Familial thoracic aortic aneurysms and dissections - studies on genotype and phenotype

Matias Hannuksela
Cover art: The term aorta originates from the ancient Greek word aorte. Aorte derives from the verb aorteo, which is the lengthened form of aiero meaning “to lift” or “to be hung up”. The word aorta has common etymological origins with the word aorter, a shoulder strap that was part of the weapons of the ancient Greek hoplites from where the sword was hanging. Hippocrates (ca. 470 - 360 B.C.) introduced the medical term aorta to describe the trachea and the bronchial tubes. One century later, Aristotle, the Greek philosopher and scientist, applied the term to describe the anatomical structure from which the heart hangs.1
To my family
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Abstract

Background:
Thoracic aortic aneurysms and dissections (TAAD) have a genetic component with an estimated 20-25% of the patients having a positive family history. An aneurysm often precedes a dissection. Acute aortic dissections are associated with high mortality and morbidity, even when operated on. Complications due to prophylactic surgery are considerably fewer. Therefore, patients at risk for dissection should be identified, followed-up and evaluated for prophylactic intervention.

Aims:
1. To establish reference values for ascending (AoA) and descending aortic (AoD) diameters measured by computed tomography. 2. To study the effectiveness of phenotypic cascade screening in families with an inherited form of thoracic aortic aneurysms and dissections (FTAAD) and to address questions that arise when screening for a genetic disorder is applied. 3. To study the agreement of aortic diameters obtained by TTE and MRI and to study aortic stiffness in individuals from families with FTAAD. 4. To perform exome sequencing in order to identify pathogenic sequence variants causing FTAAD, to characterize the phenotype, and to compare thoracic aortic diameter and stiffness in mutation carriers and non-carriers.

Results:
Paper I: The diameter of the thoracic aorta increased by 0.17 mm (0.12 – 0.20 mm) per year. The mean sex-related difference in diameter was 1.99 mm (1.28 – 2.60 mm) with men having larger aortas than women. The mean difference in aortic diameter per unit BMI was 0.27 mm (0.14 – 0.44 mm). Upper normal limits for the AoA can be calculated by the formula D (mm)=31+0.16*age and for the AoD by D (mm)=21+0.16*age.

Paper II: Of 106 individuals from families with FTAAD but without known thoracic aortic disease, 19 individuals (18%) were identified to have a dilated AoA. The expected number of individuals in this group with an autosomal dominant disease would have been 40 (p<0.0001). In first-degree relatives younger than 40, we found only one individual with a dilated aorta although the expected number of individuals with disease causing mutation would have been 10.

Paper III: Of 116 individuals investigated, 21 were identified with thoracic aortic dilatation and 95 individuals with normal thoracic aortic diameter. Aortic stiffness increased with age and diameter. The individuals with aortic dilatation were older than those without (49 vs. 37 years, p=0.001) and showed lower aortic elastic properties. The diameters measured by TTE and MRI correlated strongly (r²=0.93). The mean difference in diameters between the two methods was 0.72 mm (95% CI 0.41-1.02) with TTE giving larger diameters than MRI.

Paper IV: From exome sequencing and segregation analysis, a 2-bp deletion in the MYLK gene (c.3272_3273del) was identified to cause FTAAD. The age and the aortic diameter at dissection or rupture varied in the family.
members. We did not find any differences in aortic diameter, aortic stiffness, or pulse wave velocity between carriers and non-carriers.

**Conclusions:**
Thoracic aortic diameter increases with age, and sex and body size are also associated with the diameter.

In FTAAD, screening identifies family members with a previously unknown aortic dilatation. However, a normal aortic diameter does not exclude an individual from being a carrier of FTAAD.

TTE can be used in follow-up for the ascending aorta. Individuals identified to have a dilated thoracic aorta have increased aortic stiffness compared to individuals with normal thoracic aortic diameter.

The *MYLK* mutation (c.3272_3273del) causes thoracic aortic dissections with variable clinical expression. No differences in aortic stiffness were identified between *MYLK* mutation carriers and non-carriers.

**Keywords:** Thoracic aorta, familial aortic aneurysm, familial aortic dissection, genetics, aortic stiffness
Svensk sammanfattning

**Bakgrund:** Kroppsådern inne i bröstkorgen (thorakala aorta) kan drabbas av olika sjukdomstillstånd. De vanligaste är bråck (aneurysm) och bristning (dissektion eller ruptur). Ofta, men inte alltid, föregås en bristning av en långsam vidgning av kroppspulsådern, vilket leder till att ett bräck bildas. Ju större bräcket är desto större är risken att det brister. En bristning är ett livshotande tillstånd som kräver akut omhändertagande.

Förekomsten av bråck på thorakala aorta anges till ca 450/100 000 personer, men det är svåruppskattat eftersom ett aneurysm sällan ger några symtom innan det brister och därför ofta blir odiagnostiserat. Förekomsten av bristningar på thorakala aorta är relativt ovanligt. Incidensen uppskattas till ca 4-6/100 000 invånare och år. Det finns dock ett mörkertal, då en del av personerna avlider innan de hinner till sjukhus eller innan diagnosen fastställs.

Det är vanligare att drabbas av bråck på kroppspulsådern i buken (bukaortaaneurysm) än inom bröstkorgen (thorakala aortaaneurysm). Dessa två sjukdomstillstånd skiljer sig åt på vissa punkter. Personer med buaortaaneurysm har ofta riskfaktorer för hjärtkärlsjukdom, d v s högt blodtryck, höga blodfetter, rökning och övervikt. Thorakala aortaaneurysm är inte kopplade till dessa riskfaktorer med undantag för högt blodtryck.

En bristning som drabbar den uppåtstigande delen av kroppspulsådern kallas för typ A dissektion. I dessa fall måste dissektionen opereras akut, då risken för att kroppspulsådern brister helt och att man avlider är stor. Om dissektionen drabbar den nedåtstigande delen av kroppspulsådern kallas den för typ B dissektion. Dessa dissektioner behöver i regel inte opereras utan patienterna behandlas i första hand med blodtryckssänkande läkemedel.

Om ett bråck på kroppspulsådern upptäcks, följs det regelbundet med ultraljud, skiktröntgen eller magnetkameraundersökning. Normal diameter på den uppåtstigande delen av kroppspulsådern är ca 25-40 mm beroende på ålder och kön. Om diametern ökar till ca 55 mm, rekommenderas förebyggande operation, vid vilken man byter den vidgade delen av kroppspulsådern mot ett rör av konstgjort textilmateriel. Den nedåtstigande delen av kroppspulsådern åtgärdas om diametern når ca 60 mm. Man gör då antingen en operation eller sätter i ett så kallat stent (ett metallnät) via ljumsken på insidan av kroppspulsådern och som förhindrar aortas fortsatta tillväxt.

I ca 20-25% av fallen är de thorakala aneurysmen ärftliga. Med andra ord finns en genetisk förändring (mutation) som nedärvs och ger individen en ökad risk att drabbas av bräck eller bristning av kroppspulsådern. Denna
mutation kan vara lokaliserad i någon av de olika gener som kodar för proteiner som påverkar kärlväggens funktion. Den kan därmed orsaka en förändrad funktion hos de glatta muskelcellerna eller stödjeproteinerna i kärlväggen eller i signalsystemet som styr samspelet mellan dessa strukturer.


Avhandlingens syfte: Att bestämma gränser för när kroppspulsådern är vidgad och studera hur aortadiametern påverkas av ålder, kön och kroppstorlek. Att studera vad screening av familjer med ärflig form av aortadissektioner leder till; hur många nya individer med vidgad kroppspulsåder kan hittas och vilka frågeställningar man kan ställas inför när man screenar för en genetisk sjukdom. Att studera kroppspulsåderns elastiska egenskaper och jämföra om dessa skiljer sig mellan individer med normal och vidgad aorta och att jämföra om ultraljud och magnetkameraundersökning av kroppspulsådern ger likvärdiga resultat. Att försöka identifiera den sjukdomsorsakande mutationen i någon av de i studien ingående släkterna, beskriva den genetiska förändringen och studera skillnader mellan individer med och utan mutationen.

Resultat: Delarbete I: Vi fann att kroppspulsådern växer med stigande ålder och till viss mån även påverkas av kön och kroppstorlek. Referensvärden för kroppspulsådern i olika åldrar beräknades.

Delarbete II: Av 106 individer tillhörande släkter med ärflig förekomst av thorakala dissektioner identifierades 19 (18%) individer med tidigare okänd
vidgad thorakal aorta. Eftersom sjukdomen följer ett specifikt nedärvningsmönster beräknas antalet bärare av det sjukdomsorsakande arvsanlaget vara ca 40. Således kan man inteidentifiera alla anlagsbärare med hjälp av aortadiametern.


Original papers

This thesis is based on the following papers, which will be referred to in the text by their Roman numbers:

I  Hannuksela M, Lundqvist S, Carlberg B.  
**Thoracic aorta – dilated or not?**  
*Scand Cardiovasc J* 2006;40:175-178

II  Hannuksela M, Stattin E-L, Johansson B, Carlberg B.  
**Screening for Familial Thoracic Aortic Aneurysms with Aortic Imaging Does Not Detect All Potential Carriers of the Disease**  
*Aorta* 2015;3:1-8

III  Hannuksela M, Johansson B, Carlberg B.  
**Aortic Stiffness in Families with Inherited Thoracic Aortic Disease.**  
Submitted

**A novel variant in MYLK causes thoracic aortic dissections: genotypic and phenotypic description**  
*BMC Med Genet.* 2016;17:61

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### Abbreviations

<table>
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<th>Abbreviation</th>
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<tr>
<td>AAA</td>
<td>Abdominal aortic aneurysm</td>
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<td>AoA</td>
<td>Ascending aorta</td>
</tr>
<tr>
<td>AoD</td>
<td>Descending aorta</td>
</tr>
<tr>
<td>ARB</td>
<td>Angiotensin II receptor blocker</td>
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<td>AS</td>
<td>Aortic stiffness</td>
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<tr>
<td>BAV</td>
<td>Bicuspid aortic valve</td>
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<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>BSA</td>
<td>Body surface area</td>
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<tr>
<td>cfPWV</td>
<td>Carotid-femoral pulse wave velocity</td>
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<tr>
<td>CT</td>
<td>Computed tomography</td>
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<tr>
<td>CV</td>
<td>Cerebrovascular</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<tr>
<td>ECM</td>
<td>Extracellular matrix</td>
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<td>EDS</td>
<td>Ehlers-Danlos syndrome</td>
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<tr>
<td>vEDS</td>
<td>Vascular Ehlers-Danlos syndrome</td>
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<td>FTAAD</td>
<td>Familial thoracic aortic aneurysms and dissections</td>
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<td>LDS</td>
<td>Loeys-Dietz syndrome</td>
</tr>
<tr>
<td>MFS</td>
<td>Marfan syndrome</td>
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<tr>
<td>MMP</td>
<td>Matrix metalloproteinase</td>
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<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
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<td>MYLK</td>
<td>Myosin light chain kinase</td>
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<tr>
<td>NMD</td>
<td>Nonsense-mediated decay</td>
</tr>
<tr>
<td>PWV</td>
<td>Pulse wave velocity</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic acid</td>
</tr>
<tr>
<td>SoV</td>
<td>Sinuses of Valsalva</td>
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<tr>
<td>SMC</td>
<td>Smooth muscle cell</td>
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<tr>
<td>SNV</td>
<td>Single nucleotide variant</td>
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<tr>
<td>TAA</td>
<td>Thoracic aortic aneurysm</td>
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<tr>
<td>TAAD</td>
<td>Thoracic aortic aneurysms and dissections</td>
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<tr>
<td>TGF-β</td>
<td>Transforming growth factor-β</td>
</tr>
<tr>
<td>TEE</td>
<td>Transesophageal echocardiography</td>
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<tr>
<td>TIMP</td>
<td>Tissue inhibitor metalloproteinase</td>
</tr>
<tr>
<td>TTE</td>
<td>Transthoracic echocardiography</td>
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Prologue

Several years ago, my supervisor Doctor Bo Carlberg was examining a patient, a 70-year old man, in the medical ward at the hospital. The patient had come to the emergency unit the day before, complaining of chest pain. He was admitted to the cardiac ward for observation. During the examination, the patient collapsed and the following cardiopulmonary resuscitation was unsuccessful. The autopsy revealed an aortic dissection with a rupture to the pericardium.

Two years earlier, his grandson had died from an aortic dissection at the age of 15. The family members were now very concerned and several questions came up in their minds. What disease is this? What is known about the inheritance? Who in the family is the next one to be affected? Which examinations should be performed on the remaining family members? How can an aortic dissection be prevented?

The questions were very adequate. However, we had few answers to give and new questions arose. Which method should be used to investigate the aorta? When should the aorta be regarded as dilated? How is the thoracic aortic diameter influenced by age, sex, and body size? Is this really a genetic disease, and which is the disease-causing genetic variant in that case? How can we identify the variant and the carriers? What kind of information can screening of the aorta give? What can we tell the remaining family members after screening?

Soon, we became aware of other families in which several members had suffered from thoracic aortic dissections and aneurysms. The concern in the families was obvious and similar questions arose.

We found it important to find answers to these questions and we decided to design a research plan. Today, we have answers to some of these questions, which are presented in this thesis. Other questions still remain unanswered but the research on familial thoracic aortic dissections and aneurysms continues.


**Introduction**

**Background**

Acute thoracic aortic dissection is a life-threatening condition associated with high mortality and morbidity, even when operated on. Elective surgery for a dilated thoracic aorta is also associated with a risk for serious complications although to a much lesser extent than surgery for an acute dissection. Therefore, patients at risk for dissection should be identified and evaluated for prophylactic surgical intervention. An estimated 20-25% of patients with a thoracic aortic aneurysm or dissection (TAAD) have an inherited form of the disease (familial TAAD, FTAAD), and subsequently have relatives with the same disease. FTAAD is associated with genetic and phenotypic heterogeneity with dissections occurring at variable ages and at variable range of aortic dilatation. The main aims of this thesis were to define normal diameter ranges for the thoracic aorta related to age, sex, and body size, to evaluate screening for thoracic aortic disease, to evaluate aortic stiffness as a marker for thoracic aortic disease, and to attempt to identify the underlying genetic variant in families with FTAAD.

**The aorta**

The aorta is the main artery in the human body, originating from the left ventricle of the heart and extending into the abdomen where it splits into the common iliac arteries (Figure 1). The aorta is divided into the thoracic aorta localized above the diaphragm and the abdominal aorta localized below the diaphragm. The thoracic aorta is further divided into the aortic root, the ascending aorta (AoA), the aortic arch, and the descending aorta (AoD) (Figure 1).

![Figure 1](attachment:image.png)  
*Figure 1. The anatomy of the thoracic and the abdominal aorta. rPA = right pulmonary artery. Reprinted from [4] with permission from Oxford Journals.*
Histology of the aorta

The aortic wall consists of three layers – the tunica intima, the tunica media and the tunica adventitia (Figure 2). The intima is the inner layer consisting of a single layer of endothelial cells attached to the basement membrane. Within the tunica media, the circumferentially arranged smooth muscle cells (SMC) and the extracellular matrix (ECM) are the largest components of the aortic wall. The smooth muscle component does not alter the diameter of the aorta to a large extent, but rather serves to increase the stiffness of the aortic wall when activated and thus regulates blood flow and blood pressure. The elastic matrix dominates the biomechanical properties of the aorta. The elastic matrix forms lamellae, consisting of elastic fibres, collagen (predominately type III), proteoglycans, and glycosaminoglycans. The elastic fibres in the ECM are connected to the SMC via the integrin-receptors in the cell membrane. The adventitia is the outermost connective tissue layer. It is composed of longitudinally arranged collagenous tissue and the vasa vasorum, which is a network of small blood vessels that supplies the circulation of the aortic wall. The thoracic aorta contains more elastin than the abdominal aorta and is therefore more distensible. The distensibility of the aorta declines with age and with increasing diameter.

Figure 2: The histology of the aortic wall with the three layers - tunica intima, tunica media and tunica adventitia.
The ageing of the normal aorta

The aortic diameter increases with age. When we started work with this thesis, the normal aortic expansion rate related to age was unknown. Our study and other later studies have shown the expansion rate to be about 0.09-0.20 mm/year.\textsuperscript{5, 6} This slow dilatation is thought to be related to a higher collagen to elastin ratio and an increase in ground substance. The elastic fibres undergo thinning and fragmentation and the concentric arrangement of the laminae is disturbed leading to increased stiffness and thereby also increased pulse pressure.\textsuperscript{7-8}

Aortic aneurysms and dissections – definitions, incidence and prevalence

An aortic aneurysm is defined as an enlargement (dilatation) of the aorta to greater than 1.5 times the normal diameter.\textsuperscript{9, 10} The aortic aneurysms are most commonly located in the abdominal aorta (AAA, abdominal aortic aneurysm), but can also be located in the thoracic aorta (TAA, thoracic aortic aneurysm). An aneurysm increases the risk of rupture or dissection of the aorta.

TAA affects men two to four times more frequently than women and the mean age at diagnosis is 60-70 years.\textsuperscript{11-15} In a Swedish autopsy study, the prevalence of asymptomatic TAA was 437-489/100 000 individuals.\textsuperscript{16} The yearly incidence of thoracic aortic dissections is reported to be 4-6/100 000 and as many as 48% of patients with type A dissection die before admittance to hospital.\textsuperscript{11, 17}

Natural history of TAA

The expansion rate of thoracic aortic aneurysms is estimated to be 1.0 – 4.2 mm/year.\textsuperscript{18-22} Aortic size is a strong predictor of rupture, dissection and death. TAA is often asymptomatic until a dissection or a rupture occurs. For aneurysms greater than 60 mm in diameter, the yearly risk for rupture, dissection, and death is estimated to be approximately 15\%.\textsuperscript{19} Using aortic size index, the corresponding risk for rupture, dissection or death is 8\% per year for patients with an index of 27.5-42.4 mm/m\(^2\) body surface area (BSA) and 20\% per year for patients with an index of 42.5 mm/m\(^2\).\textsuperscript{23} In an aortic dissection, a tear in the intimal layer results in a blood flow inside the aortic wall, separating the intima from the media (Figure 3). Patients with aortic dissections in the AoA should undergo acute surgical repair meanwhile dissections in the AoD can initially be managed by meticulous control of the blood pressure.
Vascular remodelling and aneurysm formation

Vascular remodelling refers to the architectural alterations in a vessel wall in response to hemodynamic changes or vascular injury. This process maintains the vessel lumen diameter and consistent blood flow under normal physiological conditions. Histologically, aneurysmal disease has been characterized based on the alterations in the vascular extracellular matrix, primarily a pathological remodelling of collagen and elastin. This process is driven by enhanced production of extracellular proteases leading to loss and fragmentation of elastic fibres and by loss and disarrangement of vascular smooth muscle cells. The matrix metalloproteinases (MMPs) play an important role in this process. The MMPs are extracellular proteases that are capable of degrading aortic extracellular matrix components. The normal balance between tissue inhibitor metalloproteinase (TIMP) and MMP is disturbed favouring an enhanced proteolytic state and matrix degradation. This leads to loss of mechanical strength and integrity of the aortic wall and further to aortic dilatation and occasionally dissection or rupture. The former term cystic media necrosis that was used to describe histological changes in the aortic wall has been replaced by medial degeneration.
The genetic background to pathological vascular remodelling – a key-role for transforming growth factor-β (TGF-β) signalling or mechanotransduction?

The pathogenesis of the TAA is different from that of the AAA. The AAAs are related to atherosclerotic risk factors including overweight, hyperlipidaemia, hypertension and smoking. The only known common risk factor for TAA is hypertension.

TAAs have a genetic component with an estimated 20-25% of patients having a positive family history. This number is likely to be underestimated because TAA usually remains asymptomatic until a catastrophic event. During the last two decades, the understanding of the underlying genetic factors for FTAAD has increased remarkably. Several sequence variants in different genes and in different chromosomes causing FTAAD have been identified.

The genes involved in FTAAD are inherited mainly in an autosomal dominant pattern and encode for components of 1) the ECM, 2) the TGF-β signalling pathway or 3) vascular SMC (Table 1).

Table 1: The most common genes identified to cause FTAAD, the proteins encoded by the genes and the chromosome localisation.

<table>
<thead>
<tr>
<th>Name</th>
<th>Gene</th>
<th>(year)</th>
<th>Protein</th>
<th>Chromosome</th>
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<tr>
<td><strong>Genes encoding components of the extracellular matrix</strong></td>
<td></td>
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<tr>
<td>Marfan Syndrome</td>
<td>FBN1</td>
<td>(1991)</td>
<td>Fibrillin-1</td>
<td>15q21.1</td>
</tr>
<tr>
<td>Ehlers-Danlos, vascular type</td>
<td>COL3A1</td>
<td>(1989)</td>
<td>Procollagen III</td>
<td>2q32.2</td>
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<tr>
<td><strong>Genes encoding components of TGF-β signaling</strong></td>
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<tr>
<td>Loeys-Dietz Syndrome, LDS-1</td>
<td>TGFB1</td>
<td>(2005)</td>
<td>TGFB1</td>
<td>9q22.33</td>
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<td>LDS-2</td>
<td>TGFB2</td>
<td>(2006)</td>
<td>TGFB2</td>
<td>3p24.1</td>
</tr>
<tr>
<td>LDS-3, AOS</td>
<td>SMAD3</td>
<td>(2011)</td>
<td>SMAD3</td>
<td>15q22.33</td>
</tr>
<tr>
<td>LDS-4</td>
<td>TGFB2</td>
<td>(2012)</td>
<td>TGFB2</td>
<td>1q41</td>
</tr>
<tr>
<td>LDS-5</td>
<td>TGFB3</td>
<td>(2015)</td>
<td>TGFB3</td>
<td>14q24.3</td>
</tr>
<tr>
<td><strong>Genes encoding components of contractility of smooth muscle cells</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha-actin-2</td>
<td>ACTA2</td>
<td>(2007)</td>
<td>Alpha-actin-2</td>
<td>10q23.31</td>
</tr>
<tr>
<td>Myosin Light Chain Kinase</td>
<td>MYLK</td>
<td>(2010)</td>
<td>MLCK</td>
<td>3q21.1</td>
</tr>
</tbody>
</table>
The transforming growth factor-β (TGF-β) signalling pathway

TGF-β signalling has an important role in aneurysm formation both through disturbances in the signalling system itself as well as being a link between the composition of the ECM and the vascular SMC function (Figure 4).

TGF-β is a protein involved in different cellular processes including proliferation, differentiation, angiogenesis, and apoptosis. It is also described as a modifier of the structure and composition of the ECM.26

In the classical TGF-β signalling pathway, TGF-β (consisting of three different ligands – TGF-β1, TGF-β2, TGF-β3) binds to a receptor complex consisting of type 1 receptors (TGF-βR1) and type 2 receptors (TGF-βR2). TGF-βR2 activates TGF-βR1, which initiates a cascade of intercellular signalling mediated by the SMAD-proteins. SMADs transduce these extracellular signals to the nucleus where gene transcription is activated.27

In addition to the classical signalling pathway, alternative pathways exist and TGF-β effects may be mediated through pathways excluding SMAD-mediated activity.

Studies suggest that TGF-β signalling can regulate the production of critical vascular matrix proteins as well as degrading enzymes. Therefore, alterations in normal TGF-β signalling, especially overstimulation of the pathway, is associated with enhanced proteolysis of the vascular ECM.24, 25, 28, 29

Mechanotransduction

The SMC contractile unit consists of actin and myosin filaments. The actin filaments of the contractile unit interface with the cytoskeleton via integrin receptors. Integrin receptors are the principal receptors for the ECM and serve as a transmembrane link between the contractile unit and matrix microfibrils (e.g. fibrillin-1). This complex provides the interface between the contractile machinery on the interior of the cell and the ECM on the exterior (Figure 4).
From genes to disease – what are the mechanisms?

The gene variants associated with TAA can cause either a syndromic or non-syndromic trait of the disease. The most common syndromic forms are Marfan syndrome (MFS), Loeys-Dietz syndrome (LDS) – including Aneurysm-Osteoarthritis syndrome, and the vascular type of Ehlers-Danlos syndrome. The non-syndromic forms present with an aneurysm or a dissection as sole disease manifestation.

**Marfan syndrome (MFS)**

Antoine Marfan (1858-1942), a French paediatrician, first described MFS more than 100 years ago. MFS is a multisystemic disorder affecting the skeletal (overgrowth, joint laxity, vertebral column deformity), ocular (lens dislocation and myopia) and cardiovascular system (aortic root aneurysm and dissection, mitral valve disease). Other manifestations include dural ectasia, inguinal hernias, pneumothorax, and lung emphysema. The cardiovascular manifestations cause the most important morbidity and mortality in patients with MFS. The Ghent criteria, revised in 2010, are used to diagnose MFS.

The molecular basis of MFS is a mutation in the **FBN1** gene on chromosome 15 encoding for Fibrillin-1, a component of the extracellular matrix. The **FBN1** gene is large; it contains 65 exons and more than 2000 different mutations causing MFS have now been identified in the gene. Fibrillin-1 is essential for the proper formation of the ECM. Elastic fibres are
distributed throughout the body, but are particularly abundant in the aorta, ligaments and the ciliary zonules of the eye.

TGF-β plays an important role in MFS. Fibrillin-1 directly binds a latent form of TGF-β, keeping it sequestered and unable to exert its biological activity. Reduced levels of fibrillin-1 allow TGF-β levels to rise due to inadequate sequestration.

Although it is not established how elevated TGF-β levels are responsible for the specific pathology seen with the disease, an inflammatory reaction releasing proteases that slowly degrade the elastic fibers and other components of the extracellular matrix is known to occur.

**Loeys-Dietz syndrome (LDS)**

LDS was described as late as in 2005. A triad of hypertelorism, cleft palate or bifid uvula, and arterial tortuosity, combined with widespread aneurysms, characterize LDS in its most typical presentation. In the first type of the syndrome, the aortic aneurysms tend to be more aggressive than in patients with MFS, leading to dissection and rupture at smaller diameters and at younger ages. At present, four different types of LDS have been described. LDS type 1 involves the TGF-βR1 gene, LDS type 2 the TGF-βR2 gene, LDS type 3 the SMAD3 gene, and LDS type 4 the TGF-β2 gene. Recently, a mutation in the TGF-β3 gene encoding a ligand of the TGF-β pathway was identified in an individual with a syndrome presenting overlapping manifestations with MFS and LDS (Table 2).

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Gene</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDS 1</td>
<td>TGFBR1</td>
<td></td>
</tr>
<tr>
<td>LDS 2</td>
<td>TGFBR2</td>
<td>Previously known as MFS2 syndrome</td>
</tr>
<tr>
<td>LDS 3</td>
<td>SMAD3</td>
<td>Also known as Aneurysm-Osteoarthritis syndrome</td>
</tr>
<tr>
<td>LDS 4</td>
<td>TGFBR2</td>
<td></td>
</tr>
<tr>
<td>LDS 5</td>
<td>TGFBR3</td>
<td>Overlapping MFS and LDS</td>
</tr>
</tbody>
</table>

Patients with different forms of LDS show clinical pictures ranging from pure familial thoracic aortic aneurysm to severe malformative disease with a dismal prognosis.

Common for the different types of LDS is an up-regulation of the TGF-β signalling pathway. Mutations in TGF-βR1/2 genes (LDS1/2) lead to loss-of-function of the receptors for TGF-β and thus higher levels of TGF-β. SMAD3 is the first intracellular downstream effector of the TGF-β pathway and it is activated by phosphorylation by the type 1 TGF-β receptor. This leads to a loss-of-function of SMAD3 and an increase of TGF-β signalling. TGF-β2 is one of the three TGF-β cytokines, and mutations in the gene lead to increased TGF-β signalling in association with normalization of TGF-β2 expression and high expression of TGF-β1 compared to wildtype.
Ehlers-Danlos syndrome (EDS)

Ehlers-Danlos syndrome is a group of connective tissue disorders of which the vascular form (vEDS), also called EDS type 4, involves the aorta and other arteries. The syndrome is caused by mutations in the \textit{COL3A1} gene encoding for collagen type III. Collagen is an important contributor to the physical strength of tissue and counteracts deformation. The clinical phenotype is characterized by rupture of middle-sized arteries and of intestines and by aortic dissection. Although it is known that collagen production is TGF-\(\beta\) dependent, there is no evidence that mutations in \textit{COL3A1} affect TGF-\(\beta\) pathway regulation in a direct way. Nevertheless, it was suggested, that the beta-blocker celiprolol effect seen in the treatment of vascular EDS treatment might be related to its TGF-\(\beta\) suppressing effects.41

Mutations in ACTA2, MYH11, and MYLK genes

\textit{ACTA2}, \textit{MYH11}, and \textit{MYLK} genes encode for proteins involved in the contractile unit of the vascular SMC. There is evidence of increased TGF-\(\beta\) signalling in aortic tissue in patients with \textit{ACTA2} and \textit{MYH11} mutations (Figure 5).42

\textit{ACTA2} mutations

Mutations in the \textit{ACTA2} gene are responsible for 12-20\% of non-syndromic FTAAD. Approximately half of the mutations-carriers present with aortic events. Dissections occur in both the AoA and the AoD, but more often in the AoA. The median age for dissections varies between 27-36 years with type B dissections occurring at younger ages than type A dissections.43 The diameter of the sinuses of valsalva (SoV) and the AoA at the time of dissection varies widely, but an estimated 30\% of patients experience aortic dissections at diameters < 50 mm. 43 Associated manifestations including livedo reticularis, iris flocculi, stroke, and Moya-Moya disease have been described.44 However, no clear genotype-phenotype correlations can be demonstrated in the majority of \textit{ACTA2} mutation carriers.42

\textit{MYH11} mutations

\textit{MYH11} mutations were reported to cause FTAAD and to be associated with persistent ductus arteriosus.42, 46 Only a few families with \textit{MYH11} mutations have been reported and the knowledge of the phenotype is limited. Up-regulation of TGF-\(\beta\) signalling in the presence of \textit{MYH11} mutations has been reported.42
**MYLK mutations**

Clinical data from FTAAD caused by mutations in the *MYLK* gene have been described in only one previous publication. Two different families with different mutations were presented. Aortic dissection occurred at variable ages and in some cases without preceding aneurysm formation.

![Figure 5: The localisation of the action site for proteins encoded by genes associated with FTAAD.](image)

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**Bicuspid aortic valve (BAV)**

BAV is the most common congenital heart defect, occurring in 0.46-1.37% of the population. Dilatation of the AoA occurs more frequently and at younger age in patients with BAV compared to patients with tricuspid aortic valve (TAV) regardless of valve function. The risk for aneurysm formation in the presence of BAV was reported to be 80 times higher, and for aortic dissection approximately 8 times higher than in the general population. However, the incidence of BAV-associated AoA dissections is low and they occur mainly at a size range comparable to sporadic aneurysms. Mutations in the *NOTCH1* gene have been associated to the development of BAV. BAV has also been presented in a few families with mutations in the *ACTA2*, *TGF-βR1*, and *TGF-βR2* gene, but there is no obvious association between FTAAD and BAV.

**Aortic imaging modalities**

Several different techniques can be used for aortic imaging. Plain chest radiography and invasive aortic angiography have nowadays been replaced by echocardiography, computed tomography (CT), and magnetic resonance imaging (MRI) (Table 3).
**Echocardiography**

The thoracic aorta can be examined by transthoracic (TTE) or transesophageal (TEE) echocardiography. Echocardiography is based on the reflections of high frequency sound waves emitted from a transducer as short bursts. As sound waves travel in the body and meet tissues, the waves are refracted and reflected according to different densities of the tissues (acoustic impedance). The transducer also functions as a receiver and listens for the returning ultrasound reflections. Structures close to the transducer create early returning echoes while echoes from more distant structures return later. The amplitude and time delay of the returning signals are visualised as brightness, and displayed as an image on the screen of the machine.

Although TTE has limited value for the evaluation of the entire aorta, it is highly useful for the diagnosis and follow-up of proximal ascending aortic segments. TTE also permits assessment of the aortic valve, which can be involved in diseases of the AoA. TTE is an excellent imaging modality for serial measurement of aortic root diameters and timing for surgery for aneurysms in the AoA. However, there still is no international consensus of the exact way to measure aortic diameter (diastolic vs. systolic diameter, leading-edge to leading-edge vs. inner-edge to inner-edge, or at specific anatomic localisation vs. widest diameter).

TEE requires esophageal intubation and is therefore more uncomfortable than TTE for the patient. The proximity of the esophagus to the thoracic aorta and the ability to visualize AoA and AoD and parts of the arch are advantages for TEE compared to TTE.

**Computed tomography (CT)**

Computed tomography refers to a computerized x-ray imaging procedure in which a narrow beam of x-rays is aimed at the patient and quickly rotated around the body, producing signals that are processed by the machine's computer to generate cross-sectional (tomographic) images of the body. Once a number of successive slices are collected by the machine's computer, they can be digitally “stacked” together with the possibility to reconstruct three-dimensional images. ECG-gated acquisition reduces motion artefacts that are common in the proximal aorta due to movements of the heart during scanning.

The ability to view the aorta in multiple projections and orientations helps evaluating the anatomy of the aorta manifested by dilatation, tortuosity, or dissection. The rapid image acquisition and post-processing flexibility are obvious advantages of CT. A significant drawback is exposure to ionizing radiation, especially in young individuals often subjected to serial imaging. Another disadvantage is the need for an intravenous contrast agent.

Non-enhanced CT followed by contrast-enhanced angiography is recommended, particularly when intramural hematoma or aortic dissection are suspected.
Magnetic resonance imaging (MRI)

The basis of MRI is the directional magnetic field associated with charged particles. When the human body is placed in a strong magnetic field, the free hydrogen nuclei align themselves with the magnetic field creating a net magnetic moment. Then, radio-frequency (RF) pulses are applied, which cause the hydrogen nuclei to tilt. When the RF pulse stops, the nuclei return to equilibrium and the energy from the nuclei during this realignment is measured and processed to obtain MR images.

MR imaging of the aorta typically begins with spin-echo black blood sequences to outline its shape and diameter and to identify an intimal flap in the presence of a dissection. Steady-state free precession sequences may follow, demonstrating changes in aortic diameters during the cardiac cycle. Flow mapping by velocity-encoded phase-contrast sequences can be used to quantify aortic flow. Contrast-enhanced MRI with intravenous gadolinium generates 3D angiogram of the aorta and aortic branch vessels.

MR angiography is a complementary rather than competing imaging modality for the thoracic aorta. With neither ionizing radiation nor contrast required, MRI is ideal for patients with multiple follow-up.

The disadvantages of MRI include prolonged image acquisition time, inability to use gadolinium contrast in patients with renal insufficiency, and claustrophobia. MRI is contraindicated in patients with some ferromagnetic implants and older pacemakers.

<table>
<thead>
<tr>
<th>COMPARATOR</th>
<th>ULTRASOUND</th>
<th>COMPUTED TOMOGRAPHY</th>
<th>MAGNETIC RESONANCE IMAGING</th>
</tr>
</thead>
<tbody>
<tr>
<td>Operator dependent</td>
<td>Yes</td>
<td>Minimal</td>
<td>Minimal</td>
</tr>
<tr>
<td>Image acquisition</td>
<td>Reproducible</td>
<td>Volume data with multiplanar reformatting</td>
<td>Multiple plains</td>
</tr>
<tr>
<td>Spatial resolution</td>
<td>Excellent</td>
<td>Very good</td>
<td>Good</td>
</tr>
<tr>
<td>Quantification of flow</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Ionising radiation</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Specific risk / problems</td>
<td>No contrast media</td>
<td>Allergy / anaphylaxis</td>
<td>Nephrogenic systemic fibrosis / Claustrophobia</td>
</tr>
</tbody>
</table>

Table 3: The benefits and the drawbacks with ultrasound, CT and MRI in examination of the thoracic aorta.
Assessment of the aorta – aortic diameter and function

Assessment of aortic diameter

Measurement of thoracic aortic diameter is used for diagnosis and follow-up of aortic dilatation. It plays a major role in risk stratifying individuals and in timing of surgical intervention. As previously mentioned, aortic diameter correlates to age and body size and it is larger in men than in women. The upper normal limit of aorta has been defined as 2 standard deviations (SD) greater than the predicted mean diameter.

The Z-score is an alternative way to report aortic diameter and it describes how many SDs the measured aortic diameter deviate from the population mean. A Z-score that is 2 SD above the mean will have a Z-score of 2.0. Therefore, an aortic diameter is considered dilated when the Z-score is >2. The Z-score is particularly useful in evaluating growing children. In adults, Z-scores are less commonly used. There are several calculation tools available for the Z-score measurement. Unfortunately, these calculators use different formulas for body surface area (BSA) and different normograms for aortic size in the adult population, which is a limitation for the widespread use and comparison of Z-scores.

Currently, there is no standardized method for measuring the aortic diameter within or across imaging modalities (TTE, CT, MRI) even if efforts have been made to emphasize the need of uniform terminology and measurement techniques. It is also important to point out not only the methodological variance but also the inter- and intra-observer variability. In several studies, the variability of aortic diameters ranges between 1.6-5.0 mm. Therefore, on individual basis, using the same imaging method in follow-up and side-by-side comparison of serial examinations are crucial in evaluation of possible progress.

Whether aortic volume, instead of diameter, is able to assess changes in aortic size and estimate the risk of rupture has also been studied recently. Aortic volume changes may become a potential complement to diameter measurements in the future.

Aortic stiffness as a marker of aortic function

The aorta performs several functions. It distributes blood from the heart to the peripheral arteries. Its elastic properties allow it to expand in systole and recoil during diastole. This reservoir function is important for maintaining blood flow and arterial pressure throughout the cardiac cycle by buffering the stroke volume for each ventricular contraction.

As the pressure wavefront propagates down the vascular tree, reflections of these wavefronts return from the periphery and amplify the diastolic pressure within the aorta. A healthy aorta dampens the augmentation phenomenon from the reflective pressure waves that return. Arterial stiffening leads to increased systolic pressure and widened pulse pressure.
due to cushioning of the ventricular contraction as well as loss of reflective wave dampening (Figure 6). 63-65

![Cross-sectional diagram of the aorta demonstrating the effects of aortic ageing leading to increased aortic stiffness.](image)

**Figure 6:** Cross-sectional diagram of the aorta demonstrating the effects of aortic ageing leading to increased aortic stiffness. 
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Aortic stiffness (AS) should be understood as the resistance to deformation. The AS may be assessed locally by diameter or area change through the cardiac cycle in relation to pressure change or regionally by determining the velocity of the pulse wave. 65 Abnormal aortic stiffening has been demonstrated as a predictor of adverse outcome. There are several studies reporting the independent predictive value of AS. A meta-analysis of over 15,000 participants showed pulse wave velocity (PWV) to be an independent predictor of cerebrovascular (CV) events and overall mortality in the general population. 66 It was estimated that an increase of 1 m/s increased the risk of mortality 15% even after adjusting for known CV risk factors. This led to the recommendation by the European Society of Cardiology to include PWV assessment in the risk stratification of hypertensive individuals as a means of determining end-organ damage. 67
How to measure aortic stiffness

There are several local indices of aortic function (Table 4). The most common of these indices are compliance and distensibility. Compliance is the diameter or area change in relation to pulse pressure and distensibility is the relative diameter or area change in relation to pulse pressure. The pulse pressure should be measured at the same level of the aorta for which the aortic diameter is measured. In clinical practice the brachial artery pressure is used. Compliance and distensibility can be measured by TTE, CT or MRI.

**Table 4:** The definitions and the formulas for different measurements of aortic stiffness.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Definition</th>
<th>Formula</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stress</td>
<td>The force applied per unit area.</td>
<td>$\sigma = \frac{F}{A}$</td>
<td>Pascal</td>
</tr>
<tr>
<td>Strain</td>
<td>The deformation of an object in a stress force.</td>
<td>$\varepsilon = \frac{L - L_0}{L_0}$</td>
<td></td>
</tr>
<tr>
<td>Elastic modulus</td>
<td>The stress/stain ratio.</td>
<td>$\mu = \frac{\sigma}{\varepsilon}$</td>
<td>Pascal</td>
</tr>
<tr>
<td>Compliance</td>
<td>The absolute change in diameter or area for a given pressure step.</td>
<td>$C = \frac{A_0 \text{ max} - A_0 \text{ min}}{\text{pulse pressure}}$</td>
<td>cm/minHg x 10^{-2}</td>
</tr>
<tr>
<td>Distensibility</td>
<td>The relative change in diameter or area for a given pressure step.</td>
<td>$D = \frac{A_0 \text{ max} - A_0 \text{ min}}{A_0 \text{ min} \times \text{pulse pressure}}$</td>
<td>mmHg x 10^{-3}</td>
</tr>
<tr>
<td>PWV</td>
<td>The velocity of the pulse wave in the aorta.</td>
<td>$\text{PWV} = \frac{3.57}{\text{distensibility}}$</td>
<td>m/s</td>
</tr>
</tbody>
</table>

F=force, A=area, L=length, Ao max and Ao min = largest and smallest aortic diameter or area during a heart cycle, PWV=pulse wave velocity.

PWV is a regional measurement of arterial stiffness over a certain arterial length. PWV is defined as the speed of the pulse wave in the aorta. It can be determined by measuring the pulse transit time from the pressure waveforms at the 2 sites along a vascular segment. The distance is divided by the time it takes for the forward wave to reach the end measuring point.

The measurement of PWV can be made by several different methods and devices. The carotid-femoral PWV (cfPWV) is considered as the gold-standard measurement of arterial stiffness. The most common methods to measure cfPWV are by mechanotransducers (Complior®) or by applanation tonometry (SphygmoCor®). Other methods to measure PWV are the oscillometric method (Arteriograph®), echotracking, and ultrafast echography.

The main principle of PWV estimation for the Arteriograph® device (used in our studies) is to record oscillations detected on the upper-arm cuff by a special high fidelity sensor. Measurements are performed when cuff pressure exceeds systolic BP by 35–40 mmHg, with a completely occluded
brachial artery. During systole, blood volume ejected into the aorta generates a pulse wave, the so-called early systolic peak. This pulse wave reflects from the bifurcation of the aorta, creating a second wave, the late systolic peak. Return time is calculated as the difference between the first and the reflected systolic wave. Aortic PWV is calculated from the pulse transit time and the distance travelled by the pulse wave. The aortic length is estimated by measuring the distance between the jugulum (sternal notch) and the symphysis pubica of the patient. This oscillometric method has been validated against invasive measurement of PWV and against other non-invasive devices.

MRI can measure the PWV using the transit time of the flow curves between two predefined points from a phase-contrast acquisition (Figure 7). The transit time can be calculated by the up-slope approach with a post-processing software. The distance can be measured at the centreline of the aorta between the two levels studied.

![Figure 7 a-c](image.png)

**Figure 7 a-c**: Thoracic aortic distensibility and PWV measurement by MRI. The distance is measured in the sagittal view (a). Flow-curves are obtained from the flow-sequences (b) and the PWV can be calculated from the time difference ($\Delta t$) between the arrival of the pulse wave to AoA (red line) and to AoD (green line) (c). Reprinted from with permission from Elsevier.

Reference values for cfPWV were established in 2010 by The Reference Values for Arterial Stiffness Collaboration. Data from more than 16,000 subjects from 13 different centres in eight European countries were analysed. Different methodologies for PWV measurements were used in the centres, and therefore PWV values were converted to common standard using conversion formulas. Reference values for PWV were established according to age in normotensive individuals (Table 5). The differences in the methodologies and the need of conversion formulas highlight the difficulty in establishing reference values. In clinical practise, repeated measurements over time should be done with the same device and the results compared to earlier results for the subject rather than to reference values only.
Table 5: Distribution of PWV (m/s) according to age category by The Reference Values for Arterial Stiffness Collaboration.

<table>
<thead>
<tr>
<th>Age category (years)</th>
<th>Mean (±2SD)</th>
<th>Median (10-90 pc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;30</td>
<td>6.2 (4.7-7.6)</td>
<td>6.1 (5.3-7.1)</td>
</tr>
<tr>
<td>30-39</td>
<td>6.5 (3.8-9.2)</td>
<td>6.4 (5.2-8.0)</td>
</tr>
<tr>
<td>40-49</td>
<td>7.2 (4.6-9.8)</td>
<td>6.9 (5.9-8.6)</td>
</tr>
<tr>
<td>50-59</td>
<td>8.3 (5.4-12.1)</td>
<td>8.1 (6.3-10.0)</td>
</tr>
<tr>
<td>60-69</td>
<td>10.3 (5.5-15.0)</td>
<td>9.7 (7.9-13.1)</td>
</tr>
<tr>
<td>≥70</td>
<td>10.9 (5.5-16.3)</td>
<td>10.6 (8.0-14.6)</td>
</tr>
</tbody>
</table>

The human genome and genetic variation

The human genome is organized into 23 pairs of chromosomes. These are all large linear DNA molecules contained within the cell nucleus. The genome also includes the mitochondrial DNA, a small circular molecule present in each mitochondrion. The total length of the human genome is more than 3 billion base pairs.\(^76, 77\)

The content of the human genome is commonly divided into coding and non-coding DNA sequences. Coding DNA sequences are defined as those sequences that can be transcribed into mRNA and translated into proteins; these sequences occupy only a small fraction of the genome (<2%). Non-coding DNA sequences are made up of all of those sequences (>98% of the genome) that are not used to encode proteins.

Approximately 22 000 genes have been identified. Protein-coding genes are distributed unevenly across the chromosomes, ranging from a few to more than 2000 per chromosome. The sizes of genes show large variability. Even within the genes there are coding sequences, exons, and non-coding sequences, introns. Exons are transcribed into messenger RNA, as opposed to introns that are removed by splicing proteins. The exact role of most of the non-coding DNA is unclear, but part of the non-coding DNA is transcribed into functional non-coding RNA molecules (e.g. transfer RNA, ribosomal RNA, and regulatory RNAs). Other functions of non-coding DNA include the transcriptional and translational regulation of protein-coding sequences, origins of DNA replication, centromeres and telomeres.

A genetic variation is an alteration of the nucleotide sequence of the genome. It can be benign, pathogenic or of unknown significance. Earlier, pathogenic sequence variants were called mutations, but this term has nowadays been replaced by genetic variation. Variations arise as a result of errors during DNA replication or damage to DNA, but may also be a result from an insertion or a deletion of a short segment of DNA. Genetic variation within genes can have no effect, alter the product (protein) of the gene, or prevent the gene from functioning properly. Genetic variation can be classified by effect on structure as small-scale variants or large-scale variants.
Small-scale variants consist of:

- Single nucleotide variants (SNVs) in which one base pair has been replaced by another.
  - Synonymous variants – code for the same amino acid.
  - Missense variants – code for a different amino acid.
  - Nonsense variants – code for a stop codon and can truncate (shorten) the protein.

Insertions
- One or more extra nucleotides have been inserted into the DNA and can alter splicing of the mRNA (splice site mutation) or cause shift in the reading frame (frameshift).

Deletions
- One or more nucleotides have been removed from the DNA. Like insertions, these mutations can alter the reading frame.

Large-scale variants alter the chromosomal structure and consist of:

- Gene amplifications (also known as gene duplications or chromosomal duplication)

- Gene deletions of large chromosomal regions (chromosomal deletion)

Variants can also be classified by effect on function:

- Loss-of-function variants result in a gene product that has less or no function.

- Gain-of-function variants change the gene product resulting in increased activity.

- Dominant negative variants have an altered gene product that acts antagonistically to the wild-type allele.

- Lethal variants lead to the death of the organisms that carry the mutation.

Nonsense-mediated decay (NMD) is a surveillance pathway in the eukaryotes. The main function of NMD is to reduce errors in gene expression by eliminating mRNA transcripts that contain premature stop codons. Translation of these aberrant mRNAs could lead to deleterious gain-of-function or dominant–negative activity of the resulting proteing,78, 79
Genetic variation is important for evolution and the normal diversity of species. However, sometimes genetic variations give rise to a pathogenic trait or a genetic disease. Penetrance is the portion of individuals carrying a variant that also express clinical symptoms (phenotype). Incomplete penetrance means that all mutation carriers do not exhibit the phenotype. Expressivity describes the extent to which a given genotype is expressed at the phenotypic level. In variable expression, symptoms differ between individuals with the same genotype.

**Exome sequencing**

The exome consists of all the exons (protein coding regions) of a genome. Exome sequencing is a technique for sequencing the exome. It consists of selecting only the subset of DNA that encodes proteins (exons) and then sequencing that DNA. There are about 180,000 exons and approximately 30 million base pairs in the human genome. The aim is to identify an earlier unknown abnormal genetic variation. Exome sequencing is an efficient method to identify all the genetic variants in an individual’s genes, and is therefore especially effective in the study of rare inherited diseases. These diseases are most often caused by rare genetic variants that are present in a small number of individuals. Furthermore, because severe disease-causing variants are much more likely to be located in the protein coding sequences (85-90%), focusing on this 1-2% of the genome is more effective than whole genome sequencing.\(^80\)\(^-\)\(^83\)

The typical workflow required to sequence and analyse an exome is as shown in Figure 8. DNA is isolated from white blood cells. DNA is randomly shared and a so-called library is constructed of the fragments. The library is then enriched for sequences corresponding to exons. Specific probes that bind to the exons are added and the rest of the DNA is washed out. The exons are amplified and a sequencing of the exome is performed. Finally, the captured information is analysed and the sequences are compared to a reference genome in order to find pathogenic variants.\(^80\)

Even by only sequencing the exomes of a few individuals, a large quantity of data and sequence information is generated which requires a significant amount of data analysis. The captured sequences are compared to a reference genome to identify pathogenic variants. Identified variants are further compared to available databases over genetic variation in humans. This enables annotation of the variants to whether they are common, rare, or novel (not previously seen in the human population). Variants associated with disorders are either very rare, or more likely, novel.
Exome sequencing is only able to identify those variants found in the coding region of genes, which affect protein function/synthesis. It is not able to identify the structural and non-coding variants associated with the disease, which can be found using other methods such as whole genome sequencing. There remains 98-99% of the human genome that is not covered using exome sequencing. The statistical analysis of the large quantity of data generated from sequencing approaches is a challenge. False positive and false negative findings are associated with genomic sequencing approaches and are a critical issue. Genetic heterogeneity and population ethnicity are also major limitations as they may increase the number of false positive and false negative findings, which will make the identification of candidate genes more difficult.80, 82, 83
Screening for TAA

Population wide screening for TAA is not recommended. However, when a patient is identified, screening of first-degree relatives (parents, siblings and children) by aortic imaging is recommended. In case of FTAAAD, screening of both first and second-degree relatives should be undertaken, and geneticist for family investigation, counselling and genetic testing should be contacted. Repeated aortic imaging in healthy at-risk relatives is recommended every fifth year until diagnosis (clinical or by genetic testing) is established or ruled out. Family screening and information may be a challenge when families are spread over a large geographic area. Cardiovascular units with a focus on inherited diseases might organize the controls in a systematic way.

Medical treatment of TAAD

Since 1990s, beta-blockers were used as drug of choice for TAA, especially in patients with MFS. Beta-blockers were believed to reduce the progression of aortic aneurysms in the general population with aortic disease. The evidence for this treatment is, however, relatively weak and there are conflicting results in the studies.

Based on the demonstration of increased TGF-β signalling in mouse and human tissues of MFS, and that TGF-β could be inhibited by the angiotensin II receptor blocker (ARB) losartan, TGF-β was considered as a new interesting target for medical therapy. The initial studies showed reduced aortic root growth and inhibition of elastic fibre fragmentation in a mouse model and aortic growth reduction in a small study in humans. However, further larger randomized studies in humans have not been able to demonstrate a clear advantage for ARB.

The 2014 ESC guidelines for thoracic aortic disease summarize the recommendations for medical treatment of TAA as follows: “In chronic conditions, blood pressure should be controlled below 140/90 mm Hg, with lifestyle changes and use of antihypertensive drugs, if necessary. An ideal treatment would be the one that reverses the formation of an aneurysm. In patients with MFS, prophylactic use of beta-blockers, angiotensin-converting enzyme (ACE) inhibitor, and angiotensin II receptor blocker seems to be able to reduce either the progression of the aortic dilation or the occurrence of complications. However, there is no evidence for the efficacy of these treatments in aortic disease of other aetiologies.”

In clinical practice, patients with TAA, regardless aetiology, are often recommended beta-blockers and/or ARB. Low-intensive regular physical activity is recommended. However, weightlifting and other heavy exercise should be avoided.
Surgical and interventional treatment of TAAD

Aneurysms in the AoA are treated surgically while aneurysms in the AoD might be treated with endovascular stent (TEVAR) or surgery. In sporadic cases, surgery is recommended when AoA diameter is ≥ 55 mm.4 9 In MFS the threshold is defined to 50 mm, at smaller diameters before pregnancy or in patients with family history of dissections. In EDS there is no exact threshold for surgery and surgical intervention for AoA is recommended on case-by-case basis. In LDS observation in both children and adults of a widespread and aggressive arteriopathy led to the recommendation of early operative intervention at ascending aortic diameters of ≥42 mm. Aggressive surgical management of the aneurysms in patients with LDS is achieved with few complications in the absence of tissue fragility. Current management strategies for ACTA2, MYH11 and MYLK combine widespread imaging at baseline and follow-up, and surgical intervention according to family history of vascular events.4 Symptomatic aneurysms should be referred for urgent intervention irrespective of diameter.

It is important to identify patients at risk for dissection before a dissection occurs. In elective surgery for ascending aortic aneurysm repair, mortality is estimated to be 1.6-4.8%, the risk for stroke 2.4-3.0%, and the risk for myocardial infarction 1-2%.90-92 The risk for complications is dependent of the age of the patient and the type of surgery (aneurysm repair including the aortic valve, valve sparing surgery, supracoronary graft and the involvement of arch).

Complications to an acute aortic dissection are common and serious. The most frequent complications are myocardial ischemia (10-15%), aortic regurgitation (40-75%), cardiac tamponade (8-20%), stroke (8-12%), limb ischemia (10-15%), and mesenterial ischemia (4-7%).93-95 The prehospital mortality in aortic dissections is estimated to be as high as 30-48%.97 The in-hospital mortality for operated type A dissections is 10-20%.94

There were 1040 interventions (elective and acute) on the thoracic aorta in Sweden in 2015, which is almost three times more than 15 years earlier (Figure 9). This is probably due to a combination of more widespread and better imaging methods as well as more active attitudes toward surgery. It is unclear whether there is also an increase in the prevalence of aortic aneurysms.
Figure 9: Surgical interventions on the thoracic aorta in Sweden 1998–2015. Data are from the statistical database of The National Board of Health and Welfare. AoA = ascending aorta, AA = aortic arch, AoD = descending aorta
**Aims of the thesis**

The general aims of this thesis were to define normal thoracic aortic diameters, to evaluate screening programme for TAAD and to increase the knowledge of genetic and phenotypic features of FTAAD.

The specific aims of the papers were:

**Paper I**

To evaluate the influence of age, sex and body size on thoracic aortic diameter and to establish reference values for AoA and AoD measured by CT.

**Paper II**

To study the effectiveness of phenotypic cascade screening in families with an inherited form of thoracic aortic aneurysms and dissections and to address questions that arise when screening for a genetic disorder is applied.

**Paper III**

To study if thoracic aortic diameters obtained by TTE and MR are comparable, to study aortic stiffness in families with inherited thoracic aortic disease, and to study aortic stiffness in individuals identified to have a dilated thoracic aorta compared to individuals with normal thoracic aortic diameter.

**Paper IV**

To identify genetic variants causing FTAAD, to characterize the phenotype, and to compare thoracic aortic diameter and stiffness in mutation carriers and non-carriers.
Study designs and populations

Study designs

**Paper I** is a prospective observational study of consecutive patients undergoing CT examination of the thorax.

**Paper II** is a descriptive observational study of seven families with inherited form of thoracic aortic dissections undergoing clinical examination and screening of the thoracic aortic diameter.

**Paper III** is descriptive observational study of aortic stiffness of the families in paper II.

**Paper IV** is a descriptive observational study describing the identification of a novel pathogenic sequence variant causing FTAAD and describing the genotype and phenotype in carriers of the pathogenic sequence variant.

<table>
<thead>
<tr>
<th>PAPER</th>
<th>PROBLEM APPROACHED</th>
<th>STUDY DESIGN</th>
<th>PATIENTS</th>
<th>STUDY PARAMETERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal and reference values for thoracic aortic diameter</td>
<td>Prospective observational</td>
<td>77 consecutive patients undergoing CT thorax</td>
<td>The diameter of the ascending and the descending aorta</td>
</tr>
<tr>
<td>II</td>
<td>Identification of new cases with dilated thoracic aorta when screening for FTAAD</td>
<td>Descriptive observational</td>
<td>106 individuals in 7 families with FTAAD</td>
<td>Presence of thoracic aortic dilatation</td>
</tr>
<tr>
<td>III</td>
<td>Thoracic aortic stiffness in FTAAD: Comparison of aortic diameters and measurements by TTE and MRI</td>
<td>Descriptive observational</td>
<td>118 individuals in 7 families with FTAAD</td>
<td>Thoracic aortic diameter and stiffness</td>
</tr>
<tr>
<td>IV</td>
<td>To identify the disease causing mutation in a family with FTAAD and to describe the genotype and phenotype of the carriers</td>
<td>Descriptive observational, cross-sectional</td>
<td>19 individuals in a family with FTAAD</td>
<td>The genotype, Age and diameter at dissection, thoracic aortic diameter and stiffness in carriers and non-carriers</td>
</tr>
</tbody>
</table>

Table 6: Schematic summary of study characteristics in papers I-IV.
**Study populations**

**Paper I**

The study population consisted of 77 consecutive individuals above 18 years of age that were undergoing CT examination of the thorax. Aortic dissection and age were the only exclusion criteria.

**Papers II - III**

The individuals in paper II were family members in the seven first families with FTAAD referred to the Centre for Cardiovascular Genetics at Umeå university hospital, Sweden. The disease causing genetic variant was unknown in these families at the time of referral. In paper II, 106 individuals were investigated, and in paper III, 116 individuals were investigated. There were more individuals in paper III because some family members joined in the study after the first data analysis (Table 7).

**Paper IV**

The 19 individuals in this study were family members of one of the seven families in papers II - III.

**Table 7: Summary of family members included in paper II and III.**

<table>
<thead>
<tr>
<th>Individuals</th>
<th>Paper II</th>
<th>Paper III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dead in dissection or rupture</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Living family members</td>
<td>135</td>
<td>135</td>
</tr>
<tr>
<td>AoA operated acutely or prophylactically</td>
<td>11 (excluded)</td>
<td>11 (excluded)</td>
</tr>
<tr>
<td>Known aortic dilatation</td>
<td>2 (excluded)</td>
<td>21 (included)</td>
</tr>
<tr>
<td>Not participating</td>
<td>16</td>
<td>8</td>
</tr>
<tr>
<td>Study population</td>
<td>106</td>
<td>116</td>
</tr>
</tbody>
</table>
Methods

Clinical examination

Detailed medical history including medication was obtained for each family member. Clinical examination was conducted to exclude syndromic forms of FTAAD and to identify phenotypic characteristics common to family members. Height and weight were measured and BSA was calculated. The Beighton hypermobility scale was used to assess joint mobility.96

CT

The investigations in paper I were performed with a spiral CT scanner (Somatom Plus4, Siemens-Elema, Erlagen, Germany) and all examinations were evaluated on the same workstation (Magic View, Siemens-Elema, Erlagen, Germany). The diameters of the AoA were measured as inner diameters at the level of the aortic valve, 20 mm and 40 mm above the aortic valve, and at corresponding levels in the AoD.

TTE

The TTE examinations in papers II-IV were performed using a Vivid7 (GE Medical Systems, Horten, Norway) echocardiography scanner, equipped with a 2D transthoracic transducer. The aortic diameters were measured from the parasternal long-axis view using the inner-edge to inner-edge distance at the level of SoV and at the widest part of AoA. Measurements were made in M-mode in diastole. The average of three measurements in different cardiac cycles was used. Diameters were indexed to age, sex and body surface area. As a reference for the echocardiographic diameter measurements, data published by Mirea et al were used.96 Local aortic distensibility was calculated as the relative diameter change in relation to blood pressure change during systole and diastole.

MRI

The MRI examinations in papers II-IV were performed with the Achieva 3.0 T MRI system (Philips, Best, The Netherlands). The examinations were performed in the supine position and all imaging was ECG-gated and acquired during expiratory breath hold. T1 and T2 weighted spin-echo black blood sequences were obtained for diameter measurements, which were made as diastolic inner diameters at the level of pulmonary bifurcation using the double oblique technique. Velocity-encoded phase-contrast sequences were obtained to measure the cross sectional area change during systole and diastole using a semi-automated contouring method (Segment® v1.9 and v2.0 R4377, MedViso, Lund, Sweden). Aortic distensibility was calculated as relative area change in relation to blood pressure change during systole and diastole. The PWV analysis tool of the Segment® program was used to calculate the PWV from the MRI images.97 As a reference value for the MRI diameter measurements in the AoA and AoD, data published by Davis et al were used.98
Oscillometric PWV

In papers II-IV, the global aortic PWV was measured by an oscillometric method (Arteriograph®, TensioMed, Budapest, Hungary) after 5 min of rest in the supine position. The principle of the oscillometric method is described under the introduction part.

Sanger sequencing, exome sequencing, analysis of sequencing data and cDNA analysis

Sanger sequencing was performed to exclude genetic variants associated with FTAAD (FBN1, COL3A1, TGF-βR1, TGF-βR2, ACTA2). To identify the disease-causing sequence variant, exome sequencing was performed on DNA obtained from affected individuals in one family (paper IV). After exome enrichment and sonication of the DNA, fragment libraries were constructed and target enrichment was performed. Exome capture was conducted by specific probes and then extracted by magnetic beds. The captured DNA was amplified followed by sequencing. Individual libraries were labelled via a barcoding procedure. This way, libraries with large amount of individual exons were created. The next step was to identify abnormal sequence variants (mutations) in the exome. The exome of an individual was compared to a human reference genome (hg19). Single-nucleotide variants and small insertions and deletions were called by a specific algorithm (diBayes algorithm). All identified variants were then imported to a local database (CanvasDB) for filtering and for further analysis. After filtering, identified genetic variants were evaluated whether they could be disease-causing or not. Sanger sequencing was performed in affected individuals to verify the presence of identified mutation followed by segregation analysis in both affected and unaffected family members. In this way, a deletion in the MYLK gene was identified to segregate with the disease in the family.

cDNA analysis was then performed to confirm the mutation or to verify nonsense-mediated decay (degradation of cytoplasmic mRNA with premature stop codon). The instruments used in the different steps of exome sequencing are specified in paper IV.
Statistics

All statistics in paper I were calculated by SPSS Inc, version 14, Chicago, IL, USA and in papers II-IV by IBM SPSS Statistics, version 22, Armonk, NY, USA. Continuous data are given as means and confidence intervals (CI), or as medians and interquartile values. CIs are 95%. Summary statistics were compared by Student’s t-test for normally distributed continuous variables and by Mann-Whitney U-test for non-normally distributed continuous variables. p-values below 0.05 were considered significant.

Statistics – Paper I
Initially, a univariate linear regression model including age was used, followed by a multiple linear regression model that included age, sex and body size. Significant interactions were studied. The upper limit of the normal diameter was set to 2 SDs above the predicted normal value.

Statistics – Paper III
Pearson’s correlation and Bland-Altman plots were used to analyse the agreement between aortic diameters measured by TTE and MRI. Differences in distensibility and PWV between the groups were compared by an independent-sample Mann-Whitney U-test. Distensibility and PWV were analysed in separate multiple regression models to adjust for differences between the groups and two-way interactions were included.

Statistics – Paper IV
In this paper the aim was to study differences between carriers and non-carriers after the identification of the disease causing mutation. To compare aortic diameter, distensibility, and PWV between the two groups, the Mann-Whitney U-test was used. Linear regression models were used to study age-dependent progression in the above-mentioned parameters, stratified for carriers and non-carriers.
Results

Paper I

In the study, 77 consecutive individuals above 18 years undergoing CT examination of the thorax were included. Of the patients, 41 (53%) were men. The mean age was 54 years with the range being 18 – 82 years. The indications were mainly follow-up for lymphoma or search for metastasis (61%). Other indications were trauma (5%), tamponade (4%), suspected aortic dissection (4%, those with dissection were excluded) and pulmonary embolus (3%).

The AoA was wider than the AoD at every corresponding level. The diameter of the thoracic aorta increased by 0.17 mm (0.12 – 0.20 mm) per year. An age dependent dilatation could be seen in both AoA and AoD. The mean sex-related difference in diameter was 1.99 mm (1.28 – 2.60 mm) with men having larger aortas than women. BMI also showed statistical correlation in most of the levels. The mean difference in aortic diameter per unit BMI was 0.27 mm (0.14 – 0.44 mm).

We then constructed a formula to calculate the upper normal limit for AoA and AoD separately. The formulas are presented in Table 8. The normal values and the upper limits are presented in Figure 10.

Table 8: The formulas for calculating the upper normal limit for the ascending aorta (AoA) and the descending aorta (AoD).

<table>
<thead>
<tr>
<th>Level</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>AoA</td>
<td>D (mm) = 31 + 0.16*age</td>
</tr>
<tr>
<td>AoD</td>
<td>D (mm) = 21 + 0.16*age</td>
</tr>
</tbody>
</table>

Figure 10: The age-related upper normal limits (in mm) in the ascending aorta (Ascendens) and the descending aorta (Descendens).
The families consisted of 135 living individuals. Thirteen individuals had a known aortic disease; two individuals had a dilatation of the AoA, five had been operated prophylactically and six had been operated on due to an acute dissection (Table 9). Eleven individuals had died due to aortic dissection. The mean age at the onset of an acute aortic dissection in these families was 48 years. The diameter at the time of dissection varied between 44-55 mm in family members in which aortic imaging had been done. In the two youngest individuals who died due to dissection at the age of 15 and 23 years, the thoracic aortas were not dilated at autopsy.

A total of 106 individuals without known thoracic aortic disease were investigated. Nineteen individuals (18%) were identified to have dilated thoracic aorta related to gender, age and BSA. Of these, 15 were first-degree relatives and four were second-degree relatives. The expected number of individuals in this group with an autosomal dominant disease would have been 40 ($p<0.0001$) (Table 10). In the 20 first-degree relatives younger than 40, we identified only one individual with a dilated aorta although the expected number of individuals with disease causing mutation would have been 10.

For the 19 individuals with a dilated thoracic aorta, the dilatation was localised to the SoV in six individuals, to the AoA in nine individuals and in four individuals the dilatation extended over both levels. The diameter varied 35 to 50 mm (18.4-23.6 mm/m²) at the level of SoV and 30 to 46 mm (18.7-22.8 mm/m²) at the level of AoA. One individual (0.94%) was identified to have BAV.

Of the 24 individuals with previous aortic involvement, 20 (83%) were men and 4 (17%) were women. Of the 19 new cases, 11 (58%) were men and 8 (42%) were women.

**Table 9:** Summary of the phenotype of individuals in the families.

<table>
<thead>
<tr>
<th>Summary of findings</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Previously known – dead due to dissection</td>
<td>11</td>
</tr>
<tr>
<td>Previously known – dissection, alive</td>
<td>6</td>
</tr>
<tr>
<td>Previously known – prophylactic operation</td>
<td>5</td>
</tr>
<tr>
<td>Previously known – aorta dilated</td>
<td>2</td>
</tr>
<tr>
<td>New cases</td>
<td>19</td>
</tr>
<tr>
<td>Normal aortic diameter</td>
<td>87</td>
</tr>
</tbody>
</table>

**Table 10:** Number of new and expected cases in the families.

<table>
<thead>
<tr>
<th>Number</th>
<th>1-st degree relative, n</th>
<th>Dilated aorta, n</th>
<th>2-nd degree relative, n</th>
<th>Dilated aorta, n</th>
<th>Dilated aorta, total n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (expected)</td>
<td>56</td>
<td>15 (28)</td>
<td>50</td>
<td>4 (12.5)</td>
<td>19 (40.5)</td>
</tr>
</tbody>
</table>
Paper III

The diameters measured by TTE and MRI strongly correlated ($r^2=0.93$). The mean difference in diameters between the two methods was 0.72 mm (95% CI 0.41-1.02) with TTE giving larger diameters than MRI. The difference can probably be explained by the different ways the measurements were made. With MRI two cross-sectional diameters were measured at a fixed position (pulmonary artery bifurcation) and the smaller diameter was reported. With TTE the largest diameter in the AoA was measured.

The diameter of the AoA increased with age ($r^2=0.544$) meanwhile the distensibility of the AoA decreased with increasing age and with increasing diameter. This was seen with both TTE and MRI measurements. The variance of the distensibility between individuals decreased with increasing age.

PWV measured with MRI and with Arteriograph® increased with age and diameter.

There were 21 individuals with thoracic aortic dilatation and 95 individuals with normal thoracic aortic diameter. The individuals with aortic dilatation were older than those without (49 vs. 37 years, p=0.001). The individuals with dilated thoracic aorta showed lower aortic elastic properties in all parameters studied, i.e. TTEdist, MRIdist, MRIPWV, ARTPWV (Table 11). When we adjusted for age and diameter, individuals with dilated thoracic aorta still had significantly lower distensibility and higher PWV as measured by MRI. The distensibility measured by TTE, and PWV measured by Arteriograph® did not reach statistical significance.
Table 11: Demographic data, distensibility and PWV of the study population.

<table>
<thead>
<tr>
<th></th>
<th>Normal aorta</th>
<th>Dilated aorta</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>95</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>53 (80)</td>
<td>13 (20)</td>
<td></td>
</tr>
<tr>
<td>Age, y (range)</td>
<td>37 (18-73)</td>
<td>49 (26-65)</td>
<td>0.001</td>
</tr>
<tr>
<td>BSA, m²</td>
<td>1.95</td>
<td>1.93</td>
<td>ns</td>
</tr>
<tr>
<td>Arterial hypertension, (%)</td>
<td>10 (11)</td>
<td>2 (10)</td>
<td>ns</td>
</tr>
</tbody>
</table>

Independent samples t-test

<table>
<thead>
<tr>
<th></th>
<th>Normal aorta</th>
<th>Dilated aorta</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTE AoA diameter, mm (95% CI)</td>
<td>30.0 (29.0-31.0)</td>
<td>37.6 (31.5-40.1)</td>
<td>0.001</td>
</tr>
<tr>
<td>MRI AoA diameter, mm (95% CI)</td>
<td>29.5 (28.5-30.5)</td>
<td>36.3 (33.9-38.7)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Mann-Whitney U-test

<table>
<thead>
<tr>
<th></th>
<th>Normal aorta</th>
<th>Dilated aorta</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTE Distensibility, mmHg x 10⁻³ (q1; q3)</td>
<td>2.66 (1.2; 4.31)</td>
<td>1.01 (0.57; 2.63)</td>
<td>0.005</td>
</tr>
<tr>
<td>MRI Distensibility, mmHg x 10⁻³ (q1; q3)</td>
<td>4.53 (2.58; 7.25)</td>
<td>1.76 (1.11; 3.68)</td>
<td>0.001</td>
</tr>
<tr>
<td>ART PWV, m/s (q1; q3)</td>
<td>7.1 (6.5; 8.1)</td>
<td>8.2 (7.7; 8.8)</td>
<td>0.001</td>
</tr>
<tr>
<td>MRI PWV, m/s (q1; q3)</td>
<td>5.9 (5.2; 7.3)</td>
<td>6.9 (6.1; 7.9)</td>
<td>0.037</td>
</tr>
</tbody>
</table>

Between-subjects interactions

<table>
<thead>
<tr>
<th></th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRI PWV Dilatation * MRI AoA diameter</td>
<td>0.045</td>
</tr>
<tr>
<td>Dilatation * Age</td>
<td>0.007</td>
</tr>
<tr>
<td>MRI Distensibility</td>
<td></td>
</tr>
<tr>
<td>Dilatation * MRI AoA diameter</td>
<td>0.046</td>
</tr>
<tr>
<td>Dilatation * Age</td>
<td>0.041</td>
</tr>
</tbody>
</table>

BSA = body surface area, TTE AoA diameter = ascending aortic diameter measured by TTE, MRI AoA diameter = ascending aortic diameter measured by MRI, ART PWV = pulse wave velocity measure by Arteriograph®, MRI PWV = pulse wave velocity measured by MRI, Dilatation = presence of dilatation. Data are are presented as mean and 95% CI or median and first (q1) and third (q3) interquartile values.
Paper IV

Nineteen living members above 18 years in a family with FTAAD participated in the study (Figure 11). Three members had died due to an aortic dissection or rupture (I:1, I:2, III:6) and two members had survived an aortic dissection (II:2, II:3). One family member (II:8) had suffered from an intramural hematoma in the AoD.

Figure 11: Pedigree of the investigated family.

Sanger sequencing, exome sequencing and cDNA analysis

Sanger sequencing did not identify any of the investigated genetic variants associated with FTAAD (FBN1, COL3A1, TGF-βR1, TGF-βR2, ACTA2).

Exome-sequencing of three afflicted individuals (II:2, II:3, II:8) was performed, and identified variants were filtered against the human reference genome (hg19) and against a local database of exomes from more than 1000 individuals. Five potentially pathogenic sequence variants (SEMA4A, PLCZ1, FBLN5, SPG11, and RANBP2) were identified but none of them were previously associated with FTAAD. FBLN5 contributes elastogenesis and vascular development, but the variant did not segregate with the familial disease.

We then excluded one of the individuals (II:8) with a somewhat different phenotype and performed filtering in the two remaining individuals (II:2, II:3), who were brothers both with aortic dissection. This process revealed 25 sequence variants, including a 2-bp deletion in MYLK (c.3272_3273del), which results in a frame shift and a premature stop codon (p.Ser1091*). This variant was not present in any of the reference genomes and it co-segregated with the disease in the family with exception for the above-mentioned excluded individual (II:8). Analysis with MutationTaster predicted that the identified variant would cause disease due to premature stop codon and nonsense-mediated decay. Sanger-sequencing of cDNA from the two
individuals carrying the MYLK variant confirmed nonsense-mediated mRNA decay of the mutant transcript.

In the family, eleven individuals were identified to carry the pathogenic sequence variant and eight individuals were non-carriers. Three individuals in the family had died due to aortic dissection or rupture; biological material saved from two of them (I:2, III:6) indicated that both were carriers of this mutation and the third (I:1) was an obligate carrier.

**Phenotypic description**

The identified genetic variant shows variable expression. Table 12 shows characteristics of the patients with previous aortic event.

**Table 12:** Characteristics of family members with aortic event and presence of the 2-bp deletion in MYLK.

<table>
<thead>
<tr>
<th>ID</th>
<th>Gender</th>
<th>Age at event (years)</th>
<th>Dissection type</th>
<th>AoA diameter (mm)</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>I:1</td>
<td>F</td>
<td>70</td>
<td>Rupture AoA</td>
<td>u/k</td>
<td>No</td>
</tr>
<tr>
<td>I:2</td>
<td>M</td>
<td>75</td>
<td>A</td>
<td>u/k</td>
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<tr>
<td>III:6</td>
<td>M</td>
<td>23</td>
<td>Rupture AoA</td>
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<td>II:3</td>
<td>M</td>
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<tr>
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<td>M</td>
<td>55</td>
<td>A</td>
<td>48</td>
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F = female, M = male, AoA = ascending aorta, A = aortic dissection type A, u/k = unknown

The diameter of the AoA in individual III:6 was not obviously dilated at autopsy. Therefore, both age at dissection and the diameter of the AoA varies at the time of aortic event.

None of the carriers had experienced abnormal bleeding or wound healing after minor operations and the surgical scars appeared normal. There were no common symptoms from the gastrointestinal tract, urinary tract or circulatory system. No musculoskeletal, joint, or eye symptoms were evident. Four carriers had bronchial asthma. No abnormalities common to the carriers could be revealed in physical examination. One carrier had atrial fibrillation.

None of the carriers had a dilated AoA or AoD. One of the carriers had an ascending aortic diameter near the normal limit (age 60, AoA 41 mm, 19.9 mm/m²). This carrier’s AoA had a low distensibility measured by MRI (2.59 10⁻³mmHg⁻¹) and a high PWV (12.3 m/s). This individual was the brother of the two individuals that suffered aortic dissection and wanted to undergo a prophylactic operation.

Statistical analysis did not uncover any differences in aortic diameter, aortic stiffness, or PWV between carriers and non-carriers. The yearly age-dependent development in PWV appeared to be faster among carriers (0.13 m/s, 95% CI 0.00-0.30) than non-carriers (0.03 m/s, 95% CI -0.07-0.13), but did not reach statistical significance.
Histology revealed discontinuities in elastin fibres in the medial layer in the aortic specimens from the three individuals with an aortic event. No pathological findings were visible in the SMCs. An increase of arteries in the medial layer that has been described previously could not be seen in these specimens. In CT angiography of the aorta, the main branch vessels and the arteries of the brain in the carriers, two individuals had a small aneurysm in the right medial cerebral artery. Aortic branch vessels did not show obvious tortuosity.
Discussion

Thoracic aortic diameter

At the time of our first study, the knowledge of thoracic aortic dimensions in relation to age, sex and body size was quite limited. We had found only two previous papers studying these associations by CT.\textsuperscript{52, 100} There were no clearly defined reference values when the thoracic aorta should be considered dilated, especially in younger individuals. This was a clinical dilemma when we examined the first families with FTAAD.

In paper I we showed that the thoracic aortic diameter varies with age, sex and body size, and the strongest correlation was seen with age. Later studies have confirmed these associations and since then reference values for thoracic aortic diameters at different levels of the vessel have been established by CT\textsuperscript{101-104} and by other imaging modalities. However, there is still an on-going discussion of the need for a uniform way to measure aortic diameters.\textsuperscript{59}

Screening for TAAD

As previously mentioned, 20-25\% of patients with TAAD have relatives with the same disease.\textsuperscript{2, 3} As a consequence, guidelines for thoracic aortic disease recommend screening by aortic imaging for all first-degree relatives of patients with sporadic TAAD and all first and second-degree relatives in families with TAAD in which the genetic cause is not known.\textsuperscript{4, 9} In paper II, we applied these guidelines in our families and identified 18\% of the relatives with a dilated AoA and/or AoD. Consequently, a substantial number of persons with dilated thoracic aorta can be identified, and some of them with an aortic diameter close to the intervention threshold. Screening is therefore motivated.

However, the expected portion of carriers in these families with an autosomal dominant disease was 38\%. Therefore, all individuals with increased risk for aortic event cannot be identified in a single screening. In particular, few individuals below the age of 40 with aortic dilatation could be identified.

Because a single screening is not enough to exclude the presence of the disease, a follow-up must be offered even to those with normal aortic diameters. Consequently, as far as the disease-causing mutation is unknown, even non-carriers will be enrolled in a control program. Such control program will result in a large number of examinations, higher health care costs, and probably increased anxiety for individuals, some of whom would not be at risk for aortic dissection. Therefore, before a cascade screening is started, accurate information of the advantages and limitations must be given. There are also challenges in organizing screening if the family members are spread over a large geographic area. Special units with focus on inherited cardiovascular diseases might organize the controls in a systematic way.
Aortic imaging by TTE and MRI

In paper III we showed that TTE and MRI provided clinically reliable information on the aortic diameter with high correlation and minor systematic differences. MRI can be used for screening family members because it enables assessment of the diameter of both the ascending and descending aorta and the stiffness of aorta. Even aortic tortuosity, seen especially in LDS, can be assessed by MRI. A drawback for MRI is claustrophobia, which prevents the examination in approximately 1-5% of individuals. TTE can be used in subsequent follow-up because it is widely available and easy and quick to perform. However, assessment of the distal AoA, the aortic arch and AoD is difficult by TTE.

Aortic diameter and aortic stiffness in FTAAD

Aortic diameter is not an optimal marker for disease occurrence or risk stratification in FTAAD. The disease shows variable expression, and dissections can occur at different ages and from different aortic diameters. Therefore, aortic elastic properties might give more information about the presence of disease and disease progress. In paper III, we showed that aortic diameter increased with age, and aortic stiffness to increase with age and with the diameter of the vessel. Individuals identified to have dilated thoracic aortas had higher PWV and lower distensibility as measured by MRI than individuals with normal diameter, even when adjusted for age and diameter. Thus, measurement of aortic stiffness might give additional information beyond aortic diameter in individuals with TAAD, but conclusions must be approached with caution due to the limitations of the study. It is reasonable to pay special attention to young individuals with increased aortic stiffness even if the aortic diameter is normal. If we can identify the disease-causing mutation, it would be possible to differ carriers from non-carriers in the families and facilitate studies of the phenotype. Even if the TTE or the oscillometric method to measure PWV did not show significant results between individuals with dilated and non-dilated thoracic aorta, these methods, as well as MRI, should be repeated in larger cohorts in which the genotype is known.

The MYLK (c.3272_3273del, p.Ser1091*) sequence variant

In paper IV, we identified a 2-bp deletion in MYLK (c.3272_3273del, p.Ser1091*) to cause FTAAD in a large family. The pathogenic MYLK variant leads to a premature stop codon, resulting in the end of transcription of the gene. The mRNA is incomplete compared to the one from the chromosome without mutation, and will in this case be destroyed by nonsense-mediated decay. Therefore, the amount of the normal protein is only about half of the amount it would be without the mutation. This can result in insufficient activity of the MLCK-protein and possible SMC dysfunction in the aortic wall. Due to the decreased SMC contraction, the AoA may not withstand the forces from the pulsatile blood flow and blood pressure changes and may thus be at risk for rupture or dissection. The SMC dysfunction may lead to
premature decreased distensibility of the large arteries and hence to increased PWV.

Due to the identification of the disease-causing mutation, the family members could be informed, as to whether they were carriers or non-carriers of the mutation. The non-carriers did not need to be worried about an increased risk of aortic event and they had no need of recurrent follow-up. The surveillance can be focused on the mutation carriers.

The phenotype shows reduced penetrance and variable expression. There is a large variation both in age and aortic diameter in onset of the disease, regarded as rupture or dissection. Therefore, the timing of prophylactic intervention is difficult. We hypothesized that carriers might have higher aortic stiffness. Unfortunately, we could not show a difference in aortic elasticity or pulse wave velocity between carriers and non-carriers but there was a tendency for a higher age-dependent increase in PWV. In the carriers, there were no other common symptoms from any other organ system.

Due to the small number of individuals, conclusions must be approached with caution and the results should be confirmed in larger studies.

Clinical implications

As mentioned above, screening of large families with an inherited disease is challenging. The family might be spread over a large geographic area and the knowledge of this rare disease can vary in different health care centres.

The Centre for Cardiovascular Genetics at Umeå university hospital, Sweden, is a unit specialised in genetic diseases involving the heart and the aorta. A genetic counsellor at the unit is in contact with the families referred to the unit and draws a pedigree of the families. When an inherited disease, in this case FTAAD, is confirmed by medical history and medical records, the family members are invited to a family counselling. A genetic counsellor, a geneticist and a cardiologist or a physician in internal medicine gives information of the disease to the family members.

The proband is offered genetic testing by a panel of genes known to cause FTAAD. If the disease-causing variant is identified other family members are offered genetic testing (Sanger sequencing) of the identified variant. Carriers are enrolled into a control program. Aortic imaging is performed yearly or even more often if needed. The extent of other imaging and examinations depends on which genetic variant that is identified. Non-carriers do not need to be controlled by further clinical examinations but are offered participation in research on FTAAD.

If the disease-causing variant is not identified, exome sequencing of affected individuals is considered. Meanwhile, aortic imaging is performed every fifth year until 50-65 years of age in those individuals with normal thoracic aortic diameter. Imaging is performed yearly if the aorta is dilated (Figure 12).
Elevated blood pressure in the absence of aortic dilatation is treated according to current guidelines for treatment of hypertension. An unresolved question is whether patients with FTAAD and dilated thoracic aorta should be treated with beta-blockers or ARB, especially if the blood pressure is not elevated. The results from studies on beta-blockers and ARB are somewhat conflicting regarding the effects on aneurysm growth. Further, the studies are performed mainly on patients with MFS, only one study was performed on patients with EDS. The effect of antihypertensive medication on aneurysm growth in patients with FTAAD has not been studied. Our clinical praxis is that individuals with FTAAD, elevated blood pressure and dilated thoracic aorta are usually treated with ARB as first choice and beta-blockers as second choice. If the thoracic aorta is dilated but the blood pressure is normal, the benefits and disadvantages are discussed with the patient. ARB is usually first line therapy, but treatment is often limited by side effects such as ortostatism.

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**Figure 12**: Schematic illustration of the workflow in investigation of FTAAD.
Limitations

Paper I

The number of patients (n=77) and the age range (18-82 years) was enough to show an age dependent increase in the aortic diameter and to show a difference in diameter between men and women. The inclusion of BMI in the statistical model showed significant correlation with aortic diameter in most of the levels, but not all. With a larger number of patients we might have reached significance at all levels. At the time of the study, the CT examinations were not ECG-gated, and therefore not obtained at a specific point in the cardiac cycle. A substantial number of the patients suffered, or have suffered, from a malignant disease. These patients were asked for their weight before the onset of the disease but this information is not as reliable as a measured weight would have been.

Paper II

Today, there is no international consensus how the measurement of aortic diameter by TTE should be performed. Several studies have addressed reference values of normal aortic diameters but the method of measuring the diameter varies from study to study (diastolic vs systolic diameter, leading-edge to leading-edge vs inner-edge to inner-edge, at specific anatomic localisation vs widest diameter). Using another way to measure aortic diameter with other reference values might have given a slightly different result. However, the main conclusions of the study would likely have been the same.

Paper III

In this study, individuals with dilated thoracic aorta were compared to individuals with normal aortic diameter without the knowledge of the underlying genetic variant. Therefore, there are probably several individuals in the non-dilated group who are carriers of a genetic variant causing FTAAD, which might influence the comparison of the aortic stiffness between the groups.

Paper IV

Even if this is the largest family reported with FTAAD due to a mutation in the MYLK gene, the limited number of family members makes it difficult to describe the phenotype in detail or draw any conclusions of the precise phenotype and differences between carriers and non-carriers regarding aortic stiffness.
Ethical considerations

In this thesis, we study families with an inherited disease. The disease is potentially life threatening but is mainly without any symptoms. At present, we can identify the disease causing mutation in about 20-25% of the families and we can further differentiate carriers from non-carriers in these families. Nevertheless, in the majority of the families, the disease-causing mutation still remains unknown, and therefore individuals with increased risk for dissections cannot be identified.

Elevated blood pressure should be treated with antihypertensive medication. Whether this treatment is effective in preventing aneurysm growth and dissections is somewhat unclear. As mentioned above, studies on preventive effects on aneurysm growth and dissections of medical therapy have been made on patients with Marfan syndrome with partially similar phenotype regarding the thoracic aorta. However, it is unclear whether the results from these studies can be applied in FTAAD. There are elective prophylactic interventions, but the correct timing of these is unclear. In addition, surgical interventions always carry a potential risk of serious complications, but the risks are considerably higher in surgery for an acute dissection than in elective surgery.

Both screening for aortic dilatation and genetic testing for FTAAD raise several ethical considerations. A normal screening result by aortic imaging at younger ages does not exclude the patient from being a carrier of the genetic variant. Nothing can be said about the future risk for dissections. Therefore, once the first imaging is performed, there should be a follow-up on a regular basis. This will lead to several unnecessary investigations and concern during the time the genetic variant is unknown. On the other hand, identification of a dilated aorta can lead to an elective intervention and prevent an aortic dissection.

The drawbacks of positive genetic testing are stigmatisation and discrimination on one hand and the potential influence on different life decisions on the other hand.

Before screening and genetic testing is offered, the individuals should be given genetic counselling with meticulous information about the disease and what information the testing can give. The risk for false negative and false positive results must also be taken into consideration.

Papers II-IV are based on the study approved by The Regional Ethical Review Board in Umeå, Sweden (Dnr 08-092M 2008-06-10 and Dnr 2012-194-32M).
Conclusions

- Thoracic aortic diameter increases with age. Sex and body size influence the diameter, but to a lesser extent than age.

- In families with thoracic aortic disease, screening identifies family members with a previously unknown aortic dilatation.

- Normal aortic diameter does not exclude a person from being at risk for developing aortic disease in the future.

- Ascending thoracic aortic diameters measured by TTE strongly correlate with MRI measurements.

- Thoracic aortic stiffness increases with age and with increasing aortic diameter.

- Individuals identified to have dilated thoracic aorta in a screening, have lower distensibility and higher PWV than individuals with normal thoracic aortic diameter, even when adjusted for age and the aortic diameter.

- Increased aortic stiffness in young individuals with normal thoracic aortic diameter might be an indicator of thoracic aortic disease.

- The 2-bp deletion (c.3272_3273del) identified in MYLK causes thoracic aortic dissections with decreased penetrance and variable clinical expression.

- No significant differences in aortic stiffness could be identified between MYLK mutation carriers and non-carriers.
Future considerations

Acute thoracic aortic dissection is a life threatening condition with high mortality and morbidity, even when operated on acutely. Therefore, efforts must be made to identify individuals at risk. Even though the thoracic aortic diameter is important in diagnosing patients, its value as prognostic marker for dissection or rupture in FTAAD is limited. Identification of the disease-causing mutation within a family enables predictive testing of family members after genetic counselling. Non-carriers will benefit from knowledge of not being at risk and carriers can be offered surveillance and prevention. Further studies on thoracic aortic stiffness must continue in order to provide more information of correct timing of prophylactic surgery.
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References


