Chapter 4
Marfan Syndrome

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Abbreviations

ACEI	Angiotensin-converting enzyme inhibitors
Ang-II	Angiotensin-II
ARB	Angiotensin-II Receptor Blockers
AT1R	Angiotensin-II type 1 receptor
AT2R	Angiotensin-II type 2 receptor
BP	Blood pressure
CCB	Calcium-channel blockers
COX	cyclooxygenase
CT	Computed tomography
FBN1	Fibrillin-1
MFS	Marfan syndrome
MMP	Matrix metalloproteinase
MMPI	Matrix metalloproteinase inhibitor
MRI	Magnetic resonance imaging
PWV	Pulse wave velocity

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TAA Thoracic aortic aneurysm
TGF-β Transforming growth factor-β
VSMC Vascular smooth muscle cells

Introduction

Marfan syndrome (MFS) is a hereditary connective tissue disorder caused by mutations in FBN1. The gene encoding fibrillin-1 protein (FBN1) is located in chromosome 15 (position 15q21.1) and more than 1,000 different mutations have been described [1]. MFS has an autosomal dominant inheritance with high penetrance and high intra- and inter–familial variability, with an estimated prevalence of 1 case per 3,000–5,000 individuals. In approximately 75 % of cases, an individual inherits the disorder from an affected parent. The remaining 25 % result from a *de novo* mutation. Cardinal manifestations in MFS involve ocular, skeletal and cardiovascular systems. Ocular manifestations include ectopia lentis, myopia, retinal detachment and glaucoma. Skeletal involvement include scoliosis, bone overgrowth, joint laxity and chest deformities (pectus carinatum and excavatum). Cardiovascular manifestations include proximal ascending aorta dilatation, proximal main pulmonary artery dilatation and mitral valve prolapse. Aortic and/or mitral valve regurgitation related to structural primary abnormalities may be present.

Diagnosis

Despite the significant progress made in understanding the molecular and genetic basis of MFS, its diagnosis continues to depend primarily on clinical features that have been codified in the reviewed Ghent diagnostic nosology, described in 2010 [2], in which the coexistence of lens dislocation and aortic root aneurysm or dissection suffices to confirm the clinical diagnosis of MFS (Table 4.1). A family history of MFS and FBN1 mutation – known to be associated with

Table 4.1 Revised Ghent criteria for diagnosis of Marfan syndrome [2]

Absence of family history	Aortic root dilatation[a] or aortic root dissection AND ectopia lentis
	Aortic root dilatation[a] or aortic root dissection AND FBN1 mutation
	Aortic root dilatation[a] or aortic root dissection AND systemic score ≥ 7 points (see Table 4.2)
	Ectopia lentis AND FBN1 mutation that has been identified in an individual with aortic involvement
Presence of family history of Marfan syndrome	Aortic root dilatation[b] or aortic root dissection
	Ectopia lentis
	Systemic score ≥ 7 points

FBN1 fibrillin-1

[a]Aortic root Z-score ≥ 2. Z-score calculator can be found at http://www.marfan.org/dx/zscore

[b]Aortic root Z-score ≥ 2 above 20 years, ≥ 3 below 20 years

aortic manifestations – also contributes to the diagnosis. The remaining cardinal manifestations of Marfan syndrome are incorporated to a systemic score (Table 4.2). When this score is ≥7, it also contributes to the diagnosis. Therefore, a comprehensive multidisciplinary approach involving cardiac, orthopaedic, ophthalmological, and genetic consultations and testing are warranted to confirm the diagnosis.

Limitations of genetic testing include the following: (1) the mutation in the fibrillin-1 gene can cause conditions other than Marfan-like disorders; (2) none of the current methods used to find mutations in the fibrillin-1 gene identify all mutations that cause MFS; and (3) family members with the same mutation causing MFS may present a wide range of clinical manifestations.

Table 4.2 Systemic features in Marfan syndrome

System	Manifestation	Points for systemic score
Skeletal	Pectus carinatum	2 points
	Pectus excavatum	Or chest asymmetry: 1 point
	Scoliosis or spondylolisthesis	1 point
	Reduced upper to lower segment	When both are present without severe scoliosis. 1 point
	Increased arm-span to height ratio (dolicostenomelia)	
	Aracnodactilia	Wrist and thumb signs: both signs = 3 points; one sign = 1 point
	Hindfoot deformity	2 points
	Pes planus	1 point
	Protrusio acetabulae	2 points
	Reduced extension of the elbows (<170°)	1 point
	Facial appearance: dolicocephaly, malar hypoplasia, enophtalmos, retrognathia, down-slanting palpebral fissures	In the presence of 3 of the 5 = 1 point
	Highly arched palate with dental crowding	Not considered

(continued)

Table 4.2 (continued)

System	Manifestation	Points for systemic score
Ocular	Ectopia lentis	Major criteria
	Myopia	> 3 diopters = 1 point
	Retinal detachment	Not considered
	Glaucoma	Not considered
Cardiovascular	Aortic dilatation with or without aortic regurgitation	At the level of aortic root is a major criteria (see Table 4.1)
	Aortic dissection	Ascending aorta dissection is a major criteria (see Table 4.1)
	Mitral prolapse with or without mitral regurgitation	1 point
	Pulmonary artery dilatation	Not considered
	Mitral annulus calcification in individuals younger than 40 years	Not considered
Pulmonary	Spontaneous pneumothorax	2 points
	Apical blebs	Not considered
Integumentary	Stretch marks	1 point
	Recurrent or incisional herniae	Not considered
Dura	Lumbosacral dural ectasia	By CT or MR: 2 points

CT computed tomography, *MR* magnetic resonance

Complications

The main complication in patients with MFS is progressive aortic root enlargement, initially occurring at the sinuses of Valsalva. Ascending aortic aneurysm can precipitate acute type A aortic dissection or aortic rupture, and these complications were the primary cause of death before the advent of successful preventive therapies. Aortic aneurysm may develop early in children with MFS and the incidence rises during childhood and adolescence [3, 4]. Although early diagnosis has increased the median life span from around 40 to approximately 70 years, patients with MFS continue to suffer important morbidity [5]. Up to 90 % of Marfan patients will have cardiovascular events during their lifetime, including surgical repair of aortic root, aortic dissection or mitral valve surgery [6].

The current management of aortic involvement in MFS includes regular imaging follow-up to detect and quantify aortic dilation progression, and prophylactic aortic repair when aortic dilatation reaches a sufficient size sufficient to threaten dissection or cause aortic regurgitation. Prior to the era of open-heart surgery, the majority of patients with MFS died prematurely of aortic rupture, with an average life expectancy of 45 years [7]. The success of current medical and surgical treatment of aortic disease in MFS has substantially improved the average life expectancy, prolonging it up to 70 years [5, 8]. Thus, the major target for improving survival in patients with MFS is to prevent or delay aortic dissection.

Imaging Predictors of Complications

Several indices are associated with increased risk of a life-threatening aortic event. First among these is the absolute size of the proximal aorta [9, 10]. Aortic size ≥5.0 cm is strongly predictive of a high risk of aortic dissection and rupture [3], and surgical intervention at that stage is key. The "normal" diameter of the aorta is directly proportional to body size throughout normal growth and into adulthood.

Given their above average stature and therefore greater body surface area, growing individuals with MFS should have their aortic measurements indexed to body surface area [10]. This can be expressed as an aortic size ratio based on sex- and body size–related norms or expressed in relation to the normal aortic size distribution in the population as a *Z* score. When considered in these terms, patients with MFS with proximal aortic ratios ≥1.3 or *Z* scores ≥3 are at particular risk. However, Marfan syndrome has interesting nuances. For example, adiposity is often reduced in young patients; therefore, the body surface area calculated from standard formulae will underestimate the expected diameters of the proximal aorta and result in a higher *Z* score. Moreover, adults tend to accumulate central adiposity in adulthood, which will increase the calculated body surface area and reduce the apparent degree of aortic dilatation. Adults who gain weight after skeletal maturity will appear to have an improved aortic *Z* score. In such instances, focus on the absolute diameter and its changes is appropriate. In addition, the existing "aortic growth curves" are divided into children and young adults; interestingly, the curves do not overlap accurately. This poses problems for the clinician managing patients passing from adolescence to adulthood. Additionally, a common question is whether tall adults should have larger aortic diameters, even beyond those considered to be normal. Svensson et al. [11, 12] proposed an index (area of aortic root/ height >10 cm^2/m) to indicate surgery in patients with MFS. In addition to absolute aortic dimensions, the rate of change in size of the proximal aortic root over time is important. Even at relatively normal absolute aortic dimensions, a rapid increase in aortic size (>0.5 cm/year) portends an increased risk of dissection. However, to assume annual enlargement requires strict imaging quality control and re-measurement of aorta size at the same level and side by side. Additionally, a family history of early aortic complications is strongly predictive of decreased event-free survival [13]. Finally, diminished aortic compliance measured echocardiographically or by other means has been related to progressive aortic dilatation in

MFS patients [14, 15], although it is rarely measured on a routine clinical basis. Also of importance is the fact that patients with MFS can die from other cardiovascular complications, particularly severe mitral regurgitation (especially in children with a severe phenotype) and dysrhythmia [16].

Pathophysiology of Aortic Dilatation

The earliest recognition of the tissue abnormalities underlying aortic dilatation in MFS was medial layer degeneration, with fragmentation, disarray and loss of elastic lamina, and replacement by basophilic-staining proteoglycan. Electron microscopy in humans and in a mouse model of MFS demonstrated extracellular matrix disarray, with shrunken smooth muscle cell fibres, thickened basal membranes, abnormalities of collagen fibre structure and progressive fragmentation and loss of elastic lamellae [17]. The process is associated with signs of ongoing inflammation and matrix metalloproteinase activation [18, 19].

Fibrillin-1 is a major protein component of the microfibrils in the extracellular matrix and, as a result of its alteration, fragmentation and disarray of elastic fibres occur. However, not all manifestations of MFS (e.g. bone overgrowth) can be attributed to these structural abnormalities. In recent years, basic research has led to the notion that fibrillin-1 microfibrils also exert significant regulatory effect on cytokin-transforming growth factor-β (TGF-β) [20].

TGF-β molecules are cytokines synthesised and secreted by smooth muscle cells as inactive precursors in the form of a latent complex which is stored in the extracellular matrix [21, 22]. The fibrillins and latent TGF-β–binding proteins constitute a family of structurally-related proteins and participate in the sequestration of latent complexes of TGF-β and maintain them inactive. In the presence of deficient fibrillin-1, a lesser amount of TGF-β is inactivated and leads to an increase in TGF-β activity. Excessive TGF-β signalling – made evident by increased smad-2 phosphorylation – explains many of the manifestations found in Marfan syndrome: cystic lungs, mixomatous mitral valve leaflets and aortic dilatation [20, 23].

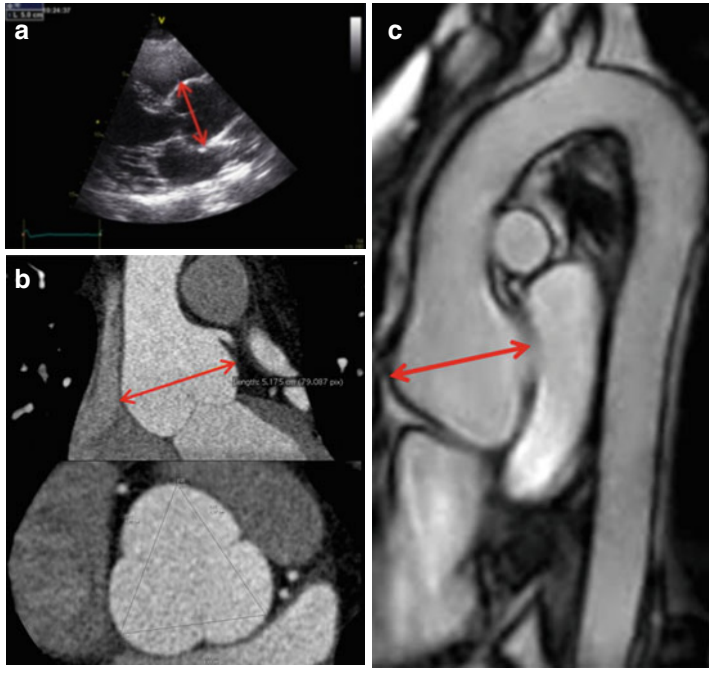

FIGURE 4.1 Imaging techniques for the study of the aorta in Marfan syndrome. (**a**) Transthoracic echocardiography; (**b**) computed tomography; (**c**) magnetic resonance imaging

Management

Although survival in these patients has improved dramatically in recent decades, mainly due to improved surgical techniques, most deaths in MFS patients are still due to aortic complications [5]. Routine aortic imaging by echo and/or MRI and CT is the recommended follow-up for these patients (Fig. 4.1), and elective aortic root surgery is considered when aortic root size is ≥50 mm [24]. However, medical treatment is needed to prevent aortic complications. As in other aortic conditions, strict blood pressure (BP) control is recommended. However, in MFS, medical treatment is considered to be prophylactic, even in the absence of high blood

pressure, with the aim of reducing haemodynamic stress. The main aim of this chapter is to depict evidences, advantages and limitations of the current knowledge of the pharmacological treatment of this disease. To this end, several drugs will be discussed: β-blockers, angiotensin receptor blockers (ARB), angiotensin-converting enzyme inhibitors (ACEI) and calcium antagonists. More recent approaches such as statins, doxycycline, will also be reported.

Pharmacological Treatment in Marfan Syndrome

Mechanisms of Pharmacological Treatment

Medical treatment aims to reduce aortic haemodynamic stress: β-blockers, ARB, ACEI, calcium channel blocker (CCB), and/or to reduce TGF-β signalling: ARB. Recently, metalloproteinase inhibitors (MMPI) or anti-inflammatory drugs have been proposed.

Biomechanical and Haemodynamic Effects

Blood pressure and biomechanical properties of the aorta such as elasticity and compliance are determinant factors in aortic diameter enlargement in MFS [14, 15]. Different studies demonstrated that aortic stiffness is significantly greater in MFS patients compared with healthy volunteers, thereby suggesting more severe wall disease in MFS [25–29].

In clinical practice, arterial stiffness can be non-invasively estimated by three principal methods: (1) estimation of pulse wave velocity (PWV) by measurement of pulse transit time, (2) analysis of the arterial pressure wave contour (i.e. augmentation index, %), and (3) direct stiffness estimation using measurements of diameter or arterial luminal cross-sectional area change during the cardiac cycle and distending pressure measured at the site of diameter changes (i.e. distensibility and compliance). Carotid-to-femoral ('aortic') PWV is

considered the gold standard [30] although PWV can also be measured at other levels.

β-blocker therapy reduces the exposure of weakened, histologically-abnormal aortic tissue to haemodynamic stressors by both inotropic and chronotropic negative effects, and thereby slows aortic dilatation progression. The use of β-adrenergic blockade to reduce haemodynamic stress in the proximal aorta in Marfan syndrome was first suggested in 1971, on the basis of findings in malignant hypertension that a reduction in the rate of increase in aortic pressure over time (dP/dt) was more effective at lowering the risk of aortic dissection than could be explained by a reduction of blood pressure alone [31]. Subsequent small studies of β-blockade effects in animal models with aortic disease and in uncontrolled studies of MFS had varying results [32]. β-blockers have proved to have little effect on central aortic pulse pressure in hypertensive patients [33], which is one of the main determinants of ascending aortic dilatation [34]. In 1989, Yin et al. [35] gave intravenous propranolol to Marfan subjects with dilated aortas during diagnostic cardiac catheterisation and found that it increased the magnitude of aortic wave reflection, reduced arterial compliance and did not reduce the maximum acceleration of blood into the ascending aorta. Other authors reported that β-blockade increases peripheral vascular resistance, which in turn may increase central aortic pressure and wall stress [36]. More recent studies also assessed the effect of β-blockers on aortic biomechanical properties: Groenink et al. [37] studied aortic properties by MRI and found a positive response of aortic distensibility and pulse wave velocity to the acute (2 weeks) treatment with metoprolol or atenolol; Rios et al. [36] found a heterogeneous response of aortic stiffness assessed by echocardiography to long-term treatment with atenolol. They defined a subgroup of patients in whom aortic distensibility improved after chronic β-blockade, with a more pronounced effect in Marfan patients with aortic root diameters below 40 mm. Furthermore, one study demonstrated that treatment with atenolol may not have an effect on the biomechanical properties of the aorta in paediatric patients with Marfan syndrome [38].

Recently, Nebivolol, a beta-1 receptor blocker with nitric oxide potentiating vasodilatory effects, has been proposed as a more appropriate choice than atenolol. In patients with hypertension, it reduces central pulse pressure and augmentation index more than atenolol, and it reduces central arterial pressure and left-ventricular hypertrophy more than metoprolol [39, 40].

Although one study assessed the role of aortic stiffness in predicting progressive aortic dilatation [14], the real clinical impact of the potential effect of β-blockade on aortic stiffness and aortic complications remains unclear.

Calcium-channel blockers reduce central aortic pressure in adult hypertensive patients [41], however similar effects have not been described in patients with MFS.

Angiotensin-converting enzyme inhibitors (ACEI) reduce angiotensin II (Ang-II) formation and are also known to reduce arterial stiffness in patients with different pathological conditions. More importantly, this ability seems to be independent of their ability to reduce BP. ACEI reduce central systolic pressure and conduit arterial stiffness, compared to β-blockers, in adults with hypertension [33].

One interesting study by Williams et al. [42] compared the haemodynamic and vascular effects of perindopril with those of two different drugs: atenolol and verapamil. Fourteen patients diagnosed of MFS were randomised (double-blinded) to receive 4 weeks of atenolol (75 mg), perindopril (4 mg) or verapamil (240 mg) in a cross-over design. Patients underwent a 2-week wash-out period prior to starting the protocol and after each treatment being switched to a new drug. Throughout the study, aortic diameter was assessed by transthoracic echocardiography, and arterial stiffness was measured as augmentation index and PWV (carotid-to-radial and carotid-to-femoral). Within-drug comparisons demonstrated that perindopril (−10.3 mmHg, $P = 0.002$), verapamil (−9.2 mmHg, $P = 0.003$) and atenolol (−7.1 mmHg, $P = 0.01$) reduced central systolic pressure and brachial pressure; central changes were the least and peripheral changes the greatest with atenolol; however between-drug comparisons were

not significant. A trend was observed for augmentation to be reduced by perindopril (–6.3 %, P = 0.05), verapamil (–5.5 %, P = 0.07) and atenolol (–3.2 %, P = 0.09). The study results prove there were no statistically-significant differences among the drugs regarding aortic stiffness parameters. Only atenolol reduced heart rate (by 16 %) and delayed expansion in the arch and abdominal aorta (by 8 % and 11 %) ($P < 0.001$, $P < 0.01$ and $P < 0.05$, respectively, for inter-drug comparisons). Unexpectedly, atenolol did reduce central arterial pressure, although to a lesser degree than that observed with ACEI and CCB. This might be explained by a reduction in cardiac output (which fell by a mean of 17 %, P = 0.24) related to the reduction in heart rate (by a mean of 16 %, P = 0.006) rather than any change in stroke volume (12 %, P = 0.22). Alternatively, a negative inotropic effect would be expected to reduce the amplitude of aortic wave reflections during systole. This study suggested that a combination of a β-blocker with an ARB or an ACEI may be the most effective: while an ARB or ACEI may lower central pressures by reducing or delaying peripheral reflections, a β-blocker may reduce reflections by an effect on the left ventricle. This combination strategy is also being tested in some ongoing trials [43].

Molecular Effects

In order to reduce pathological molecular FBN1 mutation-derived mechanisms such as excessive TGF-β activation and signalling, different classes of drugs including ACEI and ARB have been investigated.

The creation of a mouse model of Marfan syndrome has significantly helped to further understanding of this disease. Overexpression of TGF-β explains many of the manifestations found in Marfan syndrome: cystic lungs, mixomatous mitral-valve leaflets and aortic dilatation have been associated with an increase in TGF-β signalling [20, 23]. Moreover, the administration of TGF-β antagonists (polyclonal TGF-β-neutralising antibody or losartan) in mice prevented the occurrence of Marfan features [44].

Inactive TGF-β is secreted by smooth muscle cells as a large latent complex. This latent complex is sequestered by the extracellular matrix and kept inactive. Deficient fibrillin-1 leads not only to histological abnormalities in the extracellular matrix microfibrils and connective tissue weakness, but also to a decrease in TGF-β sequestration leading to excessive TGF-β activation.

TGF-β can signal either through a canonical pathway involving the signal transduction proteins, Smads [45], or through several non-canonical, Smad-independent pathways (MAP-kinase pathway). In the Smad-related pathway, elevated TGF-β levels induce Smad2 activation that regulates transcription and induce the production of MMP proteins, a family of zinc endopeptidases responsible for degradation of the extracellular matrix in aortic aneurysms. The action of this class of proteins on aortic wall weakness in Marfan syndrome exponentially improves the risk of aortic aneurysm and rupture.

Ang–II is a potent vasoconstrictor acting directly on vascular smooth muscle cells and on the sympathetic nervous system; it also stimulates secretion of the hormone aldosterone, causing volume expansion through sodium retention. At molecular level, Ang-II can promote cell migration, proliferation and hypertrophy. Most of these effects are determined by Ang-II binding to its receptors: AT receptor 1 (AT1R) and AT receptor 2 (AT2R). Although angiotensin II (AngII) mediates the progression of aortic aneurysm, the relative contribution of its type 1 (AT1R) and type 2 (AT2R) receptors remains unknown. Ang-II promotes cell proliferation and fibrosis and suppresses apoptosis when binding to its AT1R, whereas binding to its AT2R has opposite effects, including antiproliferative and anti-inflammatory effects that are beneficial in aortic wall homeostasis. The effects of AT1R stimulation are mediated, at least in part, by TGF-β. The selective AT1 receptor blocker (ARB) losartan blocks AT1R and interferes with processes that are detrimental to tissue in mice with MFS (and by extension, humans) while not affecting signalling through AT2 that produces beneficial effects.

ACEI, on the other hand, reduce Ang-II levels and therefore signalling through both receptors. Although both drugs proved to attenuate canonical TGF-β signalling in the aorta, only losartan inhibited TGF-β-mediated activation of extracellular signal–regulated kinase by allowing continued signalling through AT2.

Angiotensin-converting enzyme inhibitors (ACEI) prevent the conversion of angiotensin-I to Ang-II, thus limiting signalling through both AT receptors. On balance, however, it seems possible that the benefit of AT1-receptor antagonism achieved with ACE inhibitors could outweigh the potential negative influence of AT2-receptor blockade. Thus, although the rationale for the use of ACEI in Marfan syndrome includes their significant effect on TGF-β levels and activity, they proved to be less effective than the ARB losartan in a mouse model of MFS [46].

Treatment of affected mice with losartan, prenatally and continuing until 10 months of age, resulted in the preservation of proximal aortic elastic fibre histology and overall aortic diameter comparable to that of wild-type mice [44]. In contrast, mice with the same mutation treated with propranolol had elastic lamella disruption and dilated aortic roots comparable to those of affected mice treated with placebo [44]. When losartan therapy was initiated at 2 months of age, comparable to adolescence in humans, the histological abnormalities and dilatation were reversed. Although propranolol therapy was associated with a reduction in aortic growth rate, this effect was significantly less than that seen with losartan [44]. The results of this mouse model of MFS suggest that treatment with angiotensin receptor blockers potentially targets both the underlying tissue disorder and reduces haemodynamic stressors.

Telmisartan has the strongest binding affinity to AT1R in comparison with other ARBs including losartan [47]. Concretely, the rank order of binding affinity to AT1R is telmisartan > olmesartan > candesartan > valsartan ≥ losartan. If losartan achieves its effect on MFS through AT1R blockade mediated via downstream TGF-β signalling inhibition, telmisartan would

be expected to be the most effective ARB because of its strongest binding affinity to AT1R. Future studies should determine, however, whether telmisartan is more effective than losartan in Marfan syndrome patients [48].

Matrix Metalloproteinase Inhibitors (MMPI) and Anti-inflammatory Drugs

Multiple factors such as haploinsufficiency, FBN1 proteolysis, abnormal TGF-β signalling, increased MMP expression and changes in cell matrix interaction contribute to the complex pathogenesis of this disorder. Collagens, laminins and elastin have multiple motifs that are able to interact with cell-surface receptors on macrophages and other inflammatory cells. Evidence is accumulating in support of the notion that inflammation may also play an important role in the development of thoracic aortic aneurysm in MFS.

Statins

HMG-CoA reductase inhibitors (statins) are the most potent class of drugs used to inhibit cholesterol biosynthesis. In addition to being the mainstay of cholesterol-lowering therapy, some studies reported more beneficial cardiovascular effects unrelated to lipid reduction, the so-called pleiotropic effects [49]. Interestingly, statins exert anti-inflammatory and atherosclerotic plaque stabilisation effects by down-regulating matrix metalloproteinase (MMPs) expression [50]. Upregulation of MMP enzymes, particularly MMP-2 and MMP-9, is involved in MFS aortic wall degeneration and aneurysm formation [51].

Experimental research on a MFS animal model compared the effect of one of the statin family molecules, pravastatin, to losartan (angiotensin-2 antagonist). In that study, two Marfan genetically-modified mouse groups received, respectively, pravastatin 0.5 g/L and losartan 0.6 g/L for 6 weeks. Results from the different treated groups were compared with a third group of Marfan-modified untreated mice and a control

group without pathological mutations. Echocardiogram analysis showed a significantly beneficial effect of pravastatin in attenuating aortic root dilatation in a MFS model ($p < 0.01$) compared to a Marfan untreated group. This outcome was analogous in the losartan group ($p < 0.01$). Moreover, immunohistochemical analysis of the mural architecture of the aortic wall demonstrated that pravastatin significantly reduced the degree of elastic fibres lost in the medial layer ($p = 0.01$). However, the losartan effect on elastin preserve was greater than that of statins ($p < 0.01$). In addition, haematoxylin and eosin staining showed the presence of foci of damage (island of damage) in the aortic wall of all MFS groups. Even if the number of foci was lower in treated animals, with no statistical difference between the medical groups, this finding may suggest that aortic injury was triggered in all groups and then reduced by drugs. Statins have been shown to have a potential role in MFS therapy and, therefore, this class of drugs should be investigated as a combination therapy in MFS patients.

Doxycycline

Doxycycline, a tetracycline-class antibiotic, is a non-specific inhibitor of MMPs [52] and suppresses aneurysm formation in animal models and human abdominal aortic aneurysm [53, 54]. In Marfan syndrome, Chung et al. [55] demonstrated that long-term treatment with doxycycline, through the inhibition of MMP-2 and −9, was more effective than atenolol in preventing TAA in a mouse model of Marfan syndrome by preserving elastic fibre integrity, normalising vasomotor function and suppressing TGF-β upregulation.

Indomethacin

The complex pathogenesis of MFS involves changes in TGF-β signalling, increased MMP expression and fragmentation of the extracellular matrix. A number of studies demonstrated raised macrophage and T-cell counts in the ascending aorta of human or mouse models of MFS; however, the

efficacy of anti-inflammatory therapy in mouse MFS models has not been assessed to date. In a recent study, FBN1-underexpressing mgR/mgR Marfan mice were treated with oral indomethacin [56]. Treatment was begun at the age of three weeks and continued for 8 weeks, after which the aortas of wild type as well as treated and untreated mgR/mgR mice were compared. Indomethacin treatment led to a statistically-significant reduction in aortic elastin degeneration and macrophage infiltration, as well as lessening of MMP-2, MMP-9 and MMP-12 upregulation. Additionally, indomethacin reduced both cyclooxygenase-2 (COX-2) expression and activity in the aorta of mgR/mgR mice. COX-2-mediated inflammatory infiltrate contributed to aortic aneurysm progression in mgR/mgR mice, providing evidence that COX-2 is a relevant therapeutic target in MFS associated aortic aneurysmal disease. Therefore, COX-2-mediated inflammatory infiltration plays an important role in the pathogenesis of aortic aneurysm disease in MFS. In another paper, the same team demonstrated that the non-steroidal anti-inflammatory drug indomethacin significantly improved elastin integrity and reduced the number of macrophages in the aortic adventitia of mgR/mgR mice, which coincided with decreased MMP-2, MMP-9 and MMP-12 expression. Based on these studies, the authors speculated that the macrophage infiltration observed in the aortic wall of mgR/mgR Marfan mice participates in a kind of vicious cycle, in which matrix fragments induce deleterious effects, including upregulation of MMP activity and macrophage infiltration, which in turn reinforces the pathological processes associated with matrix degradation and defects in TGF-β sequestration [57–59].

Medical Treatment Studies

Beta-Blockers

Beta-blockers are the standard medical treatment for the prevention of aortic dilatation in Marfan syndrome. Their positive benefit relies on their haemodynamic effects: reduction

in the force of left ventricular ejection by negative inotropic and chronotropic effects leading to decreased aortic wall stress. Several studies reported that β-blockers delay aortic root dilatation (Table 4.3). However, those studies had major limitations: the majority were retrospective [5, 60–63], and others prospective but not randomised [64, 65]. The majority showed retardation of aortic root dilatation [62, 66–69], although two studies did not demonstrate this benefit [61, 70]. None of those studies convincingly demonstrated a benefit in overall morbidity and mortality. The strongest evidence comes from a prospective randomised open-label trial by Shores et al. [66] that included 70 patients with Marfan syndrome divided into a control group of 38 patients who received no treatment and a treatment group of 32 patients who received propranolol. Aortic follow-up was performed by echocardiography and aortic dilatation was evaluated with the slope of the regression line for aortic ratio evolution over time. In that study, propranolol slowed the rate of aortic dilatation compared to the control group. The authors defined *aortic ratio* as the ratio of the measured aortic diameter to the expected diameter and the slope of the regression line for the increase in aortic ratios over time. The slope for aortic ratio of the control group was 0.084 per year, whereas in the treatment group was only 0.023 per year ($p < 0.001$). Five patients in the treatment group, two of whom did not follow the propranolol regimen, and nine patients in the control group reached a composite clinical end-point, which was defined as heart failure, aortic dissection, cardiovascular surgery or death. That study supported the use of β-blockers, concretely propranolol, in patients with Marfan syndrome based on two findings: first, aortic dilatation was faster in patients in the control group than in the treatment group and second, more patients in the control group reached the composite clinical end-point than in the treatment group. The construction of a composite end-point was necessary since no single clinical end-point reached statistical significance on its own merit. Although the results were certainly promising, the authors concede that the study was neither placebo-controlled nor blind, with each patient and investigator aware of the

TABLE 4.3 Studies on β-blockers in Marfan syndrome

Author	Design	Treatment groups	Age (years)	Mean aortic root at baseline	Includes children	Includes operated patients	Follow-up	Aortic dilatation end-points	Results
Roman (1993)	Prospective observational Designed to assess the impact of aortic dilatation (no dilatation, localized to aortic root or generalized) and the presence of aortic complications	G1: β-blocker, not specified, N = 79 G2: None, N = 34	28 ± 15 yrs Range = 6 months – 66 yrs		Yes (n = 29)	No	49 ± 24 months		Similar number of complication (AD, AAS, ARP) between groups

Shores (1994)	Randomised clinical trial Open-label	G1: Propranolol, N = 32 G2: None, N = 38	G1: 15.4 yrs G2: 14.5 yrs	G1: 34.6 mm G2: 30.2 mm Significantly different	Excluded <12 yrs	No	G1 = 10.7 yrs G2 = 9.3 yrs	Aortic root by echo (M-mode) Slope of the regression line for aortic ratio over time	Lower aortic dilatation by echo (M-mode) over time in treatment group ($p < 0.001$) Composite end-point (D, AD, AAS, CHF): 5 (15.6 %) in G1 and 9 in G2 (23.7 %). No statistical comparison reported

(continued)

TABLE 4.3 (continued)

Author	Design	Treatment groups	Age (years)	Mean aortic root at baseline	Includes children	Includes operated patients	Follow-up	Aortic dilatation end-points	Results
Salim (1994)	Retrospective	G1 (Centre A or B): propranolol, atenolol, N = 100 G2: None, N = 13	G1: Centre A: 10.4 ± 3.4 yrs Centre B: 14.1 ± 3.4 yrs G2: 0.2 ± 4.6 yrs	G1: Centre A: 31.1 ± 7.0 mm Centre B 34.0 ± 5.4 mm G2: 31.3 ± 7.4 mm P = NS	Yes	No	G1: Centre A: 5.5 ± 2.7 yrs Centre B: 4.2 ± 2.1 yrs G2: 5.7 ± 1.8 yrs	Aortic root dilatation rate by echo (M-mode) in mm/year	Aortic ratios (mm/year): Centre A 1.1 ± 1.1; Centre B 0.7 ± 1.8; Control 2.1 ± 1.6 ($p < 0.006$ between centre A and control: $p < 0.003$ between centre B and control)
Silverman (1995)	Retrospective This study was designed to describe Marfan life expectancy compared to a historical cohort	G1: Atenolol, Nadolol, Propranolol, Metoprolol, N = 191 G2: None: N = 226	G1: 33 ± 14 yrs G2: 31 ± 17 yrs		Yes	Yes	5.2 ± 3.6 yrs	None	Median cumulative probability of survival 2 years longer in G1 ($p = 0.01$)

Legget (1996)	Retrospective Designed to examine the clinical and echocardiographic predictors of outcome in a cohort of patients with Marfan's syndrome	G1: β-blocker, not specified, for >12 months, N = 30 G2: None, N = 53	21 ± 13 yrs Range 1–54 yrs		Yes	No	4 yrs	Aortic ratios, by bidimensional echo	No differences in aortic root growth No differences in actuarial freedom from all events (D, AAS, AD)
Rossi-Foulkes (1999)	Prospective Non-randomized Open-label	G1: β-blockers, N = 20 G2: CCB, N = 6 G3: None, N = 27	9.4 ± 5.3 yrs	G1 + G2: 33 ± 7 mm G3: 26 ± 7 mm P < 0.01 No differences in aortic diameters indexed by BSA	Yes, exclusively	No	44 ± 24 months	Aortic root dimensions by bidimensional echo (absolute values and aortic ratios)	Medicated patients had slower aortic growth than the unmedicated patients (both absolute aortic growth rate 1.0 ± 0.8 vs. 1.7 ± 1.0, p < 0.05)

(continued)

TABLE 4.3 (continued)

Author	Design	Treatment groups	Age (years)	Mean aortic root at baseline	Includes children	Includes operated patients	Follow-up	Aortic dilatation end-points	Results
Selamet (2007)	Retrospective	G1: 27 atenolol, 1 propranolol and 1 metoprolol, N = 29 G2: None, N = 34	G1: 9.2 ± 4.0 yrs G2: 8.8 ± 4.8 yrs	G1: 29.3 ± 4.2 mm G2: 29.7 ± 7 mm P = 0.75	Yes, exclusively <18 yrs	No	G1: 76.3 ± 31.0 months G2: 81.3 ± 53.9 months	Aortic root dimensions by bidimensional echo (absolute values and z-scores)	No differences in aortic root growth Similar number of patients in each group achieved a clinical end-point (AAS, AD, D)

Ladouceur (2007)	Retrospective	G1: Atenolol (70 %), nadolol (17 %) and propranolol (6 %), N = 77 G2: None, N = 78	G1: 6.1 ± 3.2 yrs G2 7.4 ± 5.2 yrs	G1: 28.4 ± 4.8 mm G2: 27.2 ± 5.7 mm P = NS	Yes, exclusively <12 yrs	No	4.5 ± 3.7 yrs	Aortic root dimensions by bidimensional echo	Aortic root dilatation 1.05 mm/year in G1 compared to 1.15 mm/year in G2, p = 0.0001 A trend toward lower cardiac mortality, decreased need for preventive aortic surgery, and less dissection was observed

G1, G2, G3 groups of treatment, *yrs* years, *AD* aortic dissection, *AAS* ascending aorta surgery, *ARP* aortic regurgitation progression, *echo* echocardiography, *D* death, *CHF* cardiac heart failure, *NS* not significant, *CCB* calcium-channel blockers, *BSA* body surface area

patient's group. Thus, although the results did show potential for β-blockers in Marfan patients, it is highly possible that the study's results were subject to bias and a placebo effect. Furthermore, although heart failure, dissection and death are hard end-points, the decision for surgery is a softer call and might have influenced the results.

Further, the study did not have a definitive means of ensuring patient compliance; patients in the treatment group may not have followed the correct propranolol dosage, and those in the control group may have taken other medications. The major limitation of the study, however, was the small sample size. By the end of the trial, the already minimal population had decreased by 20 % owing to clinical end-points. Although the authors appropriately believed the presence of more end-points in the control group supported their conclusions, a mere four-person difference between the control and treatment groups seems unconvincing, even more so when one takes into account that two of the deaths in the control group were unrelated to aortic complications. One year later, Silverman et al. [5] published a retrospective observational study in 417 Marfan patients treated at four different Marfan clinics. Although this study was thought to describe Marfan life expectancy compared to a historical cohort [7], the authors also reported that the 191 Marfan patients treated with β-blockers (atenolol, metroprolol, nadolol or propranolol) had a median cumulative probability of survival 2 years longer than those who had never taken β-blockers, 72 vs 70 years ($P<0.01$). However, the authors themselves admitted that the design of the study precluded the assessment of the contribution of β-blockers to increased survival. Roman et al. [9] published a prospective observational study designed to assess the prognostic significance of the type of aortic dilatation (localised to aortic root or generalised to aortic root and tubular ascending aorta) and found a similar number of aortic complications between patients with or without medical treatment (mainly β-blockers but also with other blood pressure lowering medications), 33 % vs 30 %. However, that study is difficult to analyse since it was not specifically

designed to address β-blocker treatment in patients with Marfan syndrome. A paper published by Salim et al. [62] retrospectively studied 100 patients who received β-blockers (either propranolol or atenolol) at two specialised centres and compared them with a control group of 13 patients who refused treatment. The study found that patients in the treatment group had an aortic root growth rate of 1.1 mm per year, whereas patients in the control group had an aortic root growth rate of 2.1 mm per year ($P < 0.006$). The limited number of patients in the control group compared with the treatment group, however, renders it difficult to lend credence to the comparison. In 1996, Legget et al. [61] published another observational prospective study with the aim of defining a lower risk group for aortic complications depending on echocardiographic follow-up. In that study, 30 patients receiving β-blockers for least 1 year were compared with 80 patients who had not received β-blockers (or for less than 1 year) and found no differences in aortic root growth or aortic complications (death, need for surgery or aortic dissection).

Of the five previously-mentioned studies, only one [66] was a randomised clinical trial, three were not designed to study β-blocker effect on clinical outcome or aortic root growth [5, 9, 61] and one was a non-randomised prospective observational study [62].

A recent meta-analysis that included the five previous studies [5, 9, 61, 62, 66, 69] on β-blockers in Marfan concluded that there is no evidence that β-blockers have clinical benefit in patients with Marfan syndrome [71]. The above-mentioned studies mainly include young patients, so the effect of β-blockade in older ages is even less clear. On the other hand, two recent retrospective observational studies in children reported conflicting results: the first, published by Selamet et al. [63] retrospectively identified 63 Marfan patients (34 untreated and 29 treated with β-blockers) with echocardiographic follow-up and found no differences in the rates of change in aortic root measurements or aortic complications, with a mean follow-up of 81.3 vs 76.3 months in the untreated and treated groups, respectively. The second retrospective

study by Ladouceur et al. [60] included 155 children (<12 years) with MFS and compared the 77 that received β-blockers to the 78 that had never received β-blockers; they reported a lower aortic dilatation rate and a trend towards a lower cardiac event rate (mean follow-up: 4.5 ± 3.7 years) in the patients treated with β-blockers.

The role of β-blockers in certain subsets of Marfan patients is even less clear. That is the case for the subgroup of non-dilated patients or those previously operated on.

Therefore, although β-blockade is the accepted and conventional treatment for MFS, and recommended by the American and European clinical guidelines [25, 72], the evidence for these recommendations is still weak and thus prospective, multicentre clinical trials are needed to assess the real efficacy of this therapy. Moreover, while receiving treatment with β-blockers, these patients eventually present aortic dilatation or dissection; consequently, more research is required to prevent aortic complications with medical treatment.

Calcium-Channel Blockers (CCB)

Calcium-channel blockers (CCB) are sometimes prescribed for patients with Marfan syndrome when β-blockers are contraindicated, for example in asthma; however, their use has been evaluated in only one small study: Rossi-Foulkes et al. [65] reported a slower rate of aorta enlargement in 26 patients receiving treatment, compared with placebo (+0.9 vs 1.8 mm/year, $p = 0.02$), but 20 of these patients received β-blockers and only six a calcium-channel blocker (including verapamil in five). No comparisons between the drugs were reported because the numbers were too small. Since verapamil is negatively inotropic and chronotropic and also causes generalised arterial and arteriolar dilatation, there are theoretical grounds for expecting benefit in Marfan syndrome; however, the drug has not been tested adequately. Calcium antagonists reduce central arterial pressure and stiffness [41]. A dihydropyridine calcium antagonist such as

nifedipine or amlodipine might have similar effects on conduit arterial function, but might be less useful owing to the relative lack of effects on the cardiac inotropic state. However, at the American Heart Association Meeting in 2012 data were presented showing CCBs exacerbated aortic disease and caused premature lethality in MFS mice due to increased ERK activation [73]. Therefore, CCBs have to be used with caution in patients with MFS.

Angiotensin-Converting Enzyme Inhibitors

Angiotensin-converting enzyme inhibitors (ACEI) are used either alone or in combination with β-adrenoceptor blockers. The pharmacological rationale is the involvement of the renin-angiotensin system in the development of aortic stiffening, dilatation and rupture in Marfan syndrome (Fig. 4.2).

ACEI reduce central arterial pressure and conduit arterial stiffness [41]. Preliminary evidence suggests that they may be useful in Marfan syndrome. In hypertension studies, it has been suggested that perindopril may reduce large arterial stiffness by a mechanism that is independent of its direct effect on lowering blood pressure [74]. ACEI have other effects that might also be clinically useful in patients with Marfan syndrome. Activation of the Ang-II AT2 plays an important role in promoting apoptosis of VSMCs and cystic medial degeneration in Marfan syndrome [75]. A study by Nagashima et al. [76] demonstrated that an ACEI (but not an Ang-II AT1R blocker) prevented cystic medial degeneration, apoptosis of VSMCs, and aortic dissection in rats.

Different authors hypothesised that ACE inhibitors may be a useful treatment for reducing aortic dilatation in MFS patients. The first randomised, double-blind, placebo-controlled trial of ACE inhibitors in MFS patients was conducted in 2007 [77]. In that study, 10 MFS patients with normal end-diastolic aortic diameter were randomly assigned to perindopril and compared with 7 similar MFS control patients. At baseline, echocardiographic variables were similar between the two groups. Perindopril dose was raised from

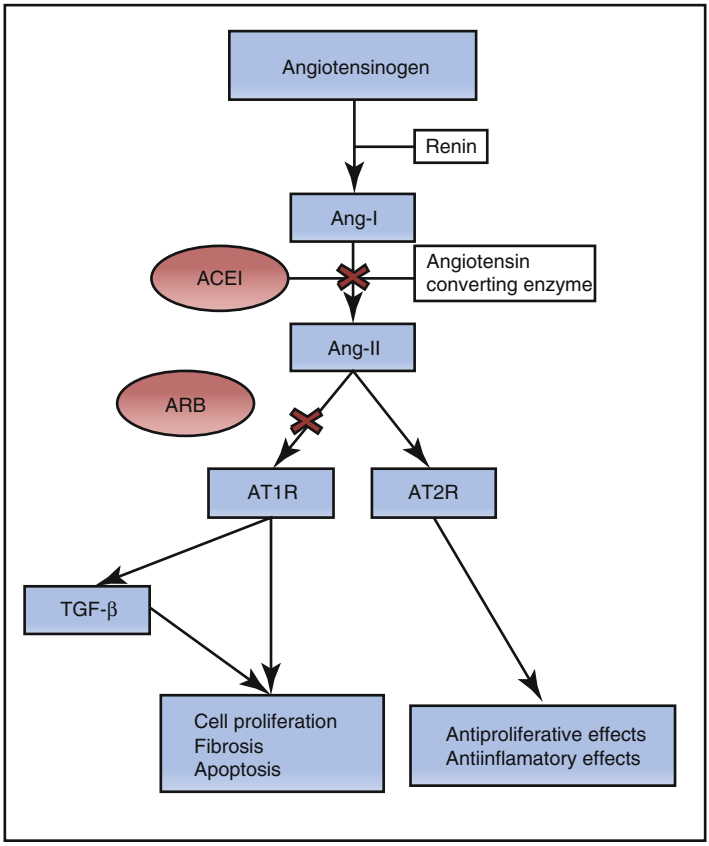

FIGURE 4.2 Renin-angiotensin system in the aorta and sites for drug treatment. *Ang-I* angiotensin-I, *Ang-II* angiotensin-II, *ACEI* angiotensin-converting enzyme inhibitors, *ARB* angiotensin-II receptor blockers, *AT1R* angiotensin-II receptor type 1, *AT2R* angiotensin-II receptor type 2, *TGF-β* transforming growth factor-β

2 to 8 mg/day over the first 3 weeks of the 24-week study. Importantly, both groups of patients were receiving long-term treatment with a β-blocker. During the study, indices of arterial stiffness were assessed by carotid tonometry, Doppler velocimetry, and pulse-wave velocity (PWV) readings.

Covariate analysis proved that perindopril significantly reduced central and peripheral PWV ($p<0.001$) and carotid pulse pressure ($p=0.03$), compared with controls. These changes in aortic stiffness parameters in perindopril group remained significant even when mean arterial pressure was included as a covariate. The main result of this study was that perindopril reduced the aortic growth rate compared to controls over a 24-week period. Aortic size was followed by two-dimensional and M-mode echocardiography. The end-diastolic aortic root diameter was significantly reduced in the perindopril group (1.2–3.0 mm/m^2) compared to the control group. Improvements in arterial stiffness and aortic diameter were independent of arterial pressure. In addition, biochemical analysis showed that perindopril reduced Ang-II production and signalling via both AT1R and AT2R-dependent pathways. Owing to the inhibition of AT1R signalling, ACE inhibitor-treated patients showed significantly reduced levels of the TGF-β cytokine ($p<0.02$) and its downstream messengers, with levels of MMP-2 and MMP-3 dropping ($p<0.001$ for both) compared with placebo at 24 weeks.

Interestingly, Williams et al. [42] reported a small but significant reduction (6 %) of the sinotubular junction aortic diameter after 4 weeks of perindopril treatment ($p=0.024$). No differences were observed at sinuses de Valsalva level.

Despite the potential usefulness of ACEI, these studies are limited because of their small sample size and short duration, therefore the results remain weak and confounding.

A recent non-randomised trial compared enalapril to either atenolol or propranolol (propranolol was given to children <12.5 kg) in 57 subjects, mean age 14.6 and 12 years, respectively [64], in the ACEI and β-blocker groups. Mean follow-up was 3.0 ± 0.2 years. Increased aortic distensibility (3.0 ± 0.3 vs 1.9 ± 0.4 cm^2/dyn; $p<0.02$) and reduced aortic stiffness index (8.0 ± 2.9 vs 18.4 ± 3.8; $p<0.05$) were seen in the enalapril group compared with the β-blocker group and this resulted in a smaller increase in aortic root diameter (0.1 ± 1.0 vs 5.8 ± 5.2 mm; $p<0.001$). Nine subjects underwent aortic root replacement during the study, two in the enalapril group

(6 %) and seven while receiving β-blockers (28 %). Marfan patients treated with ACEI had a reduced aortic growth rate and a lower event rate compared with those treated with β-blockers. However, as that study was non-randomised, treating physicians had a choice of β-blocker or enalapril, leading to a potential for confounding by indication, and the doses of drugs were not optimised by any consistent criteria. Patients with perceived lower risk could have preferentially been treated with enalapril, whereas high-risk patients would more likely have been steered toward β-blockade as "standard of care." The presence of significantly lower aortic distensibility and a higher stiffness index in the β-blocker group suggests that such a differential therapy choice did exist. The authors gave three possible mechanisms for the beneficial effect of the ACE inhibitor: the first was inhibition of VSMC apoptosis as described above; the second was a bradykinin-mediated improvement in aortic elastic tone; and the third was blocking of hyperhomocysteinaemia which increases vascular stiffness and reduction in MMP activity [78].

Angiotensin-II Receptor Blockers (ARB)

Losartan, an Ang-II AT1R antagonist, has been the object of major investigations. Losartan not only lowers blood pressure — a desirable effect in patients with aortic conditions — but has also previously demonstrated antagonism of TGF-β in animal models in different conditions [79, 80]. After the publication of results from a mouse model of MFS, a first retrospective study on the effect of ARB in children with MFS was published by the group of Dietz et al. in 2008 [81]. In that study, 18 paediatric patients (14 months to 16 years of age) were identified as having received ARB (losartan in 17 and irbesartan in 1) after other medical therapy (β-blockers with or without ACEI) had failed to prevent aortic root enlargement. ARB was added to their previous medical treatment and patients were receiving the maximal weight-based dose within 6 months after the initiation of therapy (losartan target dose was 1.4 mg/kg of body weight per day and

irbesartan 2.0 mg per kilogram of body weight per day) and received the treatment for at least 1 year. If previous treatment was ACEI, it was discontinued. With clinical and echocardiographic follow-up between 12 and 47 months, a significant reduction was demonstrated in the progression of aortic root enlargement: change in aortic root diameter decreased significantly from 3.54 ± 2.87 mm/year during the previous medical therapy to 0.46 ± 0.62 mm/year during ARB therapy ($p < 0.001$). Moreover, a statistically-significant reduction was also observed at sinotubular junction level ($p < 0.05$). The authors simultaneously identified a group of 65 Marfan paediatric patients with milder aortic root disease (aortic root diameter z-score 3.25 ± 1.52 vs. 6.52 ± 2.43 in the ARB group, $p < 0.001$) that only received β-blockers during follow-up. Mean rates of change in aortic root diameter (1.71 ± 1.24 mm per year) and in aortic root diameter z-score (0.24 ± 0.50 per year) in the patients that received β-blockers alone were significantly higher than those in severely affected patients receiving ARB therapy ($P < 0.001$ for both comparisons). However, that study had several limitations: (1) small population sample; (2) non-randomised, retrospective and observational study; (3) all patients had severe aortic root enlargement or a rapid increase in aortic diameters before ARB therapy started. However, the results were very encouraging and led to the design of many clinical trials — the majority are still ongoing — to assess the efficacy of ARB versus β-blockers, added to β-blockers, compared to no additional treatment or to placebo in Marfan patients.

The first clinical trial on ARB in Marfan syndrome was published in 2013 by Groenink et al. (COMPARE trial) [82]. This was a randomised, multicentre (four centres), open-label clinical trial with blinded assessment of end-points that included 233 Marfan patients over the age of 18 years (38 ± 13 years, 47 % females) with no history of previous aortic dissection or aortic root diameter >50 mm. Patients were randomised to receive either losartan ($n = 116$) or no additional treatment ($n = 117$) added to their previous medical treatment. Patients in the losartan group were started on

50 mg daily and this dose was doubled after 2 weeks. Maximum losartan dosage of 100 mg daily was achieved in 54 % of patients. Previous medical treatment was β-blockers in 70.1 % of the control group and 75 % of the losartan and CCB group in 2.6 and 1.7 % respectively. Mean follow-up was 3.1 ± 0.4 years.

The primary end-point was the aortic dilatation rate assessed by magnetic resonance imaging (MRI) at six predefined aortic levels from the aortic root to bifurcation. The aortic root could be evaluated in 145 patients with a native aortic root. Baseline aortic root diameters were similar between both treatment groups (43.8 ± 5.0 vs. 43.2 ± 4.4 mm, P = 0.436). The aortic root dilatation rate was significantly lower in the losartan group than in the control group, 0.77 ± 1.36 vs. 1.35 ± 1.55 mm/3 years, respectively, P = 0.014. The percentage of participants with a stable aortic root (defined as a dilatation rate ≤0 mm/3 years) was 50 % in the losartan group and 31 % in the control group (P = 0.022). The aortic dilatation rate beyond the aortic root was evaluated in 218 patients and was not significantly reduced by losartan. This study included 63 patients with previous aortic root replacement (27 in the losartan group). As expected, baseline aortic dimensions in the remaining aortic trajectory were greater in this previously operated group when compared with the total patient cohort. Although in this subgroup of patients, the aortic arch dilatation rate was significantly lower in the losartan group than in the control group (0.50 ± 1.26 vs. 1.01 ± 1.31 mm/3 years, respectively, P = 0.033), patients randomised to losartan demonstrated smaller dimensions at baseline of the aortic arch and the descending thoracic aorta at the level of the diaphragm compared with the control group (respectively, 24 ± 3 vs. 26 ± 4 mm, P = 0.029 and 21 ± 2 vs. 23 ± 4 mm, P = 0.009).

Moreover, in the overall cohort, no differences in separate clinical end-points or the composite end-point were found between groups (prophylactic aortic root surgery: 10 vs. 8, distal aortic surgical intervention: 0 vs. 1, type B aortic dissection: 0 vs. 2, for the losartan and control groups, respectively). No cardio-

vascular deaths occurred during the study. Study limitations include the open-label design of the trial.

A non-randomised interventional study with no control group was published by Pees et al. [83] in 2013; this study included 20 children and young adults (mean age 11.3 ± 6.3 years) with genetically-confirmed MFS that initiated treatment with losartan. Ten of the 20 patients received losartan monotherapy as their first medication, 8 stopped their previous treatment with β-blockers and initiated losartan and 2 received losartan plus a β-blocker. Aortic follow-up (33 ± 11 months) was performed by echocardiography and showed a significant reduction in the normalised aortic dimensions at the level of the aortic root (−3.0 ± 2.8 mm/m^2, $p < 0.001$), sino-tubular junction (−1.5 ± 2.3 mm/m^2, $p = 0.012$), and ascending tubular aorta (−2.1 ± 2.0 mm/m^2, $p = 0.001$). This last study had several major issues: (1) lack of a control group; (2) the results expressed as a reduction in indexed aortic diameters by body surface area when, in this age period, body growth may predominate over aortic growth, thereby explaining the results.

Another observational study by Mueller et al. was published in 2014 [84]. In that study, a cohort of 215 patients (mean age 9.01 ± 5.7 years) was retrospectively identified and 40 untreated and unoperated patients were selected. Clinical and echocardiographic follow-up was performed after ARB and/or β-blockers were initiated. Twenty-two patients received ARB therapy and 18 received β-blockers. Mean follow-up in the β-blocker group was 5.51 ± 3.30 years vs 1.4 ± 0.24 years in the ARB group ($p < 0.001$). Both medications showed a significant and similar reduction in sinus of Valsalva dilatation (evaluated as z-score). However, this study lacked of a control group, so it is not clear what the natural evolution of the z-score was in an untreated group of this age.

In 2013, Chiu et al. [85] published a clinical trial on a paediatric population to confirm the superiority of combined therapy with β-blockers and ARB vs the use of β-blockers alone in Marfan patients. In that study, 28 patients (aged 13.1 ± 6.3 years) with aortic root dilatation (z-score >2) were

randomised to receive β-blockers (atenolol or propanolol) or β-blockers and ARB (losartan). In the monotherapy β-blocker group, the maximum dose of atenolol or propanolol was 150 mg/day for adults and 2 mg/kg per day for children. In the combined therapy group, the adult target dosage of losartan was 100 mg/day (or the maximum tolerable dose) and the paediatric dose was started at 0.7 mg/kg/day and increased gradually up to 50 mg/day. Moreover, in the latter group, β-blocker doses were reduced (atenolol 50 mg/day, propanolol 20 mg/bid) to decrease pharmacologic cross-interactions. Patients with a history of aortic surgery or severe aortic disease (aortic root diameter at sinus of Valsalva level >55 mm, or aortic diameter growth > 1 mm/year) were excluded. The follow-up trial lasted 3 years. The aortic diameter of patients was checked every 3–4 months by transthoracic echocardiography. Emphatically, the results showed that combined therapy (β-blocker + losartan) reduced the annual dilatation rate of aortic root compared to β-blocker therapy alone (respectively 0.10 mm/year vs 0.89 mm/year, respectively; $p = 0.02$). Moreover, the study found a significant reduction in aortic diameter relative to baseline in 33 % of patients in the combined group but in none of those receiving β-blockers alone. Importantly, changes in aortic diameters were significantly less in the combined group at all ascending aorta levels (sinus of Valsalva, $p = 0.02$; aortic root z score, $p = 0.04$; aortic annulus, $p = 0.03$; and sinotubular junction, $p = 0.03$). However, no significant changes in blood pressure after medication use occurred in either group. Moreover, no changes were found either in descending aorta, aortic stiffness, and cross-sectional compliance. Even if that study was limited to a small population, it showed the potential benefit of ARB drugs added to standard therapy in Marfan patients.

Regarding losartan treatment, it is important to bear in mind that impressive results obtained in mice cannot be directly extrapolated to general medical therapy in MFS patients. It should be emphasised that, in animal models, losartan was administered in the first months of life or during pregnancy in the embryogenesis phase.

Ongoing Clinical Trials

The pharmacological prophylactic management of MFS has moved somewhat beyond the Marfan mouse stage to humans, although considerable insights are still being gained from such animal studies. With the use of losartan, an AT1R inhibitor licensed for other conditions, the translational path has been considerably shortened. The next crucial event is publication of the results of the ongoing randomised controlled trials. An increasing problem in the testing of novel hypotheses generated by new molecular insights into Marfan syndrome is that the small patient population can only sustain a limited number of trials. In this respect, there is no strong evidence to suggest that any of the AT1R antagonists are any better than losartan.

Ongoing trials are listed on the clinical trial homepage http://www.clinicaltrials.gov, see also Table 4.4.

The ***USA trial*** is comparing β-blocker therapy (atenolol) directly with losartan in an open-label, randomised trial [86]. The study will eventually include 600 patients with an age range of 0.5–20 years and a follow-up period by echo of 3 years. This study evaluates the advantages of two different first-line therapies but not the benefit of combining the two drugs compared with up-to-date standard therapy.

The ***French MARFANSARTAN trial*** **[**87] is a multicentre randomised placebo-controlled trial evaluating the efficacy of losartan in limiting aortic dilatation in MFS patients aged 10 years or older receiving standard therapy (β-blocker or calcium channel blocker if β-blocker therapy is not tolerated). Patients who had previously undergone aortic surgery were excluded. Aortic root diameter will be measured using two-dimensional echocardiography in a 3-year follow-up period. The desired number of patients included will be 300.

The ***Italian trial*** (**MaNeLo**) [43] is comparing three different approaches: β-blocker or losartan alone or the combination of both. The β-blocker being used (nebivolol) carries theoretical advantages over the non-selective propanolol used in the landmark study of Shores et al. [66] and over the

Table 4.4 Ongoing clinical trials on ARB in Marfan syndrome

First author (country)	Name and identification number	Study design and objectives	Year trial started – estimated completion	Follow-up	Estimated enrolment	Age	Aortic size at entry	Drug and daily dosage	Control drug and daily dosage
Lacro et al. (USA)	NCT 00429364	Randomized, SB Losartan vs Atenolol	Jan 2007–Feb 2014	3 years	604	6 months to 25 years	Z-score >3	Losartan 0.3–1.4 mg/kg	Atenolol 0.5–4 mg/kg
Delaint et al. (France)	MARFANSARTAN trial NCT 00763893	Randomized, DB Losartan vs placebo (+ standard therapy: propranolol)	Sep 2008–Mar 2014	3 years	303	>10 years	ND	Losartan 50 mg/day if<50 kg; 100 mg/day if>50 kg	Placebo
Gambarin et al. (Italy)	MaNeLo trial NCT 00683124	Randomized, DB Losartan vs Nebivolol or combination of both	July 2008–July 2013	4 years	291	1–55 years	Z-score ≥2; or absolute aortic root diameter >38 mm for females and >40 mm for males	Losartan 100 mg/day for adults and 1.5 mg/kg/d for children <16 years	Nebivolol alone or combined 10 mg/day for adults and 0.16 mg/kg/d for children <16 years

Mullen et al. (United Kingdom)	The AIMS trial ISRCTN 90011794	Randomized, DB Irbesartan vs placebo	Sep 2010–Mar 2017	5 years	490	6–40 years	<45 mm	Irbesartan 300 mg for patients > 50 kg and 150 mg if <50 kg	Placebo
Forteza et al. (Spain)	LO-AT-MARFAN NCT 01145612	Randomized, DB Losartan vs Atenolol	Oct 2008–Feb 2013	3 years	150	5–60 years	<45 mm	Losartan 2.5 mg/day for patients <50 kg or 25 mg/day for patients >50 kg (14 days). From day 15:50 mg/day or 25 mg/day for patients <50 kg	Atenolol 12.5 mg/day for patients <50 kg or 25 mg/day for patients >50 kg (14 days). From day 15:50 mg/day or 25 me/day for patients <50 kg

(continued)

TABLE 4.4 (continued)

First author (country)	Name and identification number	Study design and objectives	Year trial started – estimated completion	Follow-up	Estimated enrolment	Age	Aortic size at entry	Drug and daily dosage	Control drug and daily dosage
Radonic et al. (Netherlands)	COMPARE study NTR 1423	Randomized, open-label Losartan vs not-treated controls	Feb 2008–Feb 2010	3 years	330	≥18 years	Aortic root diameter <50 mm	Losartan 50 mg (first 14 days) or 100 mg (after first 14 days)	Patients taking β-blockers will continue their treatment
Chiu et al. (Taiwan)	NCT 00651235	Randomized, open-label Losartan added to β-blockers	Feb 2007–Feb 2011	3 years	44	≥1 year	Z-score >2	Losartan 100 mg/day for adults and 50 mg/day for children. Atenolol 50 mg/day; Propranolol 20 mg/twice daily for adults, 1 mg/kg/day for children	Atenolol or Propranolol. Atenolol or Propranolol is 150 mg/day for adult and 2 mg/kg/day for children

Möberg et al. (Belgium)	NCT 00782327	Randomized, DB Losartan added to β-blockers vs placebo	June 2009–Dec 2014	3 years	174	>10 years	Z-score ≥2	Losartan 50 mg for <50 kg and 100 mg if >50 kg	Placebo
Creager et al. (USA)	NCT 00723801	Randomized, DB Losartan vs Atenolol on aortic stiffness	Oct 2007–Dec 2012	6 months	50	>25 years	ND	Losartan 100 mg/day	Atenolol 50 mg/day
Sander et al. (Canada)	NCT 00593710	Randomized, DB Losartan vs Atenolol on aortic PWV	Jan 2008–Dec 2011	12 months	30	12–25 years	ND	Losartan 25 mg/day	Atenolol 25–50 mg/day

NCT ClinicalTrials.gov number, *ISRCTN* International Standard Randomised Controlled Trial Number, *NTR* Netherlands Trial Register, *PWV* pulse wave velocity, *SB* single-blind, *DB* double-blind, *ND* not described

betablocker used in the USA trial (atenolol). Its vasodilator properties could reduce the rebound wave and therefore the stress applied on the proximal aorta and enhance the haemodynamic benefit of the drug; its beta-1 selectivity should increase its tolerance and therefore compliance. Finally, the relative benefits of the two classes of drug and their combination are ideal. The drawback of having three groups is the need for a high number of patients (n: 291) to obtain the statistical power necessary to recognise differences between groups.

The ***University of Ghent trial*** [88] has a design similar to the French trial, but also evaluates the evolution of aortic stiffness over time. The objective is to include 174 MFS patients (age ≥ 10 years and z-score ≥ 2). Patients already taking β-blockers are randomised for weight-adjusted treatment with losartan versus placebo. The primary end-point is to reduce the aortic root growth rate. MRI evaluation will be made at baseline and at the end of the trial. The similar design may permit a secondary combination of the populations to increase statistical power, which is obviously an issue when the protocol aims to include such a selected population.

The English ***AIMS*** [89] (Aortic Irbesartan Marfan Study) Trial is studying the effects of another ARB, irbesartan, in Marfan patients. For this study, 490 Marfan patients (aged ≥ 6 and ≤40) will be enrolled and randomised to 2 groups: irbesartan vs. placebo. The therapeutic dose of ARB will be uptitrated to the maximum tolerated dose in 2 months (target dose 300 mg/die for patients ≥50 kg, 150 mg/die if <50 kg) and continued for 5 years. Patients with previous cardiac or aortic surgery are excluded. The primary outcome of that multicentre, prospective, randomised, double-blind trial will be evaluation of the different rate of aortic root dilatation between these groups measured by transthoracic echocardiography. Annual echocardiography follow-up will be carried out. Importantly, standard medical treatment (including β-blockers) will be given to all patients, if tolerated. Therefore, the study is not designed to evaluate the effects of irbesartan monotherapy in MSF, but rather the effects of combined

therapy. However, analysis of β-blocker-intolerant patient subgroup could also permit estimation of the effects of irbesartan alone.

The Spanish trial is a clinical trial conducted at two institutions. One hundred and fifty subjects of both sexes diagnosed with MFS, aged between 5 and 60 years, and who meet the Ghent diagnostic criteria will be included in the study, with 75 patients per treatment group. It will be a randomised, double-blind trial with parallel assignment to atenolol or losartan (50 mg per day in patients under 50 kg and 100 mg per day in patients over 50 kg). Both growth and distensibility of the aorta will be assessed with echocardiography and magnetic resonance. Follow-up will be 3 years.

Special Conditions

Medical Treatment in Operated Patients

After ascending aorta surgery, the distal aorta is still susceptible to dilatation or dissection [90]; thus, close imaging follow-up is required in these patients. Furthermore, the maintenance of long-term treatment with β-blockers and exercise restrictions must also be considered. A subgroup analysis from the COMPARE trial [82] suggested that the addition of losartan was significantly associated with a reduced dilatation rate of the aortic arch. However, this result should be interpreted with caution as baseline aortic dimensions in patients with prior aortic root replacement were not completely comparable between the treatment groups.

Medical Treatment in Pregnant Women

ACEI and ARB are contraindicated during pregnancy owing to the increased risk of fetal loss and birth defects. These deleterious effects have been confirmed in animal studies. Women of childbearing age under these treatments should be

informed of the potential teratogenic and fetotoxic risks of these drugs if they become pregnant [91]. Data related to the use of β-blockers during pregnancy are limited. All studies are observational and retrospective. Although β-blockers have been related to a higher risk of fetal growth retardation, consensus holds that β-blockers may be used during pregnancy to prevent aortic complications [92]. However, we recommend balancing the risk-benefit ratio in each individual patient, and fetal growth should be monitored if treatment with β-blockers is prescribed. A recent retrospective observational study [93] included 29 pregnancies in 21 women with MFS and compared them with 116 controls. Mean aortic root diameter pre-pregnancy was 39.5 ± 1.3 mm in the nulliparous group (n = 21). Although the study does not compare the outcome of Marfan patients with and without β-blocker treatment, it is informative of the outcome of Marfan pregnancies under this treatment since almost all patients were taking β-blockers throughout pregnancy (n = 26; 89.7 %). In this study, there were no maternal or perinatal deaths, but complications were more likely in the MFS group. Maternal complications occurred in five pregnancies (17 %) and included one type A aortic dissection, 2 aortic surgeries within 6 months of delivery and 2 patients who developed left ventricular dysfunction. Neonates in the Marfan group were more likely to be small for gestational age.

Omnes et al. [94] also published an observational retrospective study on 22 pregnancies with maternal mean aortic root at baseline 39.0 ± 3.9 mm. Again in this study, almost all patients were under β-blocker treatment (n = 19; 86.4 %). In this cohort, aortic diameter did not increase significantly during pregnancy, one aortic dissection occurred and fetal growth restriction was observed in 7 (31.8 %) pregnancies.

In 1995, Pyeritz et al. [95] published an observational study that included 28 pregnancies in Marfan patients. In that study, only 10 patients received β-blockers, but no comparison was made between both Marfan patient groups. Two patients suffered an aortic dissection: one was not treated with β-blockers and the other did receive them.

The risk of aortic dissection in Marfan patients during pregnancy has also been related to aortic dimensions. However, there is not a completely safe aortic dimension: Marfan patients with normal aortic root diameter (generally considered <40 mm) have a low risk of aortic dissection or other cardiac complications during pregnancy [96]. ESC guidelines for cardiovascular diseases during pregnancy recommend using the WHO classification to assess maternal risk in pregnant woman with cardiovascular conditions [92]. Thus, Marfan patients with normal aortic root are classified as having a WHO II risk [97] (small increased risk of maternal mortality or moderate increase in morbidity), and cardiological quarterly checks are recommended. Marfan patients with a diameter >40 mm and also patients with an increase in aortic diameters throughout pregnancy have an increased risk of aortic complications. Moreover, in the presence of an aortic diameter > 45 mm, pregnancy should be discouraged (WHO risk IV). In this scenario, some centres recommend aortic root surgery with a valve-sparing procedure (David's technique) prior to pregnancy, since the presence of a mechanical prosthetic aortic valve increases morbidity and mortality during pregnancy (WHO risk III: significant increased risk of maternal mortality or severe morbidity). However, after aortic surgery, patients remain at risk for aortic dissection in the distal aorta. Aortic root diameters between 40 and 45 mm in Marfan are generally classified as WHO risk III, but other risk factors for aortic dissection (indexed aortic root by body surface area >27 mm/m^2, family history of aortic dissection, rapid aortic growth, and aortic regurgitation) should also be taken in consideration. In these patients, monthly or bimonthly cardiological checks are recommended.

Current Recommendations

Although β-blocker therapy is currently recommended for all patients with MFS (American College of Cardiology Foundation/American Heart Association guidelines class I

recommendation for the use of β-adrenergic– blocking drugs for all patients with Marfan syndrome to reduce the rate of aortic dilation), the evidence level is B. Several studies reported that β-blockers may not produce the desired haemodynamic effects in patients with marked aortic root dilatation with a heterogeneous response. Recently, many studies have shown that additional treatment with losartan improves the efficacy to reduce aortic root and ascending aorta dilatation. Therefore, this strategy may be applied in high-risk patients with aorta dilatation and in cases where β-blocker treatment does not reach the maximum doses due to poor tolerance or side effects. Until future therapy directed at the fibrillin-1 gene or the TGF-β axis ultimately proves most effective at preventing the aortic complications of MFS, β-blocker therapy remains the "standard of care". Losartan as monotherapy would only be justified in patients with severe bradycardia, asthma or other β-blocker contraindications. Effects of pharmacological therapy should be monitored closely during the initiation phase to ensure that heart rate goals and blood pressure management are optimal. Routine monitoring of proximal aortic size and growth rate, usually with echocardiography on an annual basis, is essential in all patients. In cases in which echo is technically inadequate and/or when aortic root diameter reaches 45 mm or surgery is indicated, cardiac magnetic resonance or computed tomography of the thoracic aorta are recommended. Future research and ongoing trials should elucidate the benefits, advantages and limitations of each drug or their combinations, taking into account individual factors such as age, aortic dilatation, risk factors or genetic mutations.

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