CHAPTER 1

General introduction
Chapter 1

CONNECTIVE TISSUE DISORDERS

Hereditary connective tissue disorders are relatively rare diseases affecting the extracellular matrix (ECM). The ECM consists mainly of collagens, and has numerous functions, including supplying support for neighbouring cells and regulating cell behaviour. In the cardiovascular system, the ECM provides compliance and elasticity to the heart (valves) and vascular tree. The cardiovascular manifestations of connective tissue disorders vary greatly, from uncomplicated valve abnormalities in (familial) mitral valve prolapse (MVP) to disastrous aortic or other larger vessel dissection in, for example, Marfan syndrome (MFS), Loeys-Dietz syndrome (LDS), familial thoracic aortic aneurysm and/or dissection (FTAAD) and the vascular type of Ehlers-Danlos syndrome (EDS). There are many connective tissue disorders with cardiovascular involvement and it is beyond the scope of this thesis to discuss all of them in detail. Instead, the focus will be on the heritable connective tissue disorders MFS, LDS and familial MVP. A short general introduction to these disorders follows in the next sections. Appendix A (page 128) provides an overview of other connective tissue disorders with cardiovascular involvement.

MARFAN SYNDROME

In 1896 Antoine Bernard-Jean Marfan presented a case of a 5-year-old girl with disproportionally long limbs accompanied by long and slender fingers and toes. In the years that followed, several more cases were presented with similar characteristics. Other features were described, including cardiovascular abnormalities and dislocation of the ocular lens. As the understanding of the disease progressed, the name Marfan syndrome was coined. In more than a century of medical development, the knowledge of MFS has expanded tremendously and the patient presented by Antoine Bernard-Jean Marfan probably actually suffered from congenital contractural arachnodactily instead of MFS. Currently, MFS is known as a clinically heterogeneous disorder (due to variable gene expression) caused by mutations in the fibrillin 1 gene (FBN1), and in rare cases by mutations in the transforming growth factor (TGF)-β receptor 1 (TGFBR1) or -2 genes (TGFBR2). The estimated prevalence of MFS is approximately 1-3 in 5,000 persons and it is inherited in an autosomal dominant mode. Typical characteristics of MFS include aortic root dilatation, MVP, ectopia lentis, slender body habitus, long extremities and pectus deformities. If left untreated, aortic root dilatation can lead to aortic dissection or rupture and is the cause of premature mortality and reduced life expectancy in patients with MFS. In addition to the aortic root, other parts of the aorta are also at risk for dissection or rupture in MFS. Since 1996, a MFS diagnosis has been made when a patient fulfils the Ghent nosology, and these criteria were updated in 2010 giving rise to the revised Ghent nosology (table I). Signature characteristics in establishing a MFS diagnosis are aortic root dilatation (typically pear-shaped, figure I), ectopia lentis, a family history of MFS and mutations in FBN1. Nevertheless, MFS can also be diagnosed by evaluating other less-typical features (table I). In the Netherlands, patients suspected of MFS are seen at specialized outpatient clinics located in Groningen, Amsterdam, Nijmegen and Leiden. In these clinics, patients are systematically evaluated according...
Table I. Diagnostic criteria for MFS according to the 2010 Ghent nosology

In the absence of a family history of MFS:
1. Aortic root Z-score ≥2 AND ectopia lentis
2. Aortic root Z-score ≥2 AND an $FBN1$ mutation
3. Aortic root Z-score ≥2 AND a systemic score* ≥7 points
4. Ectopia lentis AND an $FBN1$ mutation with known aortic pathology

In the presence of a family history of MFS (as defined above):
1. Ectopia lentis
2. Systemic score* ≥7
3. Aortic root Z-score ≥2

* Points for systemic score
- Wrist AND thumb sign = 3 (wrist OR thumb sign = 1)
- Pectus carinatum deformity = 2 (pectus excavatum or chest asymmetry = 1)
- Hindfoot deformity = 2 (plain pes planus = 1)
- Dural ectasia = 2
- Protrusio acetabula = 2
- Reduced upper segment/lower segment ratio AND increased arm/height AND no severe scoliosis = 1
- Scoliosis or thoracolumbar kyphosis = 1
- Reduced elbow extension = 1
- Facial features (3/5) = 1 (dolichocephaly, enophthalmos, downslanting palpebral fissures, malar hypoplasia, retrognathia)
- Skin striae = 1
- Myopia > 3 diopters = 1
- MVP = 1

$FBN1$ fibrillin 1 gene, MFS Marfan syndrome, MVP Mitral valve prolapse, Z-score number of standard deviations above the mean

Figure I. Echocardiographic image of a pear-shaped aortic root in a patient with Marfan syndrome (parasternal long axis view).
LV left ventricle, LA left atrium
to the Ghent nosology. In cases where MFS is excluded, other connective disorders can sometimes be discovered. Currently, cardiovascular treatment of MFS consists of β-blockade to slow down aortic root growth and prophylactic aortic (root) surgery.\textsuperscript{11-13} Recently, the results of the COMPARE (COzaar in Marfan PAients Reduces aortic Enlargement) study were published.\textsuperscript{14} This was the first study to demonstrate that losartan, which antagonizes the effects of TGF-β (an important cytokine in the pathophysiology of MFS), reduces aortic root dilatation in adults with MFS.\textsuperscript{14} The results of other studies investigating the effect of losartan on aortic growth are awaited and, depending on the results, losartan might have an important role in the treatment of MFS in the future.\textsuperscript{15-18}

**LOEYS-DIETZ SYNDROME**

LDS was first described in 2005 and it is thought that its prevalence lies somewhere between that of vascular EDS (1:50,000) and MFS (1-3:5,000), although solid data are lacking.\textsuperscript{19} The clinical spectrum of LDS is also heterogeneous and has been subdivided into two types which form a continuum. LDS type I is characterized by facial dysmorphic features like a cleft palate, wide/bifid uvula, craniosynostosis (premature closure of cranial sutures) and hypertelorism (increased distance between the pupils). Neurocognitive development disorders can also be present. LDS type II does not have craniofacial abnormalities, but has striking cutaneous manifestations like a velvety skin, easy bruising and atrophic scars, although a wide/bifid uvula and hypertelorism can sometimes be present.\textsuperscript{20} Aggressive arterial aneurysms (e.g. aortic root, cerebral) and vascular tortuosity can be found in both types and are the major cause of mortality. LDS is caused by mutations in $TGFBR1$ or $TGFBR2$ and the mode of inheritance is autosomal dominant. The diagnosis is established when typical features combined with mutations in $TGFBR1$ or $TGFBR2$ are present. Aortic complications tend to occur at smaller diameters than in MFS and timely prophylactic surgery is crucial.\textsuperscript{21} Just as in MFS, there might be a role for treatment with β-blockade and losartan, however, this has not yet been investigated.

**MITRAL VALVE PROLAPSE**

MVP is a common valvular abnormality with an estimated prevalence of 2-3 in 100 individuals and it is characterized by single or bileaflet systolic billowing of the mitral valve in the left atrium (figure II).\textsuperscript{22} In addition, there is often leaflet thickening and redundancy, also known as myxomatous degeneration (Barlow disease).\textsuperscript{23} MVP can occur in isolation or as part of a connective tissue disorder, for example in MFS and LDS. In isolated MVP, sporadic and familial forms have been described. Mutations in $FLNA$ were the first, and thus far only mutations, described to cause isolated myxomatous valvular dystrophy, of which MVP is the most common form.\textsuperscript{24} Inheritance of $FLNA$ is X-linked dominant and therefore men are more severely affected than women when mutations in this gene occur. MVP can be asymptomatic, however, it can also be accompanied by complications such as significant mitral regurgitation (MR), bacterial endocarditis, thromboembolism and even sudden cardiac death due to ventricular tachyarrhythmias.\textsuperscript{12,25} Treatment depends on the clinical
situation; in case of significant MR, surgical intervention may be required and in case of ventricular arrhythmias, an implantable cardioverter defibrillator (ICD) may be indicated in addition to treatment with a β-blocker.

PATHOPHYSIOLOGY AND GENETICS
Although the clinical manifestations of the individual connective tissue disorders are diverse, there is overlap in the pathophysiology of these diseases. Dysregulation of the TGF-β-cytokine pathway is, for example, present in MFS and in LDS. TGF-β stimulates cell proliferation, inflammation and activates matrix metalloproteinases. Once TGF-β has been formed, it binds to several proteins and is secreted into the ECM as a large latent complex. In the ECM, the collagen fibrillin-1 binds the TGF-β latent complex, thereby reducing the release of free TGF-β. In MFS, mutations in FBN1 lead to abnormal fibrillin-1, which may have less affinity for TGF-β. As a consequence, more free TGF-β is present in the ECM, which then activates its receptors, and, through signal transducer- and transcriptional modulator-proteins (SMADs), transcriptional responses eventually cause the clinical manifestations of MFS. In addition to this SMAD-dependent (canonical) pathway, TGF-β also activates other (non-canonical) pathways like the RhoA and mitogen-activated protein kinase (MAPK) cascades, also leading to the transcriptional responses ultimately responsible for the characteristics of MFS (figure IIIa and IIIb).
Figure III a. Normal TGF-β pathway. TGF-β is secreted into the extracellular matrix as a large latent complex, where it is bound by fibrillin-1. Through the canonical (SMADs) and the non-canonical (for example MAPK and RhoA) pathway TGF-β can influence transcriptional responses.

Figure III b. TGF-β pathway in Marfan syndrome. Due to abnormal fibrillin-1 more free TGF-β is present in the extracellular matrix. As a consequence the TGF-β receptors are more stimulated leading to increased transcriptional responses, ultimately causing the characteristics of Marfan syndrome.
In LDS, TGF-β dysregulation takes place at the level of the TGF-β receptors since LDS is caused by mutations in the genes encoding TGF-β receptor 1 (TGFBR1) and 2 (TGFBR2). Myxomatous valve degeneration, of which MVP is an example, can be caused by mutations in FLNA and, through interaction with SMADs, abnormal filamin A also leads to dysregulation of TGF-β.

In addition to causing LDS, mutations in the TGF-β receptors can also be found in FTAAD. Other genes known to cause FTAAD are MYH11, ACTA2, FBN1, SMAD3, MYLK and NOTCH1. TGF-β upregulation was found in the aortic wall of patients with mutations in MYH11 and ACTA2, emphasizing the importance of the TGF-β pathway in connective tissue disorders with cardiovascular involvement.

Table II provides an overview of the pathophysiology and the genes involved in the discussed connective tissue disorders.

<table>
<thead>
<tr>
<th>Connective tissue disorder</th>
<th>Pathophysiology</th>
<th>Gene (chromosome)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marfan syndrome</td>
<td>TGF-β signalling, Fibrillin-1</td>
<td>FBN1(15.q21.1), TGBR1 (9q22.33),TGFBR2(3p24.1)</td>
</tr>
<tr>
<td>Loeys-Dietz syndrome</td>
<td>TGF-β signalling</td>
<td>TGFBR1(9q22.33), TGFBR2(3p24.1), SMAD3 (15q22.33)</td>
</tr>
<tr>
<td>Familial MVP</td>
<td>TGF-β signalling, Filamin A</td>
<td>FLNA (Xq28)</td>
</tr>
<tr>
<td>FTAAD</td>
<td>TGF-β signalling, SMC function</td>
<td>ACTA2 (10q23.31), TGFBR1(9q22.33), TGFBR2(3p24.1), FBN1(15.q21.1), MYH11 (16p13.11), SMAD3 (15q22.33), MYLK (3q21), NOTCH1 (9q34.3)</td>
</tr>
</tbody>
</table>

FTAAD familial thoracic aortic aneurysm and/or dissection, MVP mitral valve prolapse, SMC smooth muscle cell, TGF-β transforming growth factor beta

OUTLINE OF THE THESIS

Although much is already known about connective tissue disorders, many unresolved issues remain with regard to the pathophysiology, clinical presentation, recognition and management of these disorders. In this thesis several cardiological and genetic aspects of MFS, LDS, and familial MVPS will be addressed.

Chapter 2 investigates the diagnostic yield of the Groningen Marfan outpatient clinic and the impact of the recent revision of the diagnostic criteria for MFS (Ghent nosology).

Chapter 3 discusses the clinical characteristics and management of LDS in a group of Dutch patients with this syndrome.

Chapter 4 investigates whether familial MVP can be caused by mutations in TGFBR1 and TGFBR2.

Chapter 5 explores the detailed clinical heterogeneity of MFS and describes the largest family
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with MFS ever reported.

Chapter 6 investigates whether a relationship exists between LV dilatation in patients with MFS and the specific FBN1 genotype.

Chapter 7 investigates biventricular function and the influence of aortic elasticity in MFS by means of cardiac magnetic resonance imaging (MRI).

Chapter 8 investigates whether a protocol for prophylactic aortic root surgery in MFS based on body surface area (BSA) is effective and safe.

Chapters 9 and 10 summarize the findings of the preceding chapters and explore future perspectives.

REFERENCES


Amata S, Tavazzi L, Arbustini E. Rationale and design of a trial evaluating the effects of losartan vs. nebivolol vs. the association of both on the progression of aortic root dilation in Marfan syndrome with FBN1 gene mutations. *J Cardiovas Med* (Hagerstown), 2009;10:351-362.


