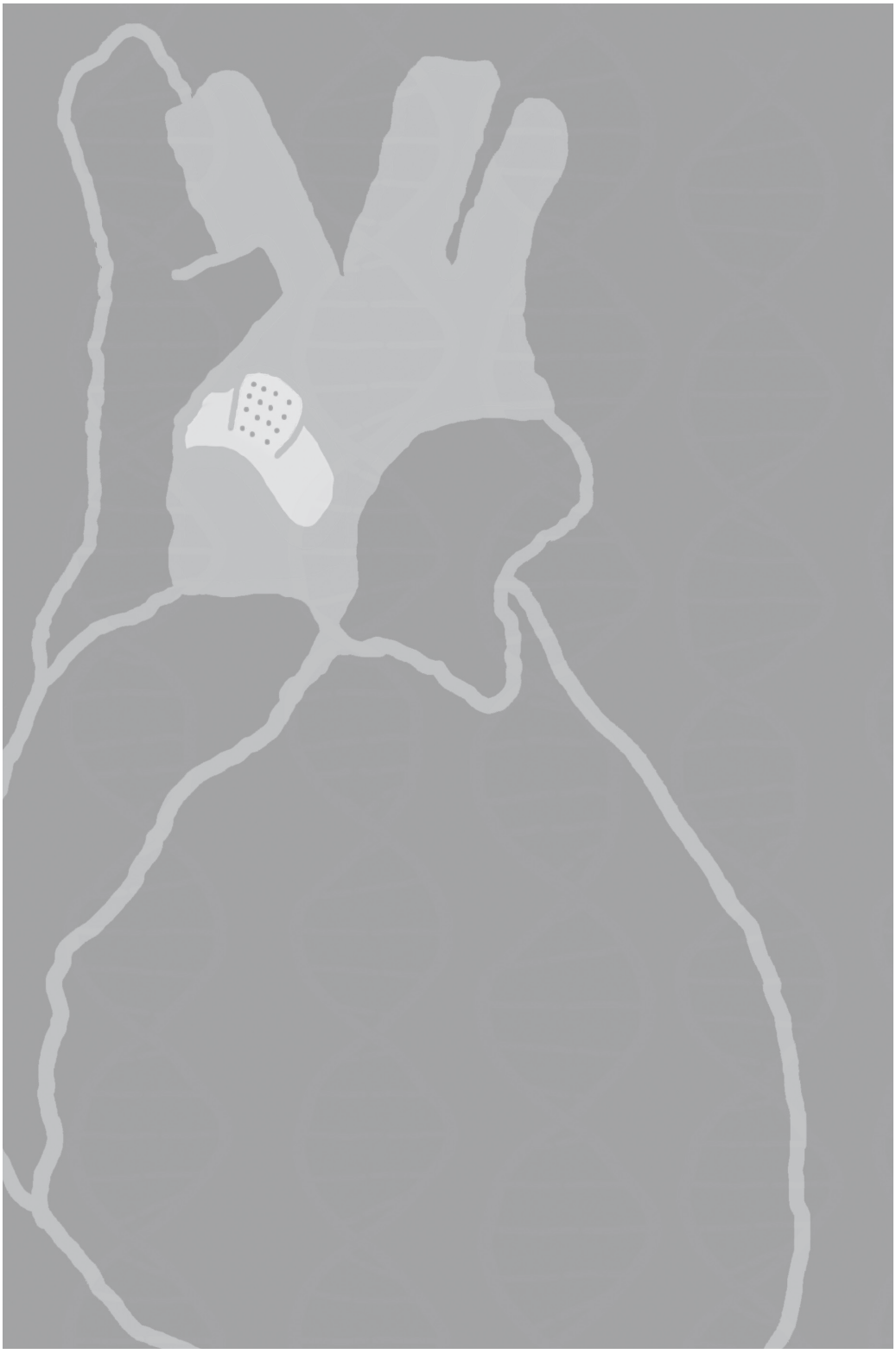
This 4-year old girl presented at the age of 2.5 years with psychomotor retardation. On examination her height was 91.5 cm (-0.4 SDS) with an arm span of 91 cm (+2.6 SDS for height) and a sitting height of 52.5 cm (normal for height). She had mild facial dysmorphisms with downslanting palpebral fissures and hypertelorism. She had arachnodactyly with positive thumb and wrist signs and flat feet with long toes. Her thorax was asymmetric and she was hypotonic. Ultrasound of heart and aorta revealed billowing of the mitral valve and tricuspid valve with grade 1 regurgitation. The aortic root measurements were on the 95th percentile. Apart from translucent irides, eye examination showed no abnormalities.

Patient 9

Patient 9 presented with psychomotor retardation with non-progressive ataxia. At the age of 4 years she had joint hyperlaxity, pectus excavatum, a very pale skin and hair without other ectodermal manifestations. She had facial dysmorphisms consisting of a brachycephalic skull, long philtrum, broad nose and prognathism. She had arachnodactyly and flat narrow feet. Her height is within normal limits. At the age of 13 years her height was 164,4 cm (+0,6 SDS) with a sitting height/height ratio of -1,1 SDS. She had a normal weight. Cardiac examination revealed a mitral valve prolapse but no aortic root dilatation. Ophthalmologic examination showed anisohypermetropia but no lens dislocation.

Patient 10

Patient 10 presented at the age of 4 years because his mother was diagnosed with MFS. Physical examination showed a tall stature, mild thoracic scoliosis, pectus carinatum, hypermobility and arachnodactyly. At the age of 8 years his height was 140.7 cm (+2 SDS) and he had an arm span of 149 cm. Cardiac examination showed an aortic root on the 95th percentile and mild mitral valve insufficiency. His mother had an normal height, an aortic root dilatation, but no ophthalmologic abnormalities. The mother was not tested for the deletion.



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An unanticipated copy number variant of chromosome 15 disrupting *SMAD3* reveals a three-generation family at serious risk for aortic dissection

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Keywords
aortic aneurysm; aortic dissection; copy number variant; osteoarthritis; *SMAD3*

Abstract

Several genes involved in the familial appearance of thoracic aortic aneurysms and dissections (FTAAD) have been characterized recently, one of which is *SMAD3*. Mutations of *SMAD3* cause a new syndromic form of aortic aneurysms and dissections associated with skeletal abnormalities. We discovered a small interstitial deletion of chromosome 15, leading to disruption of *SMAD3*, in a boy with mild mental retardation, behavioral problems and revealed features of the aneurysms-osteoartritis syndrome (AOS). Several family members carried the same deletion and showed features including aortic aneurysms and a dissection. This finding demonstrates that haploinsufficiency of *SMAD3* leads to development of both thoracic aortic aneurysms and dissections, and the skeletal abnormalities that form part of the aneurysms-osteoarthritis syndrome. Interestingly, the identification of this familial deletion is an example of an unanticipated result of a genomic microarray and led to the discovery of important but unrelated serious aortic disease in the proband and family members.

Introduction

Several genes are known to cause syndromic and non-syndromic forms of aneurysms and dissections including *FBN1*, *TGFBR1*, *TGFBR2*, *ACTA2*, *MYH11* and *MYLK*. These genes encode either structural proteins associated with connective tissue, members of the TGF-β pathway or components or regulators of the contractile unit of vascular smooth muscle cells (SMCs). Recently, mutations in *SMAD3* were shown to cause a new syndromic form of aortic aneurysms and dissections associated with skeletal abnormalities (1). The authors proposed the name aneurysms-osteoarthritis syndrome (AOS). SMAD3 is another member of the TGF- β pathway and is essential for TGF- β signal transduction (2).
We describe the molecular and clinical analysis of a three-generation family, including eight members with an interstitial deletion of the long arm of chromosome 15 containing *SMAD3*. Although the deletion was initially identified in the proband, who showed mild mental retardation, it was not related to this phenotype. Unforeseen findings resulting from untargeted diagnostic testing such as microarray analysis or whole genome sequencing will be encountered with increasing frequency and will soon represent a major

challenge for counselors. In the case of the family described here, we were able to identify additional at-risk family members and could offer them appropriate cardiovascular follow-up and treatment options.

Patients and methods

Patients

The (simplified) pedigree of the family is depicted in Fig. 1. The proband (IV-1) was a boy with mild mental retardation and behavioral problems who was eligible for SNP-array analysis as part of mental retardation screening. Following the detection of two copy number variants (CNVs), family members were approached and asked to participate in SNP-array analysis. As one of the CNVs disrupted the gene *SMAD3*, which is associated with vascular and skeletal abnormalities, affected family members underwent a thorough physical and cardiologic examination, including transthoracic echocardiography (TTE) and magnetic resonance imaging (MRI), and a skeletal survey of the hands, elbows, spine, hips, knees and feet.

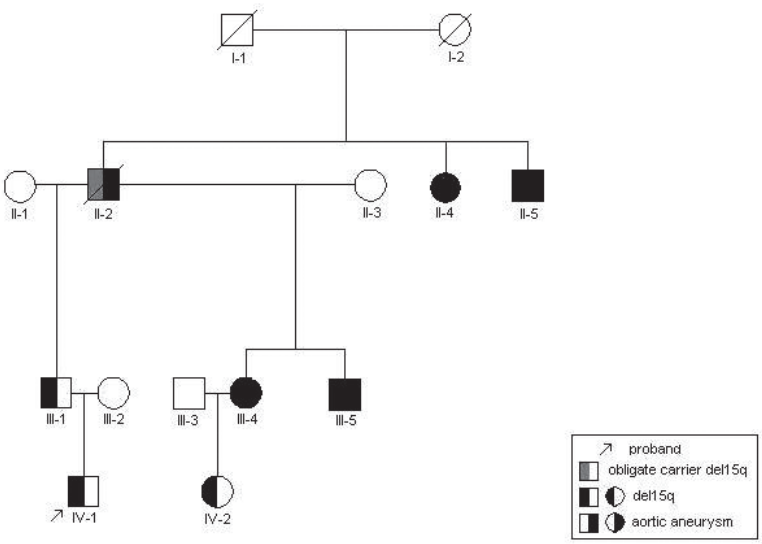

Figure 1. Pedigree of the family. Unaffected family members and family members who were not investigated are not shown.

High density microarray analyses, SNP arrays

The Affymetrix GeneChip Human Mapping 250K *NspI* array (Affymetrix, California, USA) contains 262.000 25-mer oligonucleotides with an average spacing of approximately 12 kb. Subject DNA (250 ng) was processed according to the manufacturer's instructions (www.Affymetrix.com). SNP copy number was assessed using the software program Copy Number Analyzer for Genechip (CNAG) Version 2.0 (3).

Transthoracic echocardiography

The diameters of the thoracic aorta at the level of the sinus of Valsalva, sinotubular junction and ascending aorta were measured in the left parasternal long axis view from leading edge-to-leading edge at end diastole, according to the recommendations of the American Society of Echocardiography (4). Body surface area (BSA) was calculated according to the DuBois formula (BSA (m^2) = 0.007184 × height (cm) 0.725 × weight (kg) 0.425). Measured values for adults were plotted against nomograms derived from individuals with normal cardiac findings and related to gender, age and body surface area (5). For children, Z-scores of the aortic root and ascending aorta were obtained from body surface area-related nomograms derived from normal children (6).

Magnetic resonance imaging

Imaging of the entire aorta and large arteries, including the cerebral arteries, was performed in the five adult family members (Fig. 1, II-4, II-5, III-1, III-6 and III-4).

Magnetic resonance (MR) imaging was performed on a 1.5 Tesla scanner (Philips Intera, Philips Medical Systems, Best, the Netherlands). Survey images were used for planning the scans. Body coil was used for MR angiography and 30 mL of a gadolinium-containing contrast agent (Dotarem; gadoteric acid 0.5 mmol/mL, Guerbet, Aulnay-sous-bois, France) was injected in an antecubital vein with an injection speed of 2 mL/sec. After bolus timing, MR angiography of the aorta was acquired during breath-hold. Scan parameters: 3D high resolution T1-fast field echo sequence, 75 mm coverage, field of view (FOV) 500 mm, repetition time (TR) 4.6 ms, echo time (TE) 1.3 ms, flip angle (FA) 40°, reconstructed voxel size 0.98 x 0.99 x 1.5 mm^3. Imaging of the carotid arteries (from aortic arch to head including circle of Willis) was performed using a head-neck coil and a 3D time-of-flight sequence. Scan parameters: FOV 250 mm, TR 21 ms, TE 6.9 ms, FA 20°. Reconstructed voxel size 0.82 x 0.82 x 1.0 mm^3.

Skeletal survey

Six family members underwent a radiographic skeletal survey of the whole spine, hands, elbows, knees (in weight-bearing position), ankles and feet. Osteoarthritis was

scored as positive when intervertebral disc space or joint space narrowing, osteophytes or reactive sclerosis was present. Presence of osteochondritis dissecans, spondylolysis, spondylolisthesis and scoliosis were also scored.

Results

SNP-array analysis of the proband (Fig. 1, IV-1) revealed two CNVs. The first variant was an interstitial duplication of the long arm of chromosome 22q11.2, of a maximum 3.2 Mb (130 SNP probes), from 17.020.301 bp to 20.258.915 bp (Ensembl release 54). The second variant was an interstitial deletion of the long arm of chromosome 15q22.3q23, of a maximum 194.8 kb (11 SNP probes), from 65.195.296 bp to 65.390.067 bp (Ensembl release 54) (Fig. 2a, b). The deleted region contained the three protein-encoding genes, *SMAD3*, *AC012568.7* and *IQCH* (Fig. 2c), and the proximal breakpoint was in intron 1 of *SMAD3*, resulting in the deletion of the gene from exon 2 onwards.

The proband's father (III-1) had the same copy number abnormalities as his son, and a further five family members (II-4, II-5 III-4, III-5 and IV-2) carried the interstitial deletion of chromosome 15q22.3q23 but not the interstitial duplication of chromosome 22q11.2 (Table 1).

The clinical features of the family are summarized in Table 1. Seven family members were investigated, including the proband and a 4-year-old child. Despite the fact that he could not be examined, the proband's grandfather (II-2) is an obligate carrier of the chromosome 15 deletion. The great-grandparents of the proband (I-1 and I-2) died at the age of 89 and 92 of unrelated disorders.

Of the five adults investigated, four experienced an aneurysm of the thoracic aorta. All four showed dilation of the aortic root, with three also showing dilation of the ascending aorta. MR imaging of patients II-4, II-5 and III-1 showed clear tortuosity (due to elongation) of the aorta, the carotid arteries and in III-1, of the superior mesenteric artery. The MR-angiography images of II-4 and II-5 (brother and sister) are depicted in Fig. 3a, b. No aneurysms or clear tortuosity of the cerebral arteries were detected. Although the 12-year-old proband and his 4-year-old cousin (IV-1 and IV-2) showed normal absolute aortic diameters, the Z-scores of the ascending aorta were higher compared to the Z-scores of the sinotubular junction. All five adult family members investigated for skeletal abnormalities showed scoliosis and four also exhibited intervertebral disc space narrowing. One family member (II-5) also had a grade one spondylolisthesis at lumbar level 2-3, secondary to facet artrosis. X-rays of the hands and spine (Fig. 3c-f) of a 68-year-old woman, II-4, showed both severe deforming osteoarthritis in the distal and proximal interphalangeal joints, without degeneration

of the scaphotrapezotrapezoidal (STT) and first carpometacarpal (CMC1), and severe degeneration of the whole spine. Neither spondylolysis nor ostechondritis dissecans was found in the investigated family members.

Apart from varicosis in III-1 and a soft skin in II-4, no skin abnormalities were found, and in particular, thread veins were absent. An abnormal uvula or a high narrow palate was found in four family members. Two family members showed inguinal hernias, including one that was recurrent (II-5) but possibly due to work-related physical exertion. Diaphragmatic hernias were detected by MRI in two patients (II-4 and II-5).

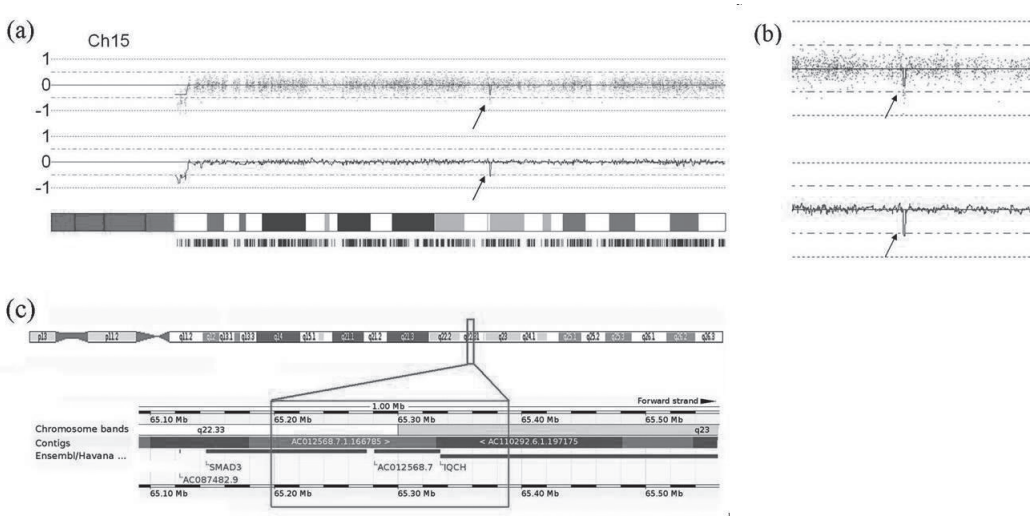

Figure 2. SNP array results – proband. (a) Chromosome 15 plot obtained by SNP array analysis, with black arrows indicating the deletion. (b) Detailed view of the 15q22.3q23 deletion. (c) Position of the deletion on chromosome 15 (Ensemble release 54, May 2009). The three known protein-encoding genes affected by the deletion, SMAD3, AC012568.7 and IQCH , are represented by the bars beneath the chromosome.

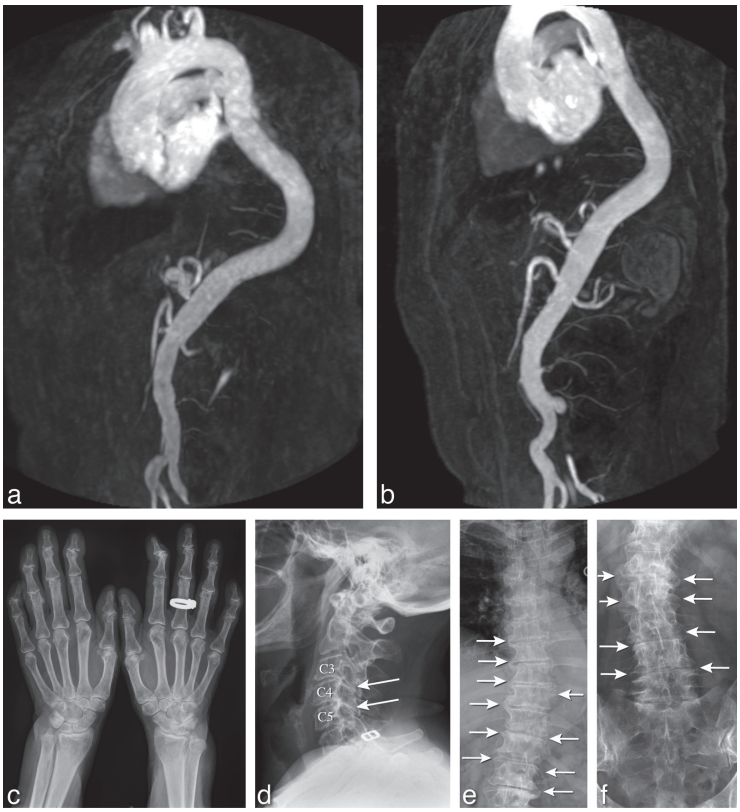

Figure 3. (a, b) Gadolinium contrast agent enhanced magnetic resonanceangiography (a, b) in a 69-year-old female patient (a, II-4)) and 64- year-old male patient (b, II-5); sister and brother. Both had severe elongation of the descending aorta with similar presentation of leftbackward bulging of the aorta (a, b). Both showed elongation of the carotid artery on the left side (not shown). (c–f) Conventional X-rays of the 69-year-old female patient (II-4) with osteoarthritic changes of the hands, showing osteoarthritic changes with narrowing of multiple interphalangeal joints and subluxation of distal interphalangeal joints 2–5 on left and 2, 3, 5 on right (c). No degenerative changes in first carpometacarpal or scaphotrapezotrapezoidal joints of wrists. Upper part of cervical spine showing narrowing of intervertebral disk spaces C3-4 and C4-5 and facetarthrosis (d, arrows). Thoracic (e) and lumbar (f) spine showing scoliosis convex to the left on thoracic level and to the right on lumbar level. Narrowing of intervertebral disk spaces and extensive degenerative spondylophytes on thoracic and lumbar levels (arrows).

Table 1. Laboratory investigations and clinical features

Pedigree number	II-2	III-1	IV-1 (proband)	III-4	IV-2	III-5	II-4	II-5
sex	M	M	M	F	F	M	F	M
age at examination; d: age at death	d54	42	12	33	4	18	69	64
BSA	u	2.2	1.9	1.9	0.8	2.3	1.9	1.9
Molecular results								
dup22q	ni	Yes	Yes	No	No	No	No	No
del15q (including *SMAD3*)	ni[1]	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Cardiovascular								
dilatation aortic root (cm)*	>2[2]	No (3.8)	No (2.9)	Yes (3.8)	No (2.1)	Yes (4.3)	Yes (4.0)	Yes (4.3)
dilatation ascending aorta (cm)*	Yes[2]	No (3.5)	No[3] (2.9)	No (2.9)	No[3] (2.0)	Yes (3.4)	Yes (4.7)	Yes (4.2)
dissection ascending aorta	Yes	No	No	No	No	No	No	No
aneurysms of other arteries	u	No	ni	No	ni	No	No	No
arterial tortuosity	u	Yes	ni	No	ni	No	Yes	Yes
mitral valve abNormalities	No	No	No	No	No	No	No	No
other heart disease	ihd	No	No	No	No	No	No	No
Skeletal								
pectus deformity	u	No	No	No	No	No	No	No
scoliosis	u	No[4]	Yes	Yes	No	Yes	Yes	Yes
joint laxity	u	No	No	No	No	Yes	No	No
Joints								
intervertebral disc degeneration	u	Yes	No	Yes	ni	No	Yes	Yes
osteoarthritis (location)	u	No	No	No	ni	No	Yes (hand, knee, hip)	Yes (hand, ankle, elbow, hip)
osteochondritis dissecans	u	No	No	No	ni	No	No	No
Craniofacial								
hypertelorism	u	Yes	Yes	No	No	No	No	No
abNormal palate or uvula	u	Broad uvula	Mild cleft uvula	High and narrow palate	No	No	No	Broad uvula
Skin/hernia's								
velvety skin	u	No	No	No	No	No	Yes	No
umbilical or inguinal hernia	u	No	No	No	No	No	Yes	Yes[5]
diaphragmatic hernia	u	No	No	No	ni	No	Yes	Yes
thread veins	u	No	No	No	No	No	No	No
varicosis	u	Yes	No	No	No	No	No	No
Other	u	Kyphosis; hearing deficit; atheroma cysts; learning difficulties; flexible hip joints; hyperkyphosis	Behavioral problems; slow motor and mental development; learning difficulties; ADHD	Striae	Strabismus	Learning difficulties; ADHD; striae		Sponylolisthesis L2-L3

*) measurements obtained by TTE; 1) obligate carrier; 2) observation during operation; 3) the Z-score of the ascending aorta was wider than the Z-score of the aortic root; 4) mild deviation of the spine; 5) recurrent inguinal hernias associated with physical labor. Abbreviations: u, unknown; ni, not investigated; ihd, ischemic heart disease; ADHD, attention deficit hyperactivity disorder.

Discussion

In this report we describe an incidental finding from an unrelated microarray analysis that led to the discovery of a serious health risk for the family involved. Microarray analysis is widely used in the diagnostic work-up of patients with mental retardation, with or without congenital abnormalities, and while much emphasis is placed in the literature on the likelihood of a pathogenic role for a CNV in the context of the patient's phenotype, the use of whole genome analysis in a diagnostic setting may detect variants unrelated to current clinical findings (7, 8). The identification of the family described here was due to a SNP-array analysis carried out as a component of mental retardation screening. The duplication of chromosome 22q11.2, found in both the proband and his father, is a frequently encountered CNV and has been associated with an extremely variable phenotype ranging from completely normal to mental retardation and congenital abnormalities. The duplication is often encountered in a parent with no or a mild phenotype (9, 10) and it is thus probable that the learning and behavioral problems of the proband are, at least in part, due to this duplication.

The interstitial deletion of chromosome 15q22.3q23 disrupts *SMAD3*, and excepting exon 1, leads to deletion of the whole coding region of *SMAD3* and haploinsufficiency. As recent reports demonstrated the role of mutations in *SMAD3* in an autosomal dominant syndromic form of aneurysms and dissections (1, 11, 12), it was decided to inform the family of the possible risk for aortic disease and to initiate a family study. In addition to the proband, seven other family members carried the deletion, including one obligate carrier. In addition to *SMAD3*, the deletion encompasses the genes *AC012568.7* and *IQCH* and has not been previously described in the normal population. All carriers showed vascular involvement and the majority also showed skeletal involvement. It is not clear whether the diaphragmatic hernia found in two carriers is due to the *SMAD3* deletion (Table 2).

Our findings are in accordance with those of previously described *SMAD3* families (1, 11, 12), including features of AOS with highly variable expression ranging from very mild features to dissection of the thoracic aorta. Thirteen *SMAD3* mutations have been described to date and are summarized in Table 2 (1, 11, 12, 13). Three reports have described 12 mutations in 11 families, with all cases recruited from TAAD families, implying a bias towards an aortic phenotype (1, 11, 12). This is further emphasized by the patient group described by van de Laar et al. (12), of which 89% showed cardiovascular anomalies, predominantly thoracic aortic aneurysms but also including high percentages of aneurysms of the abdominal aorta, large arteries and cerebral arteries. Though not ascertained due to aortic disease, the family described here shows aortic involvement in five of the six adults carrying the

Table 2. Summary of *SMAD3* mutations described in the literature

Nucleotide change	Amino acid change	Exon	Predicted effect	Family	Number of carriers	Number of obligate carriers	Phenotype	Reference
A>T	p.Asn197Ile	4	proline rich linker region; probable NMD	spor case	1	0	Knee OA no investigations for other features of AOS	Yao 2003
c.859C>T	p.Arg287Trp	6	MH2 domain	Family 1	25	5	TAAD, AAA, ICA, IAA, OA, aortic and arterial tortuosity	van der Laar 2011; van de Laar 2012
c.741–742delAT	p.Thr247ProfsX61	6	MH2 domain: premature stop in exon 7; proven NMD; removes nearly complete MH2 domain, TGFBR1 target site and residues involved in homo and heterodimer formation	Family 2	2	1		
c.782C>T	p.Thr261Ile	6	MH2 domain	Family 3	2	0		
c.313delG	p.Ala105ProfsX11	2	MH1 domain; probable NMD	Family 7	1	0		
c.539_540insC	p.Pro180ThrfsX7	4	proline rich linker region; probable NMD	Family 8	1	0		
c.788C>T	p.Pro263Leu	6	MH2 domain	Family 6	1	0		
c.1045G/C	p.Ala349Pro	8	MH2 domain	Family 4	1	0		
c.1080dupT	p.Glu361X	8	MH2 domain	Family 5	1	0		
c.652delA	p. Asn218fs	5	premature stop	TAA549	7	6	TAAD, AAA, ICA, IAA, OA, aortic tortuosity	Regaldo 2011
c.836G>A	p.Arg279Lys	6	MH2 domain, predicted to affect hydrogen bond formations and XPO4 interaction (promotion of SMAD3 nuclear transport)	TAA071	7	2		
c.836G>A	p.Arg279Lys	6	MH2 domain, predicted to affect hydrogen bond formations and XPO4 interaction (promotion of SMAD3 nuclear transport)	TAA072	2	0		
c.715G>A	p.Glu239Lys	6	MH2 domain, predicted to affect hydrogen bond formations	TAA365	3	1		
c.235C>T	p.Ala112Val	2	not conserved in fruitfly, possibly damaging	TAA115	4	1		

Abbreviations: AAA, abdominal aortic aneurysms; AOS, aneurysms-osteoarthritis syndrome; IAA, iliac arterial aneurysms; ICA, intracranial aneurysms; OA, osteoarthritis; TAAD, thoracic aortic aneurysms and dissections

SMAD3 deletion. In the 12-year-old proband (Fig. 1, IV-1) and a 4-year-old cousin (Fig. 1, IV-2), the abnormal feature of an ascending aorta wider than the sinotubular junction was seen and could be the first sign of future dilatation. All five adult carriers investigated showed remarkable scoliosis, including four with radiologically proven intervertebral disc degeneration. The 68-year-old woman (II-4) with severe distal osteoarthritis of the hands showed normal STT and CMC1 joints, in contrast to cases described in the literature (1). This might well be the classic form of osteoarthritis and not linked to the disruption of *SMAD3*.

As little is yet known of the two other genes in the deleted region, a possible role in the phenotype cannot be entirely ruled out. *AC012568.7* encodes a protein of unknown function and the *IQCH* gene is thought to be associated with tall stature (14).

We conclude that the similarities of the vascular and skeletal phenotype in the family described here to those of other families with mutations in *SMAD3* is the result of the disruption and resulting haploinsufficiency of *SMAD3*. The discovery of a serious health risk unrelated to the phenotype of the proband is a telling example of the potential impact of whole genome analysis on family members. Although current literature contains few articles describing unexpected results, this family underlines the importance of precise genetic interpretation of a CNV and the genes involved in order to provide appropriate information to the patient and family. The identification of at-risk members of this particular family allowed early detection of vascular disease, appropriate counseling and facilitated possibly life-saving follow-up and treatment.

Conflict of interest statement

None of the authors has a conflict of interest to declare.

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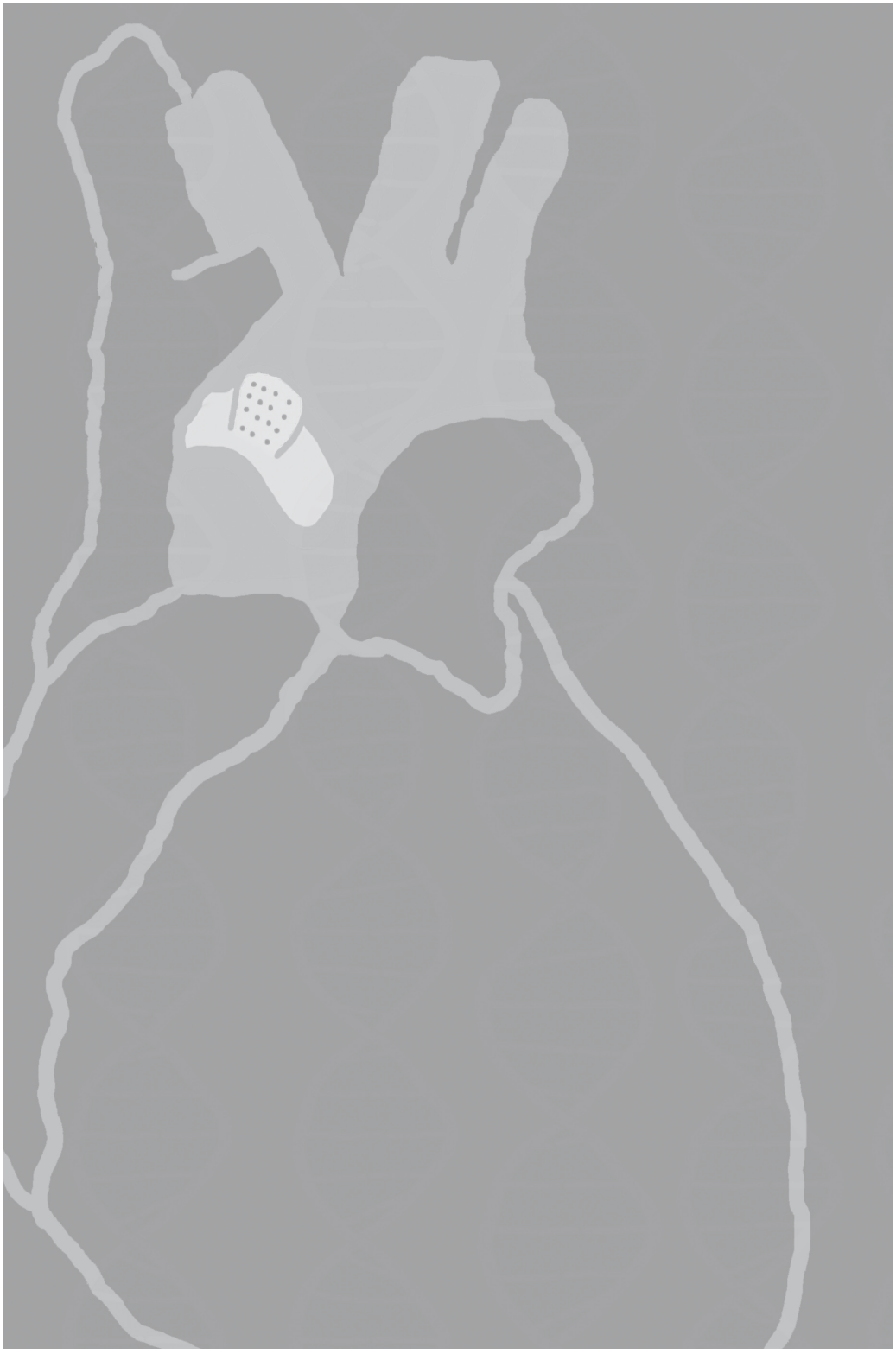

7

Intracranial hypertension in 2 children with Marfan syndrome

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Introduction

Marfan syndrome (OMIM 154700) is a connective tissue disorder with multisystemic involvement and autosomal dominant inheritance due to mutations in the fibrillin-1 gene *(FBN1)*.[1] Here we report two unrelated children with Marfan syndrome and recurrent intracranial hypertension. To our knowledge, intracranial hypertension (IH) has never been associated with Marfan syndrome.

Case report

The first case is an 11-year-old boy with Marfan syndrome who presented with headache, nausea and vomiting on several occasions. The intracranial pressure at these events was measured to be above 20 cm H_2O. His symptoms disappeared after drainage of at least 15 cc of cerebrospinal fluid (CSF). Extensive investigations revealed only mild iron deficiency anemia. Treatment of the anemia did not prevent a new episode of high intracranial pressure. Magnetic resonance imaging (MRI) of the brain showed bilateral choroid plexus cysts, slightly dilated ventricles, and a small arachnoid cyst of 1.5 cm in the cisterna magna (Figure 1A). The spinal cord MRI showed sacral dural ectasia with radicular cysts and an anterior sacral meningocele (Figure 1B and 1C). The diagnosis Marfan syndrome was made in the first year of life because of lens subluxation and a positive family history. At age 11 he has mild skeletal manifestations of Marfan (pes planus) and a mildly dilated aortic root. His length is on the 84th centile, his weight on the 80th centile and his head circumference on the 50th centile. He has had several ophthalmological operations for lens luxations and strabismus. He fulfills the international criteria for Marfan syndrome.[2] A homozygous missense mutation in exon 60 of the fibrillin-1 gene was found, leading to the amino acid substitution p.Asp2485Val. Both parents carry one copy of the mutation and exhibit very minor signs of Marfan syndrome. His affected sister also has a homozygous mutation. She has the classical manifestations of Marfan syndrome but does not have signs of high intracranial pressure.

The second case was a 7.5 year old girl who was evaluated for complaints of headache, vomiting, diplopia and a tonic clonic epileptic seizure. Apart from papil edema there were no neurological signs. After excluding intracranial mass lesions or vascular lesions, a lumbar puncture was done revealing a CSF pressure of 50 cm H_2O. Repeated lumbar punctures were necessary to alleviate her symptoms. Medical treatment was started with acetazolamide that relieved her headache and controlled the CSF pressure (8 cm H_2O). After discontinuing the medication the symptoms returned promptly. A ventricular

peritoneal drainage operation was performed with excellent result. Physical examination showed a length far above the 99th centile, arm span on the 75th centile, weight on the 80th centile and head circumference on the 90th centile. Her joints were hypermobile. Because of the high intracranial pressure and her body habitus, extensive analysis was performed for connective tissue disorders and diseases causing high intracranial pressure. Endocrinological tests, X-rays of the vertebral column, an extensive metabolic screen, including vitamine disorders, showed no abnormalities. MRI of the brain and spinal cord showed narrowing of the left transverse sinus and mild ectasia of the dural sac. At echocardiographic examination mild prolapse of the mitral- and tricuspid valves and aortic root dilation were detected. The clinical diagnosis Marfan syndrome was made according to the international criteria.[2] The diagnosis was confirmed by a de novo missense mutation in the fibrillin-1 gene (7741T>A) leading to the amino acid substitution Cys2581Ser.

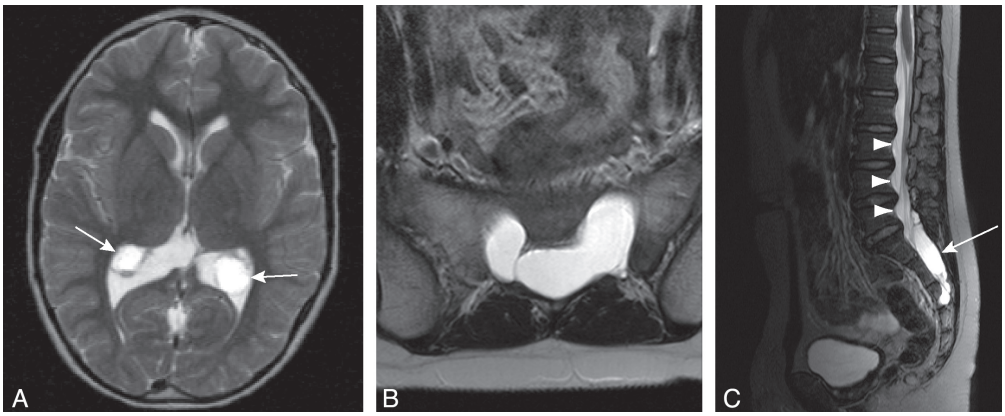

Figure 1. Magnetic resonance T2-weighted turbo spin-echo images of the brain and spine of case 1. (A) Transverse brain image demonstrating bilateral plexus choroideus cysts (arrows). (B) Transverse image showing the anterior sacral meningocele. (C) Sagittal image of the lumbosacral spine showing dural ectasia (arrow) and scalloping of the posterior lumbar vertebral bodies (arrowheads).

Discussion

Although severe headache has been reported in Marfan syndrome due to intracranial hypotension caused by CSF leakage from dural ectasia,[3,4] this is the first report of intracranial hypertension in Marfan patients. The choroid plexus cysts and the arachnoid cyst present in case 1 might have contributed to the raised intracranial pressure by impairment of CSF flow. Cerebrovenous sinus abnormalities, as present in case 2, are frequently found in idiopathic intracranial hypertension but whether such findings result from or cause the raised pressure is still unknown.[5] The intracranial lesions as described in case 1 and 2 have not been previously associated with Marfan syndrome.

Given the low incidence of both intracranial hypertension in children and Marfan syndrome, the concurrence of intracranial hypertension with Marfan syndrome in two young patients is unlikely to be coincidental.[6] Marfan syndrome, and possibly other connective tissue disorders, may confer a predisposition to developing intracranial hypertension. Therefore, patients with Marfan syndrome and unexplained symptoms of headache and vomiting should be promptly investigated for possible intracranial hypertension, and conversely, in young patients with unexplained intracranial hypertension possible signs of Marfan syndrome should be noted and, if so, further investigations instigated.

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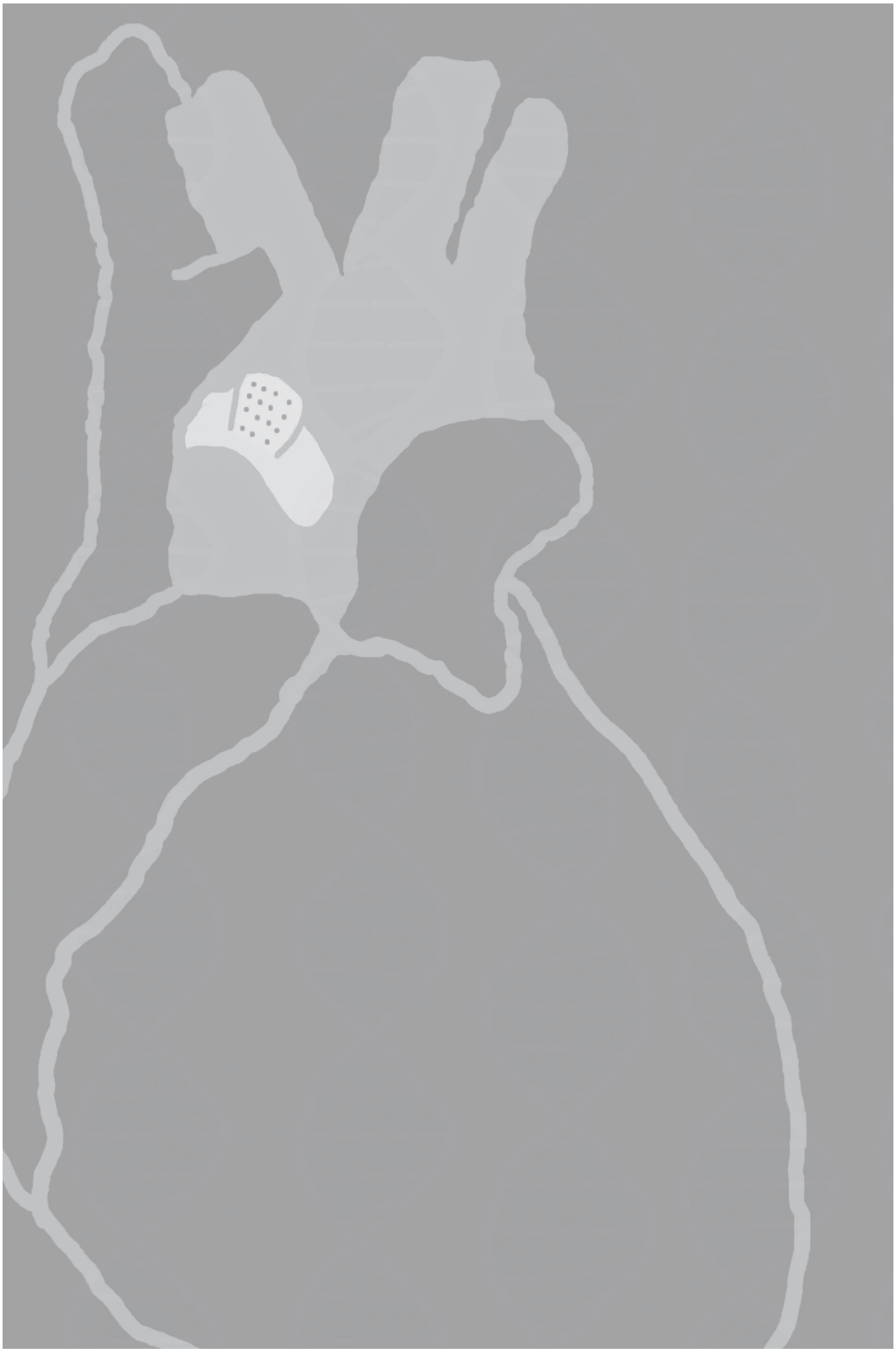

8

Summary and discussion

Summary

Thoracic aortic aneurysms and dissections have a high morbidity and mortality rate. Learning more about the aetiology and mechanisms of this aortic disease is a challenge, but may provide the opportunity to offer adequate treatment to at risk individuals. In contrast to abdominal aortic aneurysms, mutations in single genes play an important role and when a predisposing genetic factor for thoracic aortic disease has been identified in an individual, appropriate monitoring and timely surgical intervention may prevent aortic dissection. Marfan syndrome is caused by mutations in *FBN1*, the gene encoding fibrillin-1, and is one of the best known disorders with a strong genetic predisposition for thoracic aortic aneurysms and dissections at a relatively young age.

This thesis concerns the clinical and genetic aspects of thoracic aortic aneurysms and dissections, in Marfan syndrome in particular. **Chapter 1** provides a general introduction to Marfan syndrome, FTAAD and other syndromes associated with thoracic aortic aneurysms and dissections. Special emphasis is given to current knowledge of the pathophysiology of aortic disease in Marfan syndrome. **Chapter 2** presents the Dutch multidisciplinary guidelines for diagnosis and management of Marfan syndrome. These guidelines contain practical directions for referring physicians and specialists involved in the recognition, diagnosis, monitoring and treatment of Marfan syndrome. The guidelines are evidence-based when possible, but in the absence of sufficient evidence, guidelines are based on expert opinion. The revised Ghent nosology for Marfan syndrome, established by an international panel of experts, is presented in **Chapter 3**. The new nosology places greater emphasis on aortic root aneurysm or dissection and on ectopia lentis. The presence of these two cardinal features is sufficient to allow the diagnosis of Marfan syndrome. In the absence of either feature, the presence of a first-degree relative with Marfan syndrome, a pathogenic *FBN1* mutation or a combination of systemic features is required to establish a firm diagnosis.

Chapter 4 describes missense mutations in *FBN1* that are predicted to substitute the first aspartic acid of various calcium-binding Epidermal Growth Factor-like (cbEGF) fibrillin-1 domains. One of the mutations was found in a homozygous state in three cases from a large consanguineous family. Thirteen heterozygous carriers of the same mutation had no major skeletal, cardiovascular or ophthalmological features of Marfan syndrome. From literature, 14 other heterozygous missense mutations are known that lead to the substitution of the first aspartic acid of a cbEGF domain and result in a Marfan phenotype. These data show that the phenotypic effect of an aspartic acid substitution in the first position within a cbEGF domain can range from asymptomatic to a severe neonatal phenotype. The recessive nature of a specific cbEGF domain mutation in one family

suggests a threshold model in which reduced expression of *FBN1* combines with a mild functional defect due to this particular mutation. This observation underscores the importance of careful interpretation of missense mutations, as pathogenic effects can not always be easily predicted.

A series of ten patients carrying a whole-gene deletion of one allele of *FBN1* is described in **Chapter 5**. The full spectrum of marfanoid features was observed in these patients, including aortic root aneurysms needing surgical intervention and ectopia lentis. These findings show that true haploinsufficiency can result in the full spectrum of Marfan syndrome and make a major contribution to pathogenesis.

Chapter 6 describes a three-generational family at risk for serious aortic disease as a result of an interstitial deletion of chromosome 15 that disrupts *SMAD3*. This deletion was detected incidentally, through a genomic microarray performed for mental retardation. Mutations in *SMAD3* are associated with a recently discovered syndromic form of aortic aneurysms and dissections associated with skeletal abnormalities (aneurysms-osteoarthritis syndrome, AOS). In the family described, family members were not aware of their risk and several carriers of the deletion were found to have a dilatation of the thoracic aorta and skeletal manifestations of AOS, while an obligate carrier had previously died as a consequence of aortic dissection.

Chapter 7 describes two unrelated children with classic Marfan syndrome and recurrent intracranial hypertension. To the best of our knowledge, this symptom has not previously been reported in association with Marfan syndrome. Both children displayed dural ectasia, one in a severe form with an anterior sacral meningocele. However, dural ectasia has been associated with intracranial hypotension (as opposed to intracranial hypertension) caused by cerebrospinal fluid leakage. One of the children is a member of the consanguineous family described in **Chaper 4** and is a homozygous carrier of a substitution of the first aspartic acid in a cbEGF domain. The second child has a de novo mutation leading to the substitution of a cysteine in a cbEGF domain in *FBN1*.

Discussion

Victor McKusick, in 1955, was the first to classify Marfan syndrome as a connective tissue disorder and he included a review of all clinical features (McKusick, 1955). The discovery of the fibrillin-1 gene as the cause of Marfan syndrome confirmed McKusick's prediction that Marfan syndrome is caused by a defect in an extracellular matrix protein (ECM) (Dietz et al., 1991). The finding that fibrillin-1 interacts with latent-TGFβ-binding protein-1 (LTBP-1) and plays a role in the transforming growth factor β (TGFβ) bioavailability represented a

paradigm shift, moving from the concept that Marfan syndrome is caused by a structural defect of the ECM to awareness that it is a developmental disorder involving perturbation of a signalling pathway (Annes et al., 2003; Isogai et al., 2003). This insight has important implications for treatment options.

1.1 Criteria and guidelines for diagnosis and management of Marfan syndrome

From a clinical point of view, it is of the utmost importance that Marfan syndrome is recognised. Given the highly variable and pleiotropic manifestations of the disease, many clinicians may encounter an as yet undiagnosed patient. The four Marfan expertise clinics in the Netherlands together developed multidisciplinary guidelines for Marfan syndrome (**chapter 2**). These guidelines provide recommendations for Marfan clinic health workers concerning uniform diagnostic procedures, follow up and therapy. Furthermore, through the guidelines, referring specialists and general practitioners have easy access to up-to-date information concerning both the syndrome and the criteria for referral.

To establish a diagnosis of Marfan syndrome, the Dutch guidelines follow the revised Ghent criteria, formulated by an international panel of experts and published in 2010 (Loeys et al., 2010). An important revision of these criteria, opposed to the former criteria published in 1996, places greater emphasis on cardiovascular manifestations (De Paepe A. et al., 1996; Loeys et al., 2010). Although aortic root aneurysm together with ectopia lentis is sufficient for the diagnosis of Marfan syndrome, with the presence of a first-degree family member with Marfan syndrome the diagnosis can be established on the basis of an aortic root dilatation or ectopia lentis alone. Dural ectasia was a major feature in the former criteria, but is now part of a new scoring system for systemic features. If DNA analysis is available, a pathogenic *FBN1* mutation together with either an aortic root dilatation or ectopia lentis is sufficient for diagnosis. The revised Ghent criteria offer directions for further testing in case of findings that are not specific for Marfan syndrome. In cases with an alternative diagnosis, the paper includes recommendations for counselling, follow-up and treatment.

As aortic root dilation is the key diagnostic criterion, it is very important to use reliable nomograms. Although international guidelines provide recommendations for the measurement procedure, there is still controversy regarding the upper limits for the aortic root. The Roman nomograms correlate the body surface area (BSA) with the aortic root diameter and are considered to be the gold standard by many cardiologists (Roman et al., 1989). Although the regression line of the Roman nomograms appears to be linear, above 38 mm the linear relationship has to be extrapolated, with the largest aortic diameter measured at 38 mm. Later studies showed a nonlinear threshold above a certain BSA or height (Reed et al., 1993; Kinoshita et al., 2000; Pelliccia et al., 2010). Using the Z-scores advised in the revised Ghent nosology, based on the Roman nomograms, aortic root

measurements above 40 mm would be classified as normal in patients with a high BSA (>2 m^2). As patients with Marfan syndrome often have a higher than average BSA, a diagnosis may be missed. A maximum aortic root diameter of 38 mm was found in a retrospective study of 38 healthy individuals with a high BSA (Radonic et al., 2011). On the basis of these studies, the Dutch guidelines recommend considering an absolute aortic root diameter of more than 40 mm as dilated. It now appears that age, gender and body surface area should be accounted for regardless of the method used, and use of the Z-score can help in deciding whether an aortic root is or is not dilated.

Another shortcoming of the revised Ghent nosology is in the definition of pathogenicity of *FBN1* mutations. The criteria presented to define causality in the revised Ghent nosology are incomplete and need adjustment or further specification, due to the prominent role given to the presence of a *FBN1* mutation. For example, one of the criteria is presented as 'missense affecting conserved residues of the EGF consensus sequence ((D/N)X(D/N)(E/Q) Xm(D/N)Xn(Y/F) with m and n representing variable numbers of residues; D aspartic acid, N asparagine, E glutamic acid, Q glutamine, Y tyrosine, F phenylalanine'. This EGF consensus sequence formula implies that aspartic acid can be replaced by asparagine, and glutamic acid by glutamine. However, as shown in **Chapter 4**, the negatively charged aspartic acid in the first position of a cbEGF domain is crucial for calcium binding, and the substitution of aspartic acid has been shown to be deleterious in several cases (Hilhorst-Hofstee et al., 2010b).

Complete *FBN1* allele deletions were shown to cause Marfan syndrome in **Chapter 5** and should now be added to the list of causal mutations (Ades et al., 2006; Faivre et al., 2010; Hilhorst-Hofstee et al., 2010a; Hiraki et al., 2008; Hutchinson et al., 2003).

Although less specific features of Marfan syndrome were removed or given less weight in the new nosology, the scoring of systemic features remain rather subjective. No objective criteria exist for the wrist and thumb sign, pectus deformities, foot abnormalities, dural ectasia, facial features or skin striae, and the assignment of 1, 2 or 3 points for the systemic score are entirely based on expert opinion.

In most individuals with a clearly pathogenic mutation, DNA analysis will establish a definite diagnosis. However, in cases where DNA analysis is inconclusive, the clinician must rely on clinical features when seeking to decide whether a diagnosis of Marfan syndrome is justified, meaning that interpretation of clinical features will remain the core of the diagnosis. The Dutch guidelines are based on the best available evidence and provide professionals with recommendations on most of the important aspects of Marfan syndrome such as recognition, referral to expert clinics, diagnostic procedures, follow-up and therapy.

1.2 Pathogenesis of thoracic aortic aneurysms and dissections

In **Chapter 4, 5 and 6**, three different genetic causes of thoracic aortic aneurysms are described. First mutations with an expected dominant negative effect are described in **Chapter 4**. In **Chapter 5** and **6** true haploinsufficiency of *FNB1* and *SMAD3* caused by deletions are presented.

All cbEGF domains in the human *FBN1* gene begin with a codon for aspartic acid. This amino acid is crucial for calcium binding and substitutions are expected to reduce the binding of calcium, with a disturbance of the tertiary structure, susceptibility for proteolytic degradation and disturbed protein-protein interaction as a consequence. In **Chapter 4**, a series of substitutions of the first aspartic acid of a cbEGF domain are described (Hilhorst-Hofstee et al., 2010b), substitutions that are predicted to affect the binding of calcium. Calcium binding to fibrillins is necessary for structural stabilisation, protein-protein interaction and the prevention of proteolytic degradation (Cooke et al., 1987; Dietz et al., 1993; Handford et al., 1991). Around half of the mutations described were predicted to result in aberrant splicing, and in one case this was demonstrated experimentally. Mutations causing aberrant splicing and consequent exon skipping will generally result in a shorter protein rather than a frameshift, due to the fact that nearly all *FBN1* exons harbour an exact multiple of three bases. A shorter protein is expected to exert a dominant negative effect. Most of the known aspartic acid substitutions lead to a classic Marfan phenotype or even a severe neonatal phenotype. In one family, however, the substitution of the aspartic acid at cbEGF domain 39 (p.Asp2485Val) only resulted in Marfan syndrome when in a homozygous state. This is thought to be due to a relatively mild reduction in expression of the mutant allele (leading to a possibly mild functional defect), with just enough normal function in the heterozygous state but below a certain threshold in a homozygous state to express the phenotype. One explanation might be the presence of an additional variant in the promoter region in cis with the mutation, leading to reduced expression of the mutant allele.

Despite the suggestion in an earlier report that *FBN1* haploinsufficiency leads to a milder phenotype (Milewicz et al., 1995), **Chapter 5** describes 10 Marfan patients with a complete deletion of one *FBN1* allele, demonstrating that complete haploinsufficiency of *FBN1* leads to a classic Marfan phenotype including ectopia lentis and aortic root aneurysm needing surgery at a relatively young age. This accords with the observation of Marfan syndrome fulfilling the Ghent criteria in patients with a deletion of the promoter region, which was shown to result in a complete loss of transcription of the corresponding allele (Matyas et al., 2007). Three other reports have described a complete deletion of one *FBN1* allele and

marfanoid features (Ades et al., 2006; Faivre et al., 2010; Hutchinson et al., 2003). Although no mouse models with true haploinsufficiency of one *FBN1* allele are known, several studies in mice have shown that a haploinsufficiency state, due to expression of very low levels of fibrillin-1 with or without a dominant negative potential, can be associated with mild to severe phenotypes (Dietz and Mecham, 2000; Judge et al., 2004; Mariko et al., 2011; Neptune et al., 2003; Pereira et al., 1997; Pereira et al., 1999; Lima et al., 2010). Overexpression of a mutant allele in a normal mouse background does not result in a disease phenotype, while introducing a wild-type allele in a heterozygous mutant background rescues the aortic phenotype of the mutant (Neptune et al., 2003). Using a mouse model, Lima et al. showed a negative correlation between *Fbn1* expression and the severity of the phenotype (Lima et al., 2010). This protective effect of higher levels of normal fibrillin-1 has also been shown in a patient carrying a deletion and in three related patients carrying a mutation causing a premature termination codon (Hutchinson et al., 2003). These observations in humans and mice show that haploinsufficiency of fibrillin-1 is a major pathogenetic mechanism in the development of the Marfan phenotype, and that a higher level of normal fibrillin-1 correlates with a milder phenotype.

Mutations in *SMAD3* have been shown to cause an autosomal dominant syndromic form of aneurysms and dissections, the aneurysms-osteoarthritis syndrome (AOS) (van de Laar et al., 2011; van de Laar et al., 2012; Regalado et al., 2011). In **Chapter 6**, a family is described with an interstitial deletion of chromosome 15 that disrupts the *SMAD3* gene. This was an unexpected result from a microarray analysis and unrelated to the clinical features of the proband. All family members carrying the deletion showed vascular involvement and one obligate carrier died as a consequence of a thoracic aortic dissection.
The family described here showed similarities in vascular and skeletal phenotype to other families with mutations in *SMAD3*, the result of the disruption and resulting haploinsufficiency of *SMAD3*. *SMAD3* encodes a member of receptor-regulated SMADs and forms a complex with SMAD2 and SMAD4. This complex, in combination with transcription factors, regulates gene expression via activation of TGF-β receptors (Ten Dijke and Arthur, 2007; Ten Dijke and Hill, 2004) and is the mediator of most responses to activation of TGF–β (Massague and Gomis, 2006). Thirteen mutations in *SMAD3* have been described to date (van de Laar et al., 2011; van de Laar et al., 2012; Regalado et al., 2011), four of which introduce a frameshift and one a stop codon. One frameshift mutation, c.741-742delAT; p.Thr247ProfsX61, was shown to cause nonsense-mediated RNA decay and was predicted to behave as a functional knock-out (van de Laar et al., 2011). It has been suggested that all mutations lead to impaired heterotrimer formation with SMAD4, with a consequent impairment in the propagation of TGF-β signalling.

The similarities of the aortic and skeletal phenotypes in families with mutations in *SMAD3*, compared to the described family, suggests a major role for haploinsufficiency of *SMAD3*. While *SMAD3* knockout mice do show intervertebral disc abnormalities and osteoarthritis, aortic disease was not investigated (Li et al., 2009; Yang et al., 2001) and information on mice with heterozygous null alleles is unavailable.

1.3 Intracranial hypertension in Marfan syndrome

We are still puzzled by the observation of intracranial hypertension in two unrelated children with Marfan syndrome, described in **Chapter 7**. One of the patients carried a homozygous missense mutation (p.Asp2485Val) and is a member of the family harbouring a recessive mutation described in **Chapter 4**, while the second child had a cysteine substitution (p.Cys2581Ser) in *FBN1*. Both children showed the classic features of Marfan syndrome and both had dural ectasia. One of the patients had bilateral choroid plexus cysts and a small arachnoid cyst, while the other patient had a narrow left transverse sinus, frequently encountered in children with idiopathic intracranial hypertension. Dural ectasia predisposes for intracranial hypotension caused by cerebrospinal fluid leakage, rather than hypertension. Although, to date, no other reports of intracranial hypertension in combination with Marfan syndrome or another connective tissue disorder are known to us, it is important to be alert to a possible relationship between a connective tissue disorder and intracranial hypertension, in order to allow rapid and effective investigation in cases of unexplained headache and vomiting.

1.4 Counselling

Some important counselling issues can be addressed with the help of studies described in this thesis. Firstly, mutations in *FBN1* have to be carefully assessed, as shown by a seemingly pathogenetic mutation that was not as deleterious as initially expected, as described in **Chapter 4**. Secondly, careful clinical assessment of family members and the interpretation of laboratory results by an experienced clinical and molecular geneticist are a prerequisite. Although descriptions of the phenotype may be partly subjective, in most studies clinical observations and descriptions are very valuable to clinicians dealing with families with a genetic disorder. As shown in **Chapters 4, 5** and **6**, the data are of no use without the accompanying medical information. Furthermore, unexplained features such as intracranial hypertension in two unrelated patients with Marfan syndrome, as described in **Chapter 7**, require publication because such observations might lead to the recognition of health problems that were previously overlooked. With the emergence of whole genome analysis, we will soon be facing a dramatic rise in unexpected results that are unrelated to the phenotype of the individual tested. This issue is discussed in **Chapter**

6, in which an unrelated microarray analysis led to the discovery of a *SMAD3* copy number variant with serious health risks for the family, including vascular disease and osteoarthritis at a young age. The unexpected results of whole genome analysis have been described in only a handful of studies and to the best of our knowledge, vascular disease has not been previously addressed. The identification of at risk members of this particular family allowed early detection of vascular disease, appropriate counselling and will facilitate possibly life-saving follow-up and treatment.

1.5 Future perspectives

Marfan syndrome

The Dutch guidelines for Marfan syndrome aim to realise improved and better coordinated care for Marfan patients. In the process of developing the guidelines, we were faced with many issues lacking an evidence-base. While some of these issues concerned the features on which clinical diagnosis relies, others concerned treatment options such as timing of surgical repair of the aorta or medication to slow aortic dilation. Future studies to validate the clinical items in the diagnostic criteria of Marfan syndrome would therefore be valuable. On the other hand, clinical diagnosis in individual cases might not always meet the requirements of the criteria, even if all items were validated and objective. This is partly due to the variability of the phenotype. A major future challenge will be the characterisation of the individual patient based on genetic background, the modifying factors that influence the expression of the disease and the effect of certain treatment options in relation to these differences. Detailed clinical descriptions that are related to specific pathogenic mutations and studies of modifying factors are needed to provide greater insight into the causes of the variability of Marfan syndrome. Without these detailed clinical descriptions, investigations of modifying factors that determine the severity of Marfan syndrome are useless. This also applies to the characterisation of the effect of *FBN1* mutations. Validated criteria to differentiate between pathogenic mutations in *FBN*1 or neutral variants without effect on the clinical phenotype are strongly needed. Knowledge of modifying factors such as variants in the promoter region, stochastic variation in gene expression, environmental factors, or variations and mutations in modifying genes may all provide opportunities to intervene in pathogenesis and develop treatment options on an individual basis.

Many countries, including the Netherlands, have undertaken prospective and randomised studies to test the effectiveness in patients of the angiotensin II type 1 receptor blocker, losartan (Detaint et al., 2010; Forteza et al., 2011; Gambarin et al., 2009; Lacro et al., 2007; Matt and Eckstein, 2011; Moberg et al., 2011; Radonic et al., 2010). Losartan has a negative effect on the TGFβ signalling pathway and is thought to slow or halt aortic growth and other phenotypic features. While the results of these studies are still awaited,

it is expected that the possible positive effects will differ in different individuals. In order in individualise treatment, future studies should focus on factors that determine the effectiveness of certain drugs. In cases of fibrillin-1 haploinsufficiency, a method to upregulate fibrillin-1 production pharmacologically, or by gene therapy to compensate for the deficiency, might have a beneficial effect. Evident obstacles such as delivery, sustained expression and avoidance of deleterious side effects remain major challenges. Although influencing a signalling pathway seems less complicated from a pharmacological point of view, interfering with complex signalling pathways may have unwanted side effects. Close monitoring of short and long term effects are inevitable.

As the life expectancy for patients with Marfan syndrome grows, more patients will be confronted with the morbidity associated with aging. Little is known about clinical manifestations in elderly Marfan patients. A questionnaire study of 60 patients with Marfan syndrome, aged 50 or older, suggested a pattern of premature ageing (Hasan et al., 2007). Close monitoring of elderly Marfan patients in clinical and research settings is needed.

With advances in knowledge of pathogenesis, clinical guidelines should be revised on a regular basis, thus allowing the translation of scientific knowledge into useful and practical information for clinicians and patients.

Familial thoracic aortic aneurysm and dissections (FTAAD)

In contrast to Marfan syndrome, FTAAD is genetically a highly heterogeneous disease. Several genes have been found to cause FTAAD, with or without additional features. With the emergence of next generation sequencing (NGS), it will be easier and cheaper to detect mutations in known or unknown genes. In a diagnostic setting, a multigene NGS panel targeted at aortic aneurysms will provide a much more rapid diagnosis at a lower cost than conventional sequencing of individual genes. In a research setting, whole exome or whole genome sequencing has replaced the classic approach of linkage in large families and the sequencing of individual genes. One advantage of these new technologies is the possibility of gene identification in small families or even in isolated patients. Furthermore, the identification of susceptibility genes or modifying genes becomes feasible. One of the challenges of NGS is in handling the vastly increased numbers of unclassified variants that require further investigation through approaches such as segregation analysis and/or functional assays.

Similarly to Marfan syndrome, clinical studies of family members will be important in providing insights into the various phenotypes related to a specific mutated gene. Future studies of modifying factors are also expected to open new avenues to effective treatment options.

chapter
8

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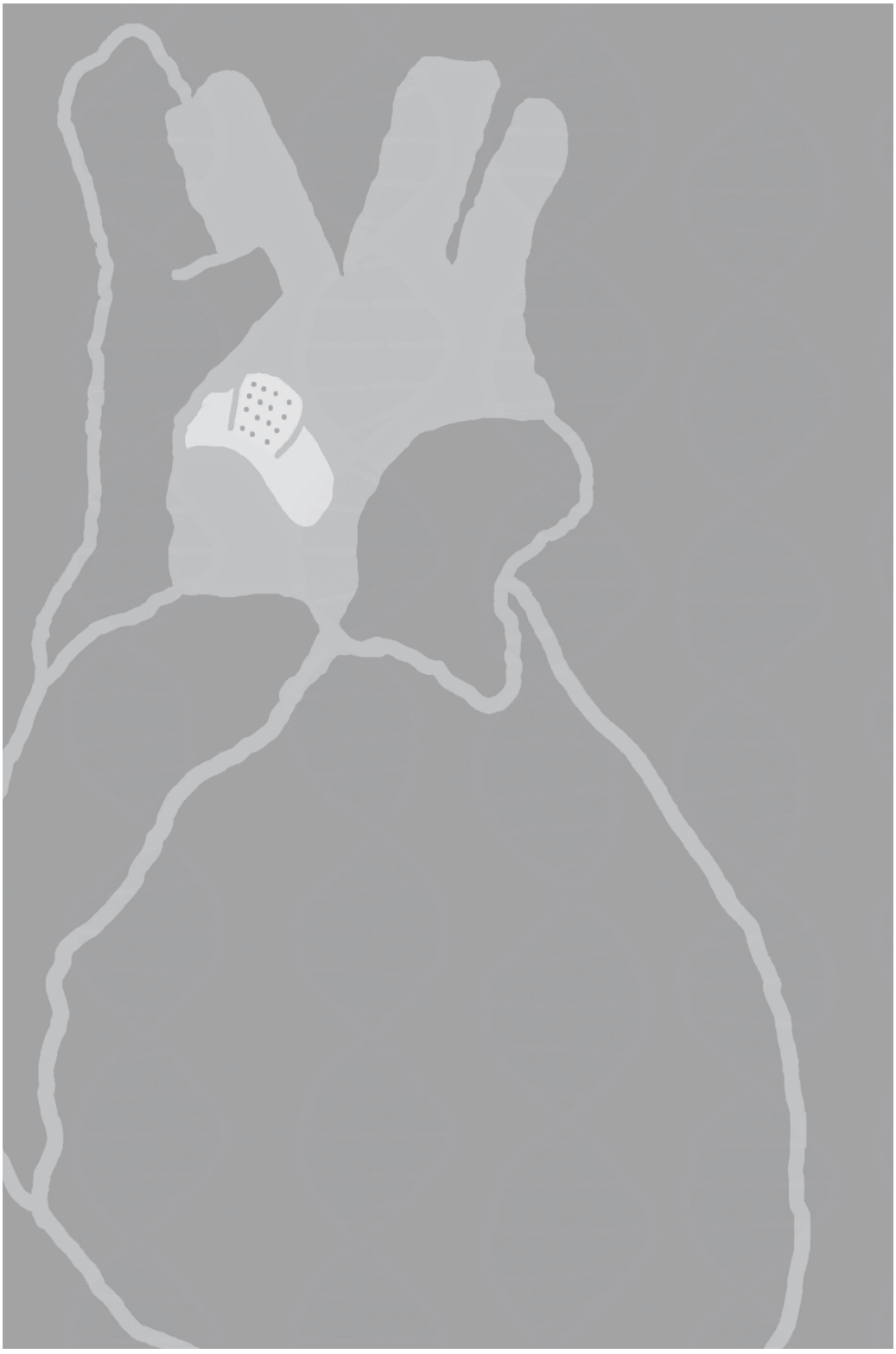

9

Nederlandse samenvatting
List of publications
Dankwoord
Curriculum Vitae
List of abbreviations

Nederlandse samenvatting

De grote lichaamsslagader ('aorta') verloopt van het hart tot aan de liesslagaders en vervoert het zuurstofrijke bloed dat via meerdere vertakkingen geleid wordt naar alle weefsels en organen. Een scheur in de binnenbekleding van de aorta ('aortadissectie') waardoor bloed tussen de binnen- en buitenbekleding stroomt wordt meestal voorafgegaan door een verwijding ('aneurysma'). Een aneurysma van de aorta kan verschillende oorzaken hebben. Bij het ontstaan van een aneurysma van de aorta in de borstkas ('thoracale aorta-aneurysma's') spelen erfelijke factoren een belangrijke rol. In tegenstelling tot thoracale aneurysma's spelen bij buikaneurysma's vooral niet-genetische factoren een rol zoals atherosclerose. Aneurysma's en dissecties van de aorta in de borstkas leiden tot ernstige complicaties en een hoge sterfte. Vroege herkenning van mensen met een verhoogde kans op een dissectie is van groot belang, omdat tijdig herkennen van de verwijde lichaamsslagader, gevolgd door operatieve reconstructie met een prothese de kans op ernstige complicaties en overlijden kan verkleinen.

Marfan syndroom is één van de bekendste aandoeningen met een sterk verhoogde kans op aneurysma's en dissecties van de thoracale aorta op relatief jonge leeftijd. Marfan syndroom wordt veroorzaakt door mutaties in *FBN1*, het *FBN1*-gen dat codeert voor fibrilline-1. Op enkele uitzonderingen na, zoals ook beschreven in Hoofdstuk 4, leidt een mutatie in één van beide kopieën van het *FBN1*-gen vrijwel altijd tot de ziekte (dominante overerving).

In dit proefschrift worden de klinische en genetische aspecten behandeld van aorta-aneurysma's en -dissecties in de borstkas, met speciale aandacht voor Marfan syndroom. **Hoofdstuk 1** is een algemene samenvatting van de stand van de kennis over Marfan syndroom, familiare thoracale aorta-aneurysma's en -dissecties (FTAAD) en andere syndromen die geassocieerd zijn met TAAD. Er wordt extra aandacht geschonken aan de ontstaanswijze van de aortaproblematiek in Marfan syndroom. **Hoofdstuk 2** is de Engelse vertaling van de Nederlandse richtlijn voor de diagnose en behandeling van Marfan syndroom. Deze richtlijn is opgesteld door verschillende specialisten die betrokken zijn bij één van de vier marfanpoliklinieken in Nederland. De richtlijn bevat praktische aanbevelingen voor verwijzers en behandelaars die betrokken zijn bij de herkenning, diagnose, controle en behandeling van patiënten met Marfan syndroom. Voor zover mogelijk is de richtlijn gebaseerd op wetenschappelijk bewijs, echter bij het ontbreken van voldoende bewijs, is uitgegaan van de ervaringen en inzichten van deskundigen ('expert opinion'). Door het beschikbaar komen van de richtlijn hebben huisartsen en andere verwijzers gemakkelijk de beschikking over recente en betrouwbare informatie over Marfan syndroom en verwijscriteria. De herziene Gentse criteria ('the revised Ghent nosology') voor de diagnose Marfan syndroom worden gepresenteerd in **Hoofdstuk 3**. Deze criteria

zijn opgesteld door een internationaal panel van deskundigen. Een belangrijke verandering ten opzichte van de eerdere criteria (Ghent nosology, 1996) is dat er meer gewicht wordt gegeven aan de twee hoofdkenmerken van Marfan syndroom, namelijk een verwijding of scheur van de aortawortel en het los liggen van de ooglens ('lens(sub)luxatie'). In de afwezigheid van één van deze twee criteria, wordt het hebben van een eerstegraads familielid, een ziekteveroorzakende *FBN1*-mutatie óf een combinatie van bijkomende (systemische) kenmerken, verlangd. Een ander belangrijk verschil is, dat de aanwezigheid van een verwijding van de ruimte waarin zich de vloeistof van het ruggenmerg bevindt ('durale ectasieën') een belangrijk kenmerk was in de voorgaande diagnostische criteria. Nu maakt dit verschijnsel deel uit van een nieuw scoringssysteem voor marfankenmerken, de systemische score.

Naast criteria voor het stellen van de diagnose Marfan syndroom worden ook mogelijke alternatieve diagnoses gegeven. In **Hoofdstuk 4** worden specifieke mutaties in het *FBN1*-gen beschreven, die ertoe leiden dat het eerste asparaginezuur van een 'calcium-binding Epidermal Growth Factor-like' (cbEGF) domein vervangen wordt door een ander aminozuur. Een dergelijke missense mutatie werd vastgesteld in beide kopieën van het gen ('homozygoot') bij drie marfanpatiënten uit een grote familie met een hoge mate van bloedverwantschap. Uit onderzoek bij familieleden bleek dat dertien heterozygote dragers (mutatie in één van beide kopieën van het gen) geen belangrijke cardiovasculaire, oogheelkundige of skeletkenmerken van Marfan syndroom hadden. Vergelijkbare heterozygote mutaties in 14 andere gepubliceerde en geobserveerde patiënten bleken te resulteren in Marfan syndroom. De verzamelde klinische gegevens laten zien dat de uiting van de mutaties op één allel kan variëren van asymptomatisch tot een ernstig, al op de babyleeftijd optredende vorm van Marfan syndroom. Asparaginezuur op deze positie in de verschillende cbEGF-domeinen is belangrijk voor de binding van het calcium dat een rol speelt bij de stabilisatie van de eiwitstructuur, de interactie tussen eiwitten en het voorkomen van eiwitafbraak. De verwachting is dan ook dat het vervangen van een asparaginezuur op deze plek in het gen door een ander aminozuur, ziekteveroorzakend zal zijn in heterozygote vorm. Dit is in tegenspraak met het ontbreken van symptomen bij de heterozygote dragers in de consanguine familie. De recessieve overervingsvorm in deze familie suggereert dat deze specifieke mutatie een mild functioneel effect heeft en mogelijk gecombineerd met verminderde expressie van het *FBN1*-gen beneden de drempelwaarde voor een klinische uitingsvorm blijft. Deze klinische bevindingen benadrukken het belang van een zorgvuldige interpretatie van missense mutaties, omdat het effect in de patiënt niet altijd goed voorspeld kan worden.

In **Hoofdstuk 5** wordt een serie van 10 patiënten beschreven met het ontbreken (deletie) van één van de twee kopieën van het gehele *FBN1*-gen. Bij deze patiënten wordt het gehele

spectrum van marfanoïde kenmerken gezien inclusief lens(sub)luxaties en aneurysma's van de thoracale aorta waarvoor operatief ingrijpen noodzakelijk was. Hieruit blijkt dat pure haploinsufficiëntie, waarbij maar één van de twee kopieën van het gen werkzaam is, voldoende is om het gehele spectrum van Marfan syndroom tot uiting te laten komen. Hieruit is geconcludeerd dat haploinsufficiëntie in belangrijke mate bijdraagt aan het ontstaan van de ziekte. Dit komt overeen met onderzoek bij muizen, waarbij één exemplaar van het *FBN1*-gen is uitgeschakeld.

Hoofdstuk 6 beschrijft een familie van 3 generaties met een genetische aanleg voor aorta-aneurysma's en -dissecties doordat een klein stukje van chromosoom 15 mist ('interstitiële deletie') met betrokkenheid van het *SMAD3*-gen. De deletie werd ontdekt tijdens chromosomenonderzoek door middel van een SNP-array, die uitgevoerd werd vanwege een verstandelijke beperking bij één familielid. De deletie houdt geen verband met de verstandelijke beperking en was een toevalsbevinding. Mutaties in *SMAD3* zijn recent geassocieerd met een syndromale vorm van aorta aneurysma's en dissecties geassocieerd met skeletafwijkingen ('AOS, aneurysms-osteoarthritis syndrome'). De familie was zich niet bewust van een verhoogde kans op aortaproblematiek. Meerdere dragers van de deletie bleken een verwijding van de thoracale aorta te hebben en skeletafwijkingen zoals beschreven bij AOS. Eén familielid was overleden aan de gevolgen van een dissectie van het eerste deel van de aorta. Hij kon niet meer onderzocht worden op dragerschap van de deletie, maar was per definitie drager vanwege zijn positie in de stamboom. Deze familie laat zien dat haploinsufficiëntie van *SMAD3* waarschijnlijk ook een belangrijke rol speelt in de ontwikkeling van AOS.

Twee ongerelateerde kinderen met Marfan syndroom en herhaalde verhoogde hersendruk ('intracraniële drukverhoging') worden beschreven in **Hoofdstuk 7**. Voor zover bekend is intracraniële drukverhoging niet eerder beschreven in associatie met Marfan syndroom. Beide kinderen hadden durale ectasieën, en één van beiden had daarbij een uitstulping van de spinale ruimte vóór het heiligbeen ('anterieure sacrale meningocèle'). Durale ectasieën zijn geassocieerd met verlaagde hersendruk (in tegenstelling tot hersendruk), veroorzaakt door lekkage van hersenvocht.

Eén van de kinderen maakte deel uit van de grote familie beschreven in Hoofdstuk 4 en heeft in beide kopieën van het gen een mutatie die leidt tot de substitutie van het eerste asparaginezuur van een cbEGF-domein. Het tweede kind had een klassieke mutatie van een cysteine in een cbEGF-domein in *FBN1*. Hoewel er geen duidelijke verklaring is voor de intracraniële hypertensie bij deze twee patiënten met Marfan syndroom, is het van belang te weten dat er mogelijk een relatie is tussen intracraniële hypertensie en bindweefselaandoeningen, zodat bij onbegrepen hoofdpijn en braken snel de correcte diagnose gesteld kan worden.

In **Hoofdstuk 8** wordt een samenvatting van dit proefschrift gegeven. Aansluitend wordt de inhoud bediscussieerd. Er wordt behandeld wat het praktisch belang is van de richtlijn voor de diagnose en behandeling van Marfan syndroom. Enkele tekortkomingen van de herziene Gentse criteria worden bediscussieerd zoals het gebruik van normaalwaardes voor de aortawortel op basis van het lichaamsoppervlak en de lengte ('Z-scores').
Tenslotte wordt in dit laatste hoofdstuk besproken welke implicaties de bevindingen in eerdere hoofdstukken hebben voor de diagnostiek, voorlichting, klinische zorg en het wetenschappelijk onderzoek gericht op patiënten met Marfan syndroom of een erfelijke vorm van thoracale aorta- aneurysma's.

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Curriculum Vitae

Yvonne Hilhorst-Hofstee werd geboren op 23 juni 1964 in Drachten. Ze behaalde haar VWO diploma in 1982. Na 1 jaar op de school voor Hoger Laboratorium Onderwijs (HLO), startte zij haar studie Geneeskunde aan de Rijksuniversiteit Groningen in 1983. Haar co-schappen deed zij in het Medisch Spectrum Twente te Enschede van 1988-1990 en zij werkte daarna ruim 1 jaar als arts-assistent kindergeneeskunde in het Streekziekenhuis Midden Twente te Hengelo (Ov). Daar werd haar interesse in de klinische genetica gewekt door het doen van een inventariserend genetisch onderzoek bij kinderen met gespleten lip- en/of gehemelte onder leiding van Drs. N. Kors en Prof. Dr. R.C.M. Hennekam. In 1992-1993 verrichtte zij wetenschappelijk onderzoek naar het fragiele X syndroom aan de Universiteit van Tsukuba (Japan) onder leiding van Prof. H. Hamaguchi. In 1993 startte zij haar opleiding in Leiden en sinds november 1996 werkt zij als klinisch geneticus in het Leids Universitair Medisch Centrum onder leiding van Prof. Dr. M.H. Breuning en sinds kort onder Prof. Dr. C.A. van Asperen. Zij houdt zich met name bezig met bindweefselaandoeningen in het bijzonder Marfan syndroom en familiaire thoracale aorta aneurysma's en dissecties. Haar klinische werk aan dit onderwerp onder leiding van Prof.Dr. M.H. Breuning als promotor en Dr. G. Pals als copromotor heeft geresulteerd in dit proefschrift. Daarnaast heeft zij speciale belangstelling voor de genetische aspecten van ontwikkelingsachterstand en/of aangeboren afwijkingen. Zij is getrouwd met Richard Hilhorst en heeft 3 kinderen, Rixt van 14 jaar, Jelle en Sjoerd van 13 jaar.

chapter
9

List of abbreviations

AAA	Abdominal Aortic Aneurysm
ACE	Angiotensin Converting Enzyme
ADCL	Autosomal Dominant Cutis Laxa
ADHD	Attention Deficit Hyperactivity disorder
ADPKD	Autosomal Dominant Polycystic Kidney disease
AGREE	Appraisal of Guidelines for Research & Evaluation
AOS	Aneurysms-Osteoarthritis syndrome
ARCL	Autosomal Recessive Cutis Laxa
AT1	Angiotensin II type 1
AT2	Angiotensin II type 2
ATS	Arterial Tortuosity syndrome
BAV	Bicuspid Aortic Valve
BSA	Body Surface Area
cbEGF	calcium-binding Epidermal Growth Factor-like
CCA	Congenital Contractural Arachnodactyly
CGH	Comparative Genomic Hybridization
CMC	Carpometacarpal
CMR	Cardiovascular Magnetic Resonance
CNV	Copy Number Variant
CSF	Cerebrospinal fluid
CT	Computed Tomography
DE	Dural Ectasia
DHPLC	Denaturing High Performance Liquid Chromatography
EBRO	Evidence Based Richtlijn Ontwikkeling (evidence based development of guidelines)
ECG	Electrocardiography
ECM	Extracellular matrix
EDS	Ehlers-Danlos syndrome
EDS-CT	Ehlers-Danlos syndrome, classic type
EDS-HT	Ehlers-Danlos syndrome, hypermobile type
EDS-KST	Ehlers-Danlos syndrome, kyphoscoliosis type
EDS-VT	Ehlers-Danlos syndrome, vascular type
EL	Ectopia Lentis
ELS	Ectopia Lentis syndrome
ERK	Extracellular signal-Regulated Kinase
FISH	Fluorescence In Situ Hybridization

FraX	Fragile-X syndrome
FTAAD	Familial Thoracic Aortic Aneurysms and Dissections
HHC	Hyperhomocysteinuria
HHT	Hereditary Hemorrhagic Telangiectasia
IAA	Iliac Arterial Aneurysm
ICA	Intracranial Aneurysm
IH	Intracranial Hypertension
IHD	Ischemic Heart disease
IVF	In Vitro Fertilization
LAP	Latency Associated Peptide
LDS	Loeys-Dietz syndrome
LLC	Large Latent Complex
LMWH	Low Molecular Weight Heparin
LS	Lower Segment
LTBP	Large Latent TGFβ-binding Protein
MASS	Myopia-Mitral valve prolapse-borderline and non-progressive Aortic root dilatation-Skeletal findings-Striae
MFS	Marfan syndrome
MLCK	Myosin Light Chain Kinase
MLPA	Multiplex Ligation-Dependent Probe Amplification
MR	Mental Retardation
MRA	Magnetic Resonance Angiography
MRI	Magnetic Resonance Imaging
MVPS	Mitral Valve Prolapse syndrome
NF1	Neurofibromatosis type 1
NGS	Next Generation Sequencing
NMD	Nonsense Mediated Decay
OA	Osteoarthritis
OAC	Oral Anticoagulants
OI	Osteogenesis Imperfecta
PA	Pulmonary Artery
PCR	Polymerase Chain Reaction
PDA	Patent Ductus Arteriosus
PGD	Preimplantation Genetic Diagnosis
PH	Periventricular Heterotopia
PND	Prenatal Diagnosis
PTC	Premature Termination Codon

RAAS	Renin-Angiotensin-Aldosteron system
RCT	Randomised Controlled Trial
SGS	Shprintzen-Goldberg syndrome
SMC	Smooth Muscle Cell
SNP	Single Nucleotide Polymorphism
ST	Sinotubular
STT	Scaphotrapezotrapezoidal
TAAD	Thoracic Aortic Aneurysms and Dissections
TGF	Transforming Growth Factor
TGFBR1	Transforming Growth Factor, β Receptor 1
TGFBR2	Transforming Growth Factor, β Receptor 2
TTE	Transthoracic Echocardiography
UMD	Universal Marfan Database
US	Upper Segment
WMS	Weill-Marchesani syndrome