Novel diagnostic and therapeutic targets in Marfan syndrome
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Aortic aneurysm in Marfan syndrome is multi-factorial
Introduction

Marfan syndrome (MFS) is a heritable connective tissue disorder with clinical features in several organ systems. The incidence of MFS is about 2-3 in 10,000 people.[1] Progressive aortic root dilatation is the most relevant clinical feature of MFS as it is responsible for most of the morbidity and the mortality of MFS patients [2–5]. It affects the sinuses of Valsava resulting in a typical pear-shaped aneurysm which has an abnormally weak wall, prone to dissection and rupture. Aortic root dilatation generally develops during adolescence and early adulthood, with most disease progression in the 3rd and 4th decade of life, which may lead to aortic dissection and sudden death if untreated [4]. Other clinical characteristics of MFS are dislocation of the ocular lens, skeletal features resulting from overgrowth of long bones[6], lung emphysema[7], mild myopathy, osteopenia[8] and cardiac ventricular dysfunction [9] [chapter 4].

MFS is caused by mutations in FBN1 gene which encodes for the fibrillin-1 protein.[10,11] The fibrillin protein forms microfibrils and elastic fibers of the connective tissue and extracellular matrix throughout organ systems[12]. Despite the increasing knowledge of the genetic background and pathogenesis, MFS remained a clinical diagnosis[13,14] due to extraordinary clinical variability and weak genotype-phenotype correlations[15].
Molecular genetics of MFS

In up-to 99% of patients, a FBN1 mutation can be demonstrated [16]. FBN1 is a large gene on chromosome 15q21.1 and consists of 65 exons. It codes for a fibrillin-1 protein which is highly abundant in many tissues and contains 47 epidermal growth factor-like domains, including 43 binding calcium, and seven TGF-β binding protein-like domains[17], (Figure adapted from Ramirez F. et al.[18]).

Cystein-residues are present in a high percentage and have an important role in providing the three-dimensional structure of the protein by forming disulphide bonds[19]. Six highly conserved cystein residues in growth factor-like domains are of particular importance as they also warrant the stability of the protein[20]. Fibrillin-1 polymerizes into microfibrils and forms elastic fibers in combination with elastin.

There are more than 1000 mutations in FBN1 described and many families have a unique mutation. Genotype-phenotype correlations appeared to be robustly weak [15]. Main genotype-phenotype correlation observed was a severe phenotype in patients with a mutation in the middle part of the gene (exons 24-32). Even when patients with neonatal MFS were excluded from the analysis, mutations in this region resulted in a severe cardiovascular phenotype. Another observation was that patients harboring a missense mutation which alters the number of cysteine-residues, have a higher chance of developing lens dislocation. [15]

In general, mutations in FBN1 gene are thought to have two main effects on the fibrillin-1 protein: dominant-negative effect and haplo-insufficiency[21]. The dominant-negative effect is based on
the incorporation of a mutant fibrillin-1 during the polymerization with a normal other fibrillin-1. Mutant fibrillin-1 then disturbs the structure and function of the whole polymer. Haplo-insufficiency results from the degradation or low production of the mutant fibrillin-1. In that way the patient has only lower levels of normal fibrillin-1. The latter mechanism emerged from a mouse model of MFS (C1039G mice) which faithfully recapitulates the disease [21]. Recently, this was confirmed in human MFS patients. Genomic deletions of even the whole FBN1 gene, which also result in a haplo-insufficiency, were associated with classical MFS[22]. Genotype-phenotype correlation studies in MFS patients based on the effect of the mutation on protein level are lacking. This approach might provide stronger correlations than genomic mutations only and come closer to explaining the large clinical variability. It requires, however, extensive studies on fibrillin-1 RNA level extracted from skin biopsies in a large patient population. In chapter 8 we found that patients, harboring a mutation with a predicted dominant-negative effect on fibrillin-1 protein, have higher circulating TGF-β. This might be explained by a poisoning effect of incorporation of the mutant protein into microfibrills. In the majority of patients, however, these predictions were made using different prediction programs in lack of patient material (fibroblast culture). These findings offer an interesting basis for further research on this matter.

**Histological findings in the aortic tissue**

At a histological level, the normal aorta consists of three layers: the tunica intima, tunica media, and tunica adventitia. The tunica media contains elastin, smooth muscle cells, collagen, and ground substance. The predominance of elastic fibers in the aortic wall and their arrangement as circumferential lamellae distinguish the elastic artery from the smaller muscular arteries[23]. A lamellar unit is made up of two concentric elastic lamellae, smooth muscle cells, collagen, and ground substance contained within. The thoracic aorta incorporates a thickness of 35 to 56 lamellar units and the abdominal aorta about 28 units[24]. These elastic lamellae enable the aorta to dilate during systole and propagate the blood bolus during elastic recoil in diastole[25]. Surrounding the tunica media is the tunica adventitia, which is
composed of loose connective tissue, including fibrillin-1, fibroblasts, collagen fibers, elastin, and ground substance. The adventitia is essential for the "strength" of the aorta and provides its solidity mainly because the abundant presence of collagen. This is reflected in a “J-shaped” stress-strain curve of the aorta: the elastic medial layer is responsible for the flat, horizontal part of the curve and the adventitial knitting results in the steep arm of stress–strain curve[26].

The main histological finding in the aorta of MFS is mucoid degeneration in the tunica media (also known as ‘cystic media necrosis’): areas of degradation of elastic fibers, aggregation of hyaluronans, proteoglycans and smooth muscle cell apoptosis. It is not specific for MFS; it only appears at younger age than in other types of thoracic aneurysms. Because of the histological abnormalities, MFS has always been viewed as a disease of the aortic media. Only in recent years the focus is shifting to adventitia for several reasons:

1. In genetic disorders of elastin such as pseudoxanthoma elasticum, aortic aneurysm is a very rare manifestation, suggesting that the elastic breaks seen in MFS are rather secondary to aortic dilatation, than the primary cause. [27]

2. Fibrillin-1, considered to be the genetic basis of the disease, is more abundant in the adventitia than in the media [28].

3. The aortic media is providing only the elasticity of the vessel and can sometimes be totally removed (during endarterectomy for example) without subsequent dilatation and aneurysm formation as long as the adventitia remains intact.

Lindeman et al. found that collagen of the adventitia in MFS showed normal biochemical properties but its microarchitecture was disturbed with complete absence of the normal collagen fibril organization and deposition. Functional analysis of the adventitial layer revealed an impaired collagen network behavior with inability of individual fibers to transfer the stress and strain to the neighboring fibers. These factors
together may contribute to aortic aneurysm formation with elastic degeneration in the tunica media as a consequence.[29]

**Aneurysm formation and role of TGF-β: lessons from animal models and related disorders**

In recent years MFS raised attention of a broad scientific public after many studies were published in several well established mouse models of the disease [30–32].

First, mgΔ mice were created by invoking an in-frame deletion of the middle part of Fbn1 gene in order to replicate the dominant negative effect of FBN1 mutation in humans[33]. Mice homozygous for this mutation (Fbn1 mgΔ/mgΔ) produce shorter fibrillin-1 protein in lower levels (10-fold lower than normal). This, however, did not interfere with the production of elastic fibers, which were structurally and quantitatively normal at birth in these mice. Thus, elastic fibers can assemble in absence of normal fibrillin-1 protein. These mice are born normal but develop severe aortic dilatation and die from dissection approximately three weeks after birth. Histological findings in the dilated parts of the aorta showed elastic fiber segmentation, accumulation of medial degeneration, recruitment of inflammatory cells and proliferation of adventitial cells. Interestingly, elastic fibers remained normal in not-affected parts of the aorta. Another mouse model is the mgR/mgR mouse that produces about a quarter of the normal amount of fibrillin-1 and display phenotypic features in the skeleton and the aorta similar to those of patients with classic MFS[34]. These mice are also born normal but die from aortic dissection at the age of approximately 4 months. Elastic lamellae in the tunica media were normal at birth and vascular disease began with focal linear calcification of intact elastic lamellae as early as 6 weeks of age. Calcified segments increased in frequency and expanded with intimal hyperplasia and deposition of abnormal and exuberant collagen and elastin. Monocytic infiltration became evident and the amount of infiltration correlated with loss of elastin content. The adventitial layer showed inflammation with fibroblast hyperplasia and macrophage infiltration. These macrophages produced MMP12 with the specific capability of fibrillin-1 binding. Taken together, these findings suggest that secondary events initiated by
a relative deficiency in fibrillin-1 may cause a dynamic and progressive loss of microfibrillar abundance, integrity, and function in both the aortic tunica media and the aortic adventitia.

These secondary events were contributed to altered TGF-β signaling. Extracellular microfibrils normally bind the large latent complex of TGF-β, composed of the mature TGF-β, latency-associated peptide and one of three latent transforming growth factor-β binding proteins. This interaction is proposed to suppress the release of free and active TGF-β. Due to mutant or deficient fibrillin-1, failure of matrix sequestration of the large latent complex promotes the activation of TGF-β. TGF-β was found to contribute to the distal alveolar septation which results in lung emphysema in these mice. [32] Inhibition of TGF-β by TGF-β-neutralizing antibodies improved the lung phenotype. TGF-β was found to be increased while its ligand which binds it to the extracellular matrix was found to be decreased. It was concluded that increased sequestration of TGF-β precursors leads to its increased signaling in the lung. Similar rescue of the phenotype by TGF-β neutralizing antibody was demonstrated in the aorta[30], mitral valve[35] and muscles [36] of a third and most widely used mouse model of MFS is Fbn1 C1039G/+ mouse. This mouse model harbors a heterozygous missense mutation of Fbn1 resulting in a haploinsufficiency, representative of the most common class of mutations causing MFS[15]. Losartan, an angiotensine II receptor 1 blocker achieved comparable phenotype rescue as TGF-β antibodies [30,35,36]. In addition, its positive effects on the aortic root dilatation were demonstrated in a small observational study in MFS patients with severe aortic root disease. We and other groups initiated clinical trials investigating effects of losartan in MFS patients[chapter 2] [37–39].

TGF-β is a remodeling cytokine which generally plays a role of the “master switch” between inflammation and fibrosis after injury in the cardiovascular system. In cardiac injury, i.e. infarcted heart, TGF-β signaling suppressed inflammatory reaction by macrophage deactivation and promoted myofibroblast transdifferentiation and matrix preservation[40]. This was supported by TGF-β inhibition experiments which, however, showed phase-related effects of TGF-β. In an early injury TGF-β inhibition
resulted in increased mortality, enhanced neutrophil infiltration and increased pro-inflammatory cytokine and chemokine gene expression[41]. In contrast, late TGF-inhibition attenuated adverse remodeling and decreased collagen deposition[42]. In abdominal aortic aneurysm similar effects of TGF-β are observed. In contrast to MFS mice, TGF-β attenuation resulted in aneurysm rupture and death in AngII -induced mouse model of abdominal aneurysms [43]. Although TGF-β seems to have differential effects in these cardiovascular diseases, losartan exerts similar positive effects both in myocardial infarction, abdominal aneurysms and MFS[44,45]. Losartan acts through inhibition of the Ang-II receptor 1 (AT1) which suggests that the angiotensine II (Ang-II) pathway plays an important role in MFS, similarly as in myocardial infarction or abdominal aneurysms. In MFS mice Ang-II was found to play a role in aneurysm formation through ERK-signaling[31]. Inhibition of the AT1 receptor and preservation of signaling through angiotensine receptor AT2 (as done by selective AT1 inhibitor losartan) attenuated aortic disease. In an Ang-II induced abdominal aortic aneurysm mouse model, ascending aortic aneurysms developed as well in response to Ang-II infusion[46]. Interestingly, in this mouse model, abdominal aneurysms and ascending aortic aneurysm were different in pathological substrate. Abdominal aneurysms formed rapidly as a consequence of a highly localized transmural elastin disruption that colocalized with focal medial macrophage accumulation[47]. Adventitial thrombi formed and promoted an intense inflammatory response with the recruitment of macrophages. In contrast, ascending aortas exhibited extensive elastin fragmentation following infusion of AngII, but not transmural in media and adventitia as seen in abdominal aneurysms. Regions of greatest elastin disruption and intra-laminar expansion were associated with macrophage accumulation on the adventitial side of the vessel, as seen in human MFS aortic root material [chapter 6]. These differences in response to Ang-II between different parts of aorta might be explained by functional diversity of smooth muscle cells based on their embryological origin.[48] The ascending aorta to just distal of subclavian artery (a predilection place for type B dissections in MFS patients) is populated by smooth muscle cells from neural crest origin while smooth muscle cells of distal aorta originate from the mesodermal layer. Interestingly, ascending aneurysm formation in these mice
was attenuated by CCR2 whole body deficiency[46]. CCR2 is a receptor for MCP-1 protein, a potent macrophage attractant. These findings together indicate that Ang-II might contribute to MFS aneurysm formation and that inflammatory cells play a role in this process. Only this process is less acute than that observed in Ang-II induced abdominal aneurysms. This might also explain contrary effects of TGF-β inhibition in MFS and abdominal aneurysms, similarly to contrary effects of TGF-β inhibition in acute and chronic phase of myocardial infarction. In this light, increased TGF-β in MFS might be a remodeling effort of the aortic wall in response to structural damage and Ang-II, just as in many other cardiovascular states. We found in chapter 6 that patients without significant aortic dilatation, regardless of the age, had remarkably low circulating total TGF-β levels. In chapter 8 circulating TGF-β levels also correlated with aortic root diameter, suggesting that circulating TGF-β level may reflect the degree of aortic root damage.

Interestingly, our further observation in chapter 8 was that only about 30% of MFS patients demonstrated a decrease of circulating TGF-β after initiation of losartan. These patients had higher levels of circulating TGF-β prior to the therapy and harbored mutations with dominant negative effect on fibrillin-1 level. There is little known about the mechanism of TGF-β reduction by losartan, it has been suggested it reduces the expression of TGF-β. This could be downstream of Ang-II and therefore the reduction of TGF-β might be a time consuming process. In addition, this molecular response to losartan might be dependent on losartan doses as it is metabolized in the liver and some patients might require higher doses in order to reach its molecular effects. This might be of particular importance for adult patients who are being treated with beta-blockers and do not tolerate higher doses of losartan on top of it.

On a histological level, increased levels of stored TGF-β were found in outer layers of media and inner adventitia of all types of ascending aortic aneurysms, also the non-syndromic forms: MFS, bicuspid valve and degenerative aortic aneurysms[49]. Thus, increased stored TGF-β levels are not specific for MFS syndrome and can be extended to all types of ascending aneurysms, in line with the notion that it might be a secondary remodeling response. Another common pathway that all these aneurysm types share
is increased Smad2 signaling. It was long thought that enhanced phosphorylated Smad2 intracellular signal in MFS was part of the canonical TGF-β signaling mainly because it was increased in MFS mice aortas and decreased after administration of losartan or TGF-β neutralizing antibodies[30]. This increased Smad2 signaling in thoracic aneurysms was specific for aortic smooth muscle cells, i.e. not found in fibroblasts or other cell types in the aortic wall. If TGF-β is released by a simple sequestration of the extracellular matrix, it would be expected that it affects all cells equally. In contrast, this increased Smad2 signaling was found to be dissociated from TGF-β in MFS and other types of thoracic aortic aneurysms. It correlated with the degree of elastic fiber degradation. Together, these observations indicate that Smad2 signaling might also be secondary to structural damage or another, still unknown process and that it is not a direct consequence of TGF-β in the aortic wall. It is known that Smad2 can be activated by Ang-II independently of TGF-β[50].

In conclusion, although increased TGF-β signaling plays an important role in MFS vascular disease, this might be a secondary remodeling process, as in other cardiovascular diseases including non MFS aortic aneurysms. The Ang-II pathway may have an important role in MFS aortic aneurysm pathogenesis and its role is still insufficiently investigated.

Inflammation in MFS aneurysm

Evidence about the functional role of inflammation in MFS aneurysm development is limited. Inflammation has an established role in abdominal aortic aneurysm which is characterized by an extensive inflammatory cell infiltrate in the adventitia. In MFS, inflammatory cell infiltrates in media and adventitia are also observed. In chapter 6 we found increased levels of CD4+ T-cells and macrophages on the adventitial side of aortic media where most of mucoid degeneration was present. In the adventitia, increased counts of CD8+ cells were observed. He et al. also found increased levels of T-cells and macrophages in MFS aortas which correlated negatively with the age of the patients [51]. This might indicate that inflammation enhances aneurysm development. In line with these findings, using a
microarray approach we found that the expression of Human Leukocyte Antigen (HLA)-DRB1 and HLA-DRB5 correlated with the rate of the aortic dilatation [chapter 6]. These genes code for the heavy chain of the major histocompatibility complex on antigen presenting cells which is recognized by CD4+ T-cells involved in T helper 1 (Th1) immune response. Increased levels of these genes indicate an inflammatory process in the connective tissue of patients with progressive aortic disease. Thus, the severity of the aortic root dilatation is associated with Th1 cell immune response [chapter 6]. Other studies also describe a similar adaptive immune response in thoracic aorta aneurysm tissue. Aneurysm tissue had increased expression of the prototypical Th1 cytokine, interferon (IFN)-gamma, and undetectable Th2 cytokines. Specimens displayed robust production of IFN-gamma, induction of the IFN-gamma-inducible chemokines IP-10 and Mig, and recruitment of lymphocytes bearing their cognate receptor CXCR3. In addition, He et al. found a T-cell associated vasculitis of vasa vasorum and increased expression of leukocyte adhesion molecules by the endothelial cells of the vasa vasorum. These findings suggest that the pathway for T-cell migration into the media is from the adventitia. Thus, T-cells and macrophages infiltrate the aortic media and adventitia of MFS patients and play an important role in disease progression.

These findings raise the question what triggers this immune response in the aorta. One of the fibrillin-1 domains contains an aminoacid sequence Arg-Gly-Asp, also known as RDG, that mediates binding to several integrins and thereby plays a role in adhesion and migration of cells. Fibrillin-1 additionally contains three Gly-x-x-Pro-Gly (GxxPG) motifs similar to elastin (Val-Gly-Val-Ala-Pro-Gly), which is known for its chemotactic activity to fibroblasts and monocytes. This effect is mediated by binding to the elastin binding protein present on the surface of mononuclear phagocytes. Guo et al. found that ascending aortic extracts from the mgR/mgR MFS mice as well as a GxxPG-containing fibrillin-1 fragment significantly increased macrophage chemotaxis compared with extracts from wild-type mice. This chemotactic response was driven by an elastin binding protein sequence on macrophages. Similar results were found with samples from the ascending aorta of patients with MFS. Increased chemotactic
ability of ascending aortic aneurysm extracts is due to the increased concentrations of elastin and fibrillin-
1 degradation products. In line with the important role of macrophages in the aortic dilatation in MFS, we
found that the circulating levels of the cytokine macrophage colony stimulating factor (M-CSF) in MFS
patients were associated with the severity of the aortic root dilatation [chapter 6]. Together, fibrillin-1 and
elastin degradation fragments are immunogenic and might be the main triggers of immune response in
MFS.

In our previous work we explored the underlying pathways which could explain the large clinical
variability in MFS patients. Patients within MFS group were compared in order to investigate which
genes and pathways distinguish patients carrying certain disease features from those not. In chapter 6,
expression of inflammatory genes was found to be associated with numerous features of MFS: aortic
disease severity (HLA-DRB1 and HLA-DRB5), dislocation of ocular lens (RAET1L, CCL19 and HLA-
DQB2) and specific skeletal features (HLA-DRB1, HLA-DRB5, GZMK) except chest deformities in
which growth-related genes were up-regulated. These genes belong to both the adaptive and innate
immune responses indicating a complex inflammatory process. Inspired by these findings we
hypothesized that patients with progressive aortic disease have distinct genetic inflammatory profiles.
Inflammation is a part of a normal healing process, but in some patients it might be more excessive and
lead to more tissue damage. In chapter 7 we found that patients with progressive aortic disease had
distinct genetic variants in two genes: Sialic Acid Binding Ig-like Lectin 7 (SIGLEC7) and in interleukine
16 (IL16). The SIGLEC7 gene controls innate immune response to tissue damage, while the IL16 gene is
a chemoattractant of various pro-inflammatory cells and induces expression of HLA-DR genes in
monocytes. These interesting findings are in line with our hypothesis that patients with progressive aortic
disease have distinct genetic variants in inflammatory genes which might enhance the normal healing
mechanism of damaged aortic tissue and enhances aortic aneurysm formation. Translated to the clinical
practice, only a distinct group of patients might benefit from anti-inflammatory interventions although
further research on this matter is needed. Interestingly, in chapter 9 we found that quality of life scores in
MFS patients were also associated with variants in inflammatory genes, independently of disease features and severity. Quality of life is an emerging parameter of patients’ well-being and it is thought that it has a genetic basis because it is independent of disease severity and therapy. MFS is a chronic multi-system disorder and impaired quality of life is expected. We found that physical quality of life was impaired and associated with the presence of scoliosis. Mental quality of life, however, was normal in MFS patients. This normal variability in mental quality of life was associated with expression (CXCL9, CXCL11 and IFNA6) and genetic variants (IL4R) in inflammatory genes. This association might be extrapolated to the normal population as mental quality was comparable to that of healthy population, although a validation is necessary.

Functional studies exploring the exact role and type of inflammatory response in MFS are lacking. There is, however, largely indirect evidence that inflammation aggravates aortic root dilatation in MFS mice. Beneficial effects of treatment with agents with anti-inflammatory properties have been described. Doxycycline, inhibitor of proteolytic enzymes matrix metalloproteinases (MMPs) produced by macrophages, reduced aortic dilatation in MFS mice[51–53]. Combination treatment of doxycycline and losartan showed even better results on the aortic diameter and preservation of the aortic wall structure. [54]Similarly, pravastatin, a statin with pleiotropic anti-inflammatory effects, also reduced aortic root dilatation in these mice[55]. Even though inflammatory infiltrates and expression of inflammatory genes accompany most of MFS features, inflammation might still be a necessary part of the healing process in damaged aortic wall, as has been shown in abdominal aortic aneurysms [56,57]. Abdominal aneurysms are characterized by a general proinflammatory response accompanied by excess matrix turnover. Excessive cytokine expression and inflammatory cell infiltrate include interleukine (IL)-6 and IL8, T-cells, B-cells, macrophages, mast-cells and other type of inflammatory cells[56,58,59]. However, several cohorts and case reports of patients with immunesuppression after solid organ transplantation were described whereby patients develop accelerated aortic growth up to 2 mm/yr and a rupture of aneurysm[60]. Lindeman et al. described a patient with an extremely malicious dilatation of the aortic
aneurysm after a kidney transplantation in whom a 13-fold reduction of interferon-γ was observed in the aortic tissue and a complete absence of T-cells, B-cells and neutrophils [61]. Other factors which could contribute to the excessive aneurysm dilatation (collagen type I and III; IL-6, IL-8, tumor necrosis factor α; matrix metalloproteinase types 2 and 9) were comparable with controls. This points to a diminished adaptive immune response in this patient. This and other reports on the aggressive clinical behavior of aneurysms in patients with a solid-organ transplant question the current hypothesis of the role of inflammation in aneurysmal disease.

Together, these findings indicate that the historically degenerative disease profile of MFS is promoted by inflammation, which plays an important modulatory role in MFS. Further mechanistic studies are needed before anti-inflammatory interventions can be implemented in the clinical practice.

**Aortic disease in MFS as a clinical problem**

Aortic root aneurysm develops in approximately 80-90% of patients [62], mostly between 20-40 years of age [4], although the spectrum of the aortic disease is wide and may affect children and neonates as well [63]. It is progressive and leads to dissection or rupture if untreated [2, 64]. In terms of risks, high blood pressure and hemodynamic stress are thought to accelerate the development of aortic dilatation in MFS patients. Therefore, accurate blood pressure control is indicated. MFS patients are also advised to avoid activities which result in an abrupt hemodynamic stress (i.e. collision sports) [65]. Medicament treatment of aortic disease includes beta-adrenergic blockers which have negative ionotropic and chronotropic effects in the aortic root and in that way might slow down the aortic dilatation [66–68], although their utility remains controversial [69]. Yearly monitoring of the aortic diameters is necessary and prophylactic aortic root replacement is considered when diameters reach sufficient size to threaten a dissection or rupture. This is according to the current guidelines at aortic root diameter of 45-50 mm. [65]
Aortic root surgery involves two major techniques: total aortic root replacement (also called Bentall operation)[70] and valve sparing aortic root replacement[71–73]. Total aortic root replacement involves a composite mechanical valve conduit implantation and requires lifelong anti-coagulation. It was considered a “gold standard” for a long time. However, a significant proportion of patients experienced complications of long term anticoagulation[74]. In the past decade valve sparing aortic root replacement emerged as an alternative to Bentall operation. It preserves native aortic valves and therefore requires functionally normal aortic valve leaflets. Valves can be preserved using two techniques: reimplantation or remodeling of aortic leaflets. Advantage of valve sparing is that it avoids long-term anticoagulation and its complications in a relatively young patient population as in MFS. Main complication of valve sparing technique is reoperation due to the failure of aortic valves which carry the defective fibrillin-1[74]. In a large meta-analysis the valve sparing aortic root replacement was found associated with a fourfold risk of reintervention compared to total aortic root replacement[74]. Reimplantation of aortic leaflets is preferred because it carries less risk for reintervention than remodeling.

In a substantial percent of patients the distal aorta becomes also affected as aortic root surgery prolonged life expectancy of MFS patients [75–78]. In a retrospective study of adults with MFS distal aortic aneurysm was found in up-to 30% of patients[77]. These patients were more likely to have cardiovascular risk factors (smoking, hypertension, and hyperlipidemia) than those without peripheral aortic disease. Interestingly, the authors found that MFS patients without peripheral aortic disease used Angiotensine Converting Enzyme (ACE) inhibitors more often which suggests this medication has protective effects.

Despite a tremendous progress in operative techniques and intensive care which prolonged the life expectancy in MFS patients[79], most of the morbidity is still associated with aortic root dilatation and operation[62]. Because of the large impact on patients’ health and wellbeing, aortic aneurysm was placed central in the new diagnostic criteria for MFS[14]. According to the revised Ghent criteria, the
diagnosis of MFS can be established only in presence of aortic root dilatation or an evident risk for developing it (i.e. mutation which is described to be associated with aortic dilatation in another patient). The definition of aortic dilatation is based on a Z-score of which the normograms of Roman et al.[80] are widely used. This however, can lead to several problems. We found in chapter 3 that Z-scores can underestimate the aortic root dilatation in adult MFS patients [39] as it was based on a relatively small population of healthy volunteers and proposes a linear relationship between the aortic root diameter and body surface area (BSA). This relationship is, however not linear [chapter 3 and [81]] and can be disturbed by high body mass index (BMI) in obese patients. High BMI, for example, can result in a large BSA and a falsely normal Z-score. Aortic root diameter of 40 mm and more should always be considered as dilated. Another problem is that, even though the aortic dilatation was placed central in the novel criteria, the diagnosis of MFS can be made in its absence if a first grade family member has aortic root dilatation or a \( FBN1 \) mutation which is described to be associated with it. However, clinical variability between the members of the same family and carriers of the same mutation, even within one family, is large and precludes the accurate risk stratification for aortic root dilatation. Actually, risk stratification for aortic root dilatation and dissection is largely lacking and the current guidelines use a “one size fits all” strategy for operation indication based on the aortic root diameter only [65].

Degeneration of the aortic wall is reflected in reduced elasticity of the aortic wall. Two main derivatives of the elasticity are distensibility and pulse wave velocity, both measured by means of magenetic resonance imaging (MRI). MFS patients have reduced elasticity of the aorta even prior to aneurysm formation suggesting it is a long process and the dilatation is just an end-stage of the aortic disease[82,83]. Interestingly, aortic elasticity was a major predictor of thoracic descending aortic dilatation[84]. This is the predilection place for the dissection type B in MFS patients which occurs suddenly without previous aneurysm formation[85]. Reduced elasticity of the thoracic aorta is therefore a promising predictor of the aortic dissection. Prospective studies investigating this matter might gain interesting results.
Conclusions

Although MFS is a monogenetic heritable disorder, aortic dilatation, its main clinical characteristic, can be described as a multi-factorial disease. This might explain the large clinical variability observed in this patient group. Structural weakness, altered collagensesis of adventitia, haemodynamic stress, perturbations in TGF-β pathway, Ang-II and inflammation play important roles in its pathogenesis. An integrative approach in further research and its implementation in the clinical practice might be most appropriate.
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