General introduction

Cardiovascular disease (CVD) represents a major global health problem: it is the leading cause of death and disability around the world, in both industrialized and developing countries [1-3]. CVD comprises a broad spectrum of disorders affecting the heart and blood vessels. The work presented in this thesis focuses on rare inherited CVD, including thoracic aortic aneurysms and dissections, congenital heart disease and cardiomyopathies. In the past decade, many of the genetic factors that contribute to CVD have been identified. Clarification of the underlying genetic architecture is important to identify relatives at increased risk of developing CVD, who should undergo regular cardiovascular surveillance, and helps to determine optimal clinical and surgical management. In addition, establishing the underlying molecular mechanisms is pivotal for the development of new treatment strategies. Lessons learned from studying CVD caused by variation in a single gene (i.e. monogenic inheritance) may also improve our understanding of molecular processes resulting in more common and complex forms of CVD.

Thoracic aortic aneurysms and dissections

Definition and classification

An arterial aneurysm is defined as a localized dilatation of an artery [4]. Most aneurysms involve the thoracic or abdominal aorta. In this thesis, we will focus on thoracic aortic aneurysms (TAA). TAA are classified according to their anatomic location (aortic root, ascending aorta, aortic arch and descending aorta) (**Figure 1A**) and may involve more than one aortic segment. An aortic root diameter ≥40 mm is generally considered enlarged [5]. However, this value may

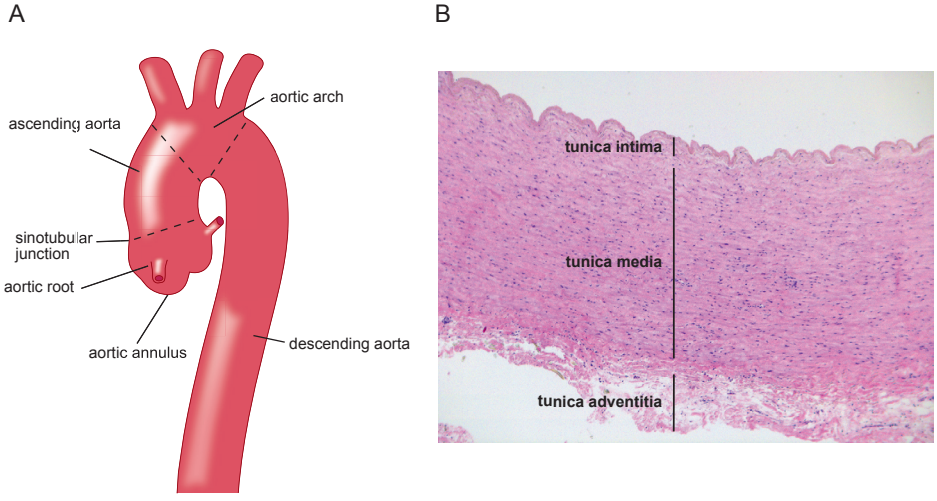

Figure 1. Anatomy of the thoracic aorta. (A) Segmental division of the thoracic aorta. (B) Histologic features of the normal ascending aortic wall stained with hematoxylin and eosin. Image provided by Jan von der Thüsen.

need to be adjusted for gender, age and body surface area. Without prophylactic surgical repair, progressive enlargement of the aorta might lead to rupture or dissection. Thoracic aortic dissections are classified according to the anatomic location of the intimal tear in the aortic wall: Stanford type A dissections involve the ascending aorta; type B dissections include only the descending aorta, without involvement of the ascending aorta [6]. The location of the dissection is important for determining the need for surgical or endovascular procedures [5].

Epidemiology

The true prevalence and incidence of TAA is not known, because patients may remain asymptomatic for many years. Men are more likely to suffer from TAA than women. A nationwide study involving all individuals diagnosed with TAA or dissections in Sweden reported an incidence of 16.3 per 100,000 per year for men and 9.1 per 100,000 per year for women [7]. Women, on the other hand, are less likely to undergo surgical treatment, and have worse outcomes and higher early and late mortality following open surgical repair than men [8-10]. These gender-related differences are poorly understood. Possible explanations include genetic or hormonal factors, atypical disease presentation (i.e. women more often present with congestive heart failure and altered mental status) [11], or underestimation of the relative aortic size in women.

Pathophysiology

The aortic wall is composed of three layers: the intima, media and adventitia (**Figure 1B**). The intima consists of a single layer of endothelial cells on top of a basement membrane, and a subendothelial layer of loose connective tissue. The media is the thickest layer and is composed of numerous concentric layers of elastic laminae interspersed with smooth muscle cells, collagen, and proteoglycans [12]. The adventitia is the outer layer which consists of collagen, fibroblasts, and vasa vasorum. Ascending TAA often result from medial degeneration, which is characterized by varying degrees of mucoid extracellular matrix accumulation, elastic fiber fragmentation and/or loss, smooth muscle cell nuclei loss, and laminar medial collapse [13]. The most important risk factors for developing TAA include ageing and hypertension [14, 15]. However, approximately one in five patients with TAA have a positive family history for arterial aneurysms, indicating that genetic predisposition plays a major role in the development of TAA. Familial TAA is associated with younger age at presentation and faster growth rate [16].

Genetics

The identification of genes involved in the pathogenesis of TAA is hampered by non-Mendelian patterns of inheritance with incomplete penetrance and variable expressivity. Nonetheless, the number of genes associated with TAA and dissections has increased rapidly over the past two decades. The majority of these genes code for proteins involved in the extracellular matrix, the transforming growth factor beta (TGF-β) signaling pathway, and the vascular

smooth muscle cell (VSMC) contractile apparatus (**Table 1**) [17]. Copy number variants (i.e. submicroscopic gains or losses of chromosomal material) may also predispose to TAA. Though individually rare, significantly higher numbers of copy number variants are found in both familial and sporadic TAA [18, 19]. Recent evidence suggests that different forms of genetically triggered TAA share a common epigenetic mechanism involving the HDAC9-BRG1-MALAT1 chromatin-remodeling complex [20].

TAA can be subdivided into two categories: syndromic (associated with abnormalities in other organ systems) and non-syndromic (isolated finding). There is, however, considerable overlap in the genetic basis between these categories, since variants in the same gene can result in both syndromic and non-syndromic TAA [21, 22]. Marfan syndrome is undoubtedly the most well-known and intensively studied syndromic form of TAA. Patients typically present with aortic root enlargement, ectopia lentis, and skeletal features [23]. Heterozygous variants in *FBN1*, encoding the extracellular matrix protein fibrillin-1, can be found in over 90% of patients that fulfill diagnostic criteria [24, 25]. Marfan syndrome shows considerable clinical overlap with other heritable connective tissue disorders, including Loeys-Dietz syndrome, Shprintzen-Goldberg syndrome, and vascular Ehlers-Danlos syndrome [26]. Compared to Marfan syndrome, vascular abnormalities in Loeys-Dietz syndrome tend to be more severe and widespread. Other features that distinguish Loeys-Dietz syndrome from Marfan syndrome include hypertelorism, bifid uvula or cleft palate, craniosynostosis, clubfoot, joint contractures, and cervical spine instability [27]. TAA can also be a (rare) manifestation of a number of other syndromes, including Turner syndrome, arterial tortuosity syndrome, cutis laxa syndromes, Alagille syndrome, polycystic kidney disease, Alport syndrome, osteogenesis imperfecta, hereditary hemorrhagic telangiectasia, Noonan syndrome, neurofibromatosis type 1, and tuberous sclerosis.

Variants in *ACTA2*, encoding the smooth muscle cell specific isoform of alpha-actin, are an important cause of non-syndromic TAA. Mutation detection rates in familial TAA vary between 3% and 21% [28-32]. Variants in ACTA2 not only predispose to TAA and dissections, but also to premature coronary artery disease, stroke, and Moyamoya-like disease. Other genes, such as the myosin heavy chain 11 (*MYH11*) gene, the myosin light chain kinase (*MYLK*) gene, and the cyclic guanosine monophosphate-dependent protein kinase (*PRKG1*) gene, each account for less than 1% of non-syndromic TAA (**Table 1**) [22]. Identification of the specific genetic cause helps guide medical and surgical management of the disease, e.g. the extent of vascular imaging and the threshold for prophylactic surgical intervention [33].

In this thesis, we provide recommendations for genetic testing in TAA. We discuss which patients should be referred for genetic counseling, and how family screening should be performed (**Chapter 2.1**). Special attention is paid to the genetic counseling process in Loeys-

Table 1. Genes contributing to thoracic aortic aneurysms and dissections

Gene	Locus	Protein	Main features	Contribution	Refs
Genes encoding components of the extracellular matrix					
BGN	Xq28	Biglycan	Early-onset aortic aneurysm and dissection, hypertelorism, pectus deformity, joint hypermobility, contractures, mild skeletal dysplasia	Rare	[77]
COL1A1	17q21.33	Type I collagen, alpha-1 chain	Skin hyperextensibility, dystrophic scarring, joint hypermobility	Rare	[78]
COL3A1	2q32.2	Type III collagen, alpha-1 chain	Arterial rupture without preceding dilatation, bowel perforations, uterine rupture during pregnancy, thin and translucent skin, easy bruisability, acrogeria	Rare	[79]
COL4A5	Xq22.3	Type IV collagen, alpha-5 chain	Progressive renal failure, sensorineural hearing loss, anterior lenticonus	Rare	[80]
COL5A1	9q34.3	Type V collagen, alpha-1 chain	Skin hyperextensibility, dystrophic scarring, joint hypermobility	Rare	[78, 81]
COL5A2	2q32.2	Type V collagen, alpha-2 chain	Skin hyperextensibility, dystrophic scarring, joint hypermobility	Rare	[78]
EFEMP2	11q13.1	EGF-containing fibulin-like extracellular matrix protein 2	Multiple arterial aneurysms and tortuosity, emphysema, inguinal and diaphragmatic hernia, skin hyperlaxity, downslanting palpebral fissures	Rare	[82]
ELN	7q11.23	Elastin	Supravalvular aortic stenosis, peripheral arterial stenosis, skin hyperlaxity, premature aged appearance, gastrointestinal diverticula, inguinal hernia	Rare	[83]
FBN1	15q21.1	Fibrillin-1	Aortic root aneurysm, ectopia lentis, myopia, pectus deformity, arachnodactyly	2-3%	[84, 85]
FBN2	5q23.3	Fibrillin-2	Joint contractures, arachnodactyly, scoliosis, crumpled ears	Rare	[86, 87]
LOX	5q23.1	Lysyl oxidase	Aortic root aneurysm, ascending aortic aneurysm, bicuspid aortic valve	1-2%	[88]
LTBP3	11q13.1	Latent transforming growth factor-beta-binding protein 3	Aortic aneurysms and dissections, dental abnormalities, shorts stature	Unknown	[89]
MFAP5	12p13.31	Microfibrillar-associated protein 5	Aortic root aneurysm, paroxysmal atrial fibrillation	<1%	[90]
PLOD1	1p36.22	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 1	Congenital muscle hypotonia, early-onset kyphoscoliosis, joint hypermobility	Rare	[91]
Genes encoding components of the TGF-β pathway					
SKI	1p36.33-p36.32	Ski oncogene	Craniosynostosis, hypertelorism, micrognathia, high palate, arachnodactyly, joint contractures, hypotonia, developmental delay	Rare	[92, 93]
SMAD2	18q21.1	Mothers against decapentaplegic homolog 2	Aortic root aneurysm, arterial aneurysms and dissections, valve abnormalities, hypertelorism, pectus deformity, scoliosis, osteoarthritis, hernias	1%	[94, 95]
SMAD3	15q22.33	Mothers against decapentaplegic homolog 3	Widespread and aggressive arterial aneurysms and dissections, arterial tortuosity, early-onset osteoarthritis, osteochondritis dissecans, hypertelorism, bifid uvula	2%	[96, 97]
SMAD4	18q21.2	Mothers against decapentaplegic homolog 4	Gastrointestinal hamartomatous polyps, cutaneous and mucosal telangiectasia, epistaxis, arteriovenous malformations	Rare	[98]

Table 1. Genes contributing to thoracic aortic aneurysms and dissections (*continued*)

Gene	Locus	Protein	Main features	Contribution	Refs
SMAD6	15q22.31	Mothers against decapentaplegic homolog 6	Bicuspid aortic valve, thoracic aortic aneurysm	2-3%	[55]
TGFBR1	9q22.33	TGF-beta receptor type-1	Widespread and aggressive arterial aneurysms and dissections, arterial tortuosity, hypertelorism, cleft palate, bifid uvula, pectus deformity, scoliosis, club feet	1%	[99]
TGFBR2	3p24.1	TGF-beta receptor type-2	Widespread and aggressive arterial aneurysms and dissections, arterial tortuosity, hypertelorism, cleft palate, bifid uvula, pectus deformity, scoliosis, club feet	4%	[99]
TGFB2	1q41	Transforming growth factor beta-2	Thoracic aortic aneurysm and dissection, arterial tortuosity, mitral valve prolapse	1%	[100]
TGFB3	14q24.3	Transforming growth factor beta-3	Aortic aneurysm and dissection, mitral valve prolapse, hypertelorism, cleft palate, bifid uvula, pectus deformity, scoliosis	2%	[101]
Genes encoding component of the vascular smooth muscle cell contractile apparatus					
ACTA2	10q23.31	Aortic smooth muscle actin	Iris flocculi, livedo reticularis, premature coronary artery disease and stroke	3-21%	[28-32]
FLNA	Xq28	Filamin-A	Periventricular heterotopia, epilepsy, joint hypermobility, patent ductus arteriosus	Rare	[102, 103]
MYH11	16q13.11	Myosin heavy chain-11	Aortic dissection, patent ductus arteriosus	1%	[104]
MYLK	3q21.1	Myosin light chain kinase	Ascending aortic aneurysm and dissection	1%	[105]
PRKG1	10q11.23-q21.1	Cyclic GMP-dependent protein kinase	Early-onset aortic dissection, coronary artery aneurysm and dissection	1%	[106]
Other genes					
ABL1	9q34.12	Tyrosine-protein kinase ABL1	Atrial and ventricular septal defects, aortic root aneurysm, pectus excavatum, scoliosis, finger contractures, failure to thrive, gastrointestinal problems	Rare	[107]
FOXE3	1p33	Forkhead box protein E3	Ascending aortic aneurysm and dissection	1-2%	[108]
GATA5	20q13.33	GATA-binding factor 5	Bicuspid aortic valve	Rare	[109]
MAT2A	2p11.2	Methionine adenosyltransferase 2A	Aortic root aneurysm, ascending aortic aneurysm, bicuspid aortic valve	1%	[110]
NOTCH1	9q34.3	Neurogenic locus notch homolog protein 1	Bicuspid aortic valve, calcific aortic stenosis	2-3%	[55, 111]
ROBO4	11q24.2	Roundabout homolog 4	Bicuspid aortic valve, thoracic aortic aneurysm	1-2%	[112]
SLC2A10	20q13.12	Solute carrier family 2, facilitated glucose transporter member 10	Generalized arterial tortuosity, arterial aneurysms and stenosis, joint laxity, skin hyperextensibility, inguinal and diaphragmatic hernia, elongated face, micrognathia	Rare	[113, 114]

Dietz syndrome type 3 (**Chapter 2.2**). In **Chapter 2.3**, we describe a newly discovered subtype of Loeys-Dietz syndrome. Finally, in **Chapter 2.4**, we applied RNA sequencing to identify new pathways involved in the pathogenesis of TAA.

Congenital heart disease

Definition and classification

Congenital heart disease (CHD) is classically defined as a structural malformation of the heart or intrathoracic great vessels present at birth that is actually or potentially of functional significance [34]. This definition encompasses a broad spectrum of structural abnormalities, ranging from a small, often spontaneously closing ventricular septal defect to a severe malformation that requires extensive surgical repair. Several classification schemes exists for describing CHD. For etiologic studies, CHD are often classified according to the underlying developmental mechanism [35].

Epidemiology

CHD is the most common major structural birth defect in human, affecting nearly 1% of live births [36]. This corresponds to approximately 1,400 children born with a heart defect in the Netherlands each year. In addition, another 1-2% of the population have a bicuspid aortic valve (BAV) instead of the normal tricuspid aortic valve (TAV). Although BAV is often asymptomatic and undiagnosed in infancy, it is associated with serious complications and sudden cardiac death later in life [37-39]. BAV can be part of a larger spectrum of left-sided CHD, also referred to as left ventricular outflow tract obstruction (LVOTO), including aortic valve stenosis, coarctation of the aorta, and in its most severe form, hypoplastic left heart syndrome.

Advances in cardiothoracic surgery and transcatheter interventions have dramatically improved survival: more than 90% of children born with CHD now reach adulthood [40]. The growing population of adults with (surgically corrected) CHD poses new challenges with regard to pregnancy-related complications and increased risk of CHD in their offspring. The recurrence risks vary according to the type of CHD [41]. In addition, the recurrence risk is generally higher in the offspring of affected mothers compared with affected fathers [41]. The latter might be explained by a threshold model with sex dimorphism (i.e. females may require a higher genetic load to develop the disease, a phenomenon known as the Carter effect) [42], maternally imprinted CHD genes, or mitochondrial inheritance [43].

Pathophysiology

The human heart is the first organ to function during embryonic development. Heart development begins with the formation of two endothelial tubes which fuse in the midline to form

the primitive heart tube (**Figure 2A**). The heart tube subsequently develops into five distinct regions: the sinus venosus, primitive atrium, primitive ventricle, bulbus cordis, and truncus arteriosus (**Figure 2B**). As the primitive heart tube elongates, it undergoes rightward looping and folding (**Figure 2C**). As a result, the ventricle moves caudally and the atrium cranially, assuming their final adult positions (**Figure 2D**). Further heart development involves remodeling of the chambers and the formation of septa and valves to form a four-chambered heart [44]. Heart development is completed by 9 weeks of gestation. Perturbation of any step of the complex biological processes involved in heart development can lead to CHD. The spectrum of left-sided CHD, for example, may result from altered intracardiac hemodynamics (e.g. impaired blood flow and shear stresses), leading to underdevelopment of left heart structures [45]. This concept is supported by experimental animal models and high-resolution imaging showing that, besides intrinsic patterning, mechanical forces are essential drivers of normal cardiac morphogenesis [46]. Familial clustering of different types of left-sided CHD also suggest a shared genetic etiology.

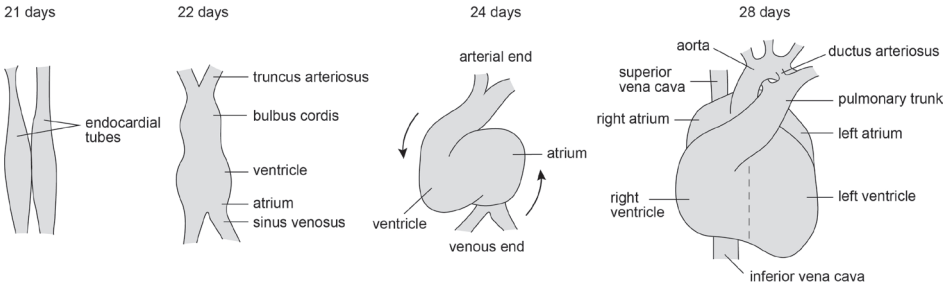

Figure 2. Development of the human heart during the fourth week. Artwork by Tom de Vries Lentsch.

Genetics

CHD often occurs as an isolated finding (non-syndromic). About 15% of newborns with CHD have extracardiac manifestations [47]. Aneuploidies, such as trisomy 21 (Down syndrome), trisomy 13 (Patau syndrome), trisomy 18 (Edwards syndrome) and monosomy X (Turner syndrome), account for approximately 10% of CHD in newborns. Smaller copy number variants, including both microdeletions or duplications, contribute to 10-15% of CHD. Microdeletion of the chromosome 22q11.2 region, encompassing over 30 genes, is the most frequently detected submicroscopic anomaly in CHD. Patients typically present with conotruncal heart defects, such as tetralogy of Fallot, pulmonary atresia, interrupted aortic arch, and truncus arteriosus. Haploinsufficiency of both *TBX1* and *CRKL* appears to contribute to the cardiovascular phenotype [48, 49]. In approximately 5% of newborns the CHD is part of a monogenic syndrome. Noonan syndrome is the most frequently encountered, with an estimated prevalence between 1:1000 and 1:2500. This syndrome is caused by variants in several genes that encode components of the Ras/MAPK pathway, with variants in the *PTPN11* gene account-

ing for approximately half of all cases. CHD is present in 60-90% of patients with Noonan syndrome. Pulmonary valve stenosis is the most common heart defect but hypertrophic cardiomyopathy is also frequently encountered. Additional features may include dysmorphic facies, short stature, pectus deformity, and developmental delay. Rare monogenic syndromes associated with CHD include Alagille syndrome, Kabuki syndrome, CHARGE syndrome, Holt-Oram syndrome, and Cornelia de Lange syndrome, among many others.

In most patients with non-syndromic CHD, the underlying cause is unknown. The majority occurs sporadically, with presumed multifactorial inheritance [50]. Environmental factors that increase the risk for CHD include teratogenic exposure and maternal illnesses [51]. Rare inherited and *de novo* variants, particularly affecting genes involved in chromatin modification and transcription regulation, may also contribute to non-syndromic CHD [52]. Families with clear monogenic (autosomal dominant, autosomal recessive or X-linked) inheritance of CHD are scarce. Left-sided CHD are an important exception: these heart defects are highly heritable, and approximately 20% of patients have at least one affected first-degree relative [53]. Variants in *NOTCH1*, encoding a transmembrane receptor protein in the highly conserved Notch signaling pathway, have been associated with congenital aortic valve defects and valvular calcification later in life [54]. Rare variants in *SMAD6*, which codes for a negative regulator of the TGF-β and bone morphogenetic protein (BMP) signaling cascades, can be found in 2.5% of BAV with concomitant TAA [55].

In this thesis, we focus on families with left-sided CHD. In **Chapter 3.1**, we show that variants in *NOTCH1* account for 7% of familial and 1% of sporadic left-sided CHD. In **Chapter 3.2**, we describe the new association between *PKP2* and hypoplastic left heart syndrome.

Cardiomyopathies

Definition and classification

Cardiomyopathy is defined as a myocardial disorder in which the heart muscle is structurally and functionally abnormal, in the absence of coronary artery disease or abnormal loading conditions sufficient to cause the observed myocardial abnormality [56]. Cardiomyopathies are classified according to their morphological and functional features into five different phenotypes: hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), arrhythmogenic right ventricular cardiomyopathy (ARVC), restrictive cardiomyopathy (RCM), and unclassified cardiomyopathy, which includes left ventricular noncompaction (LVNC) and Takotsubo cardiomyopathy (**Table 2**). The international MOGE(S) classification system adds information on the genetic background of cardiomyopathies [57].

Table 2. Classification of cardiomyopathies

Type of cardiomyopathy	Characteristics	Prevalence	Refs
Hypertrophic cardiomyopathy (HCM)	Increased LV wall thickness (≥15 mm in adults or z-score >2 in children in one or more LV myocardial segments) that is not explained by abnormal loading conditions	1:200-500	[56]
Dilated cardiomyopathy (DCM)	LV or biventricular systolic dysfunction (LV ejection fraction <40%) and dilatation that are not explained by abnormal loading conditions or coronary artery disease	1:250-2,700	[115]
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	Progressive fibrofatty replacement of the RV myocardium, with or without LV involvement	1:2,000-5,000	[116]
Restrictive cardiomyopathy (RCM)	Diastolic dysfunction with restrictive filling pattern affecting either or both ventricles, and normal or near-normal chamber size and systolic function	rare	[117]
Left ventricular noncompaction (LVNC)	Prominent LV trabeculae with deep intertrabecular recesses and thin compacted layer	rare	[118]
Takotsubo cardiomyopathy	Transient systolic dysfunction of the apical and/or midventricular LV segments in the absence of coronary artery disease, often provoked by physical or emotional stress	rare	[119]

LV, left ventricular; RV, right ventricular.

Epidemiology

HCM and DCM are the most common types of cardiomyopathy. HCM affects approximately 1 in 500 adults [58, 59]. However, when taking into account the high prevalence of HCM-causing variants in the general population and the enhanced clinical detection with advanced imaging techniques, the prevalence might be as high as 1:200 [60]. DCM might also be more common than previously anticipated. An early study reported a prevalence of approximately 1 in 2,700 individuals in the general population [61]. A more recent study, however, estimated the true prevalence of DCM at 1:250 [62].

In this thesis, we will particularly focus on cardiomyopathy in childhood. Cardiomyopathy in children is rare, with an annual incidence of approximately 1 per 100,000 children in Western countries [63-65]. The majority of children are diagnosed within the first year of life. Pediatric HCM generally has a good outcome: the overall survival is 97% at 5 years and 94% at 10 years after presentation. Heart failure is the leading cause of death; sudden death is rare. In contrast, 40% of children with DCM die or undergo cardiac transplantation within 5 years after diagnosis [66, 67]. In addition to the specific categories described above, children often display a mixed cardiomyopathy phenotype.

Pathophysiology

The heart wall consists of three layers: the endocardium, myocardium and epicardium. The myocardium is the thickest layer, and is composed of striated muscle tissue and loose endomysial connective tissue. Individual cardiac muscle cells (cardiomyocytes) are interconnected through highly specialized cell junctions called intercalated discs. There are three main junc-

tional complexes within the disc: fascia adherens, desmosomes and gap junctions (**Figure 3**). These junctions are essential for mechanical and electrical coupling between adjacent cells. Cardiomyocytes are composed of numerous bundles of myofibrils, that contain thick (myosin) and thin (actin) myofilaments. These filaments are organized in repeated subunits, called sarcomeres, which represent the fundamental contractile units of the cardiomyocytes. Binding of calcium ions to troponin C induces conformational changes in the actin-myosin complex, resulting in muscle contraction.

HCM is characterized by left ventricular hypertrophy, typically most pronounced at the basal anterior septum, and predominant diastolic dysfunction. The main histopathological hallmarks are the triad of cardiomyocyte hypertrophy, disarray, and interstitial fibrosis. DCM is characterized left ventricular or biventricular enlargement and systolic dysfunction. The histopathological changes associated with DCM include myocyte nuclear hypertrophy, myofibrillary loss, and interstitial fibrosis [68]. The causes of cardiomyopathy are diverse, particularly in children with DCM, and include environmental (e.g. infections) and genetic etiologies.

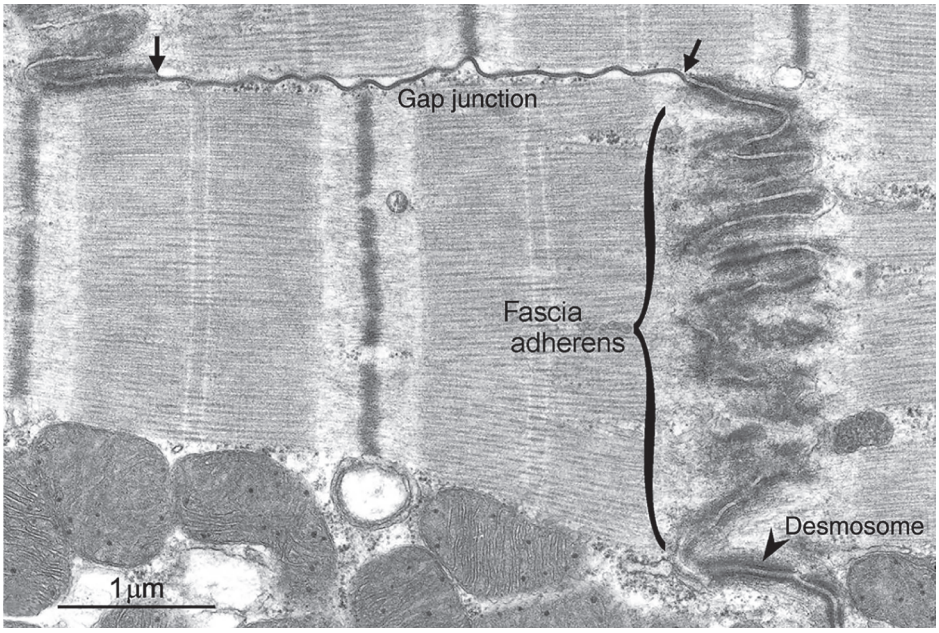

Figure 3. Organization of intracellular connections in the human heart. Electron microscopy of cardiac muscle showing different components of the intercalated disc: fascia adherens, desmosome and gap junction. From Severs, N.J. *BioEssays* 2000;22:188–199. Reprinted with permission of John Wiley & Sons, Inc.

Genetics

Genetic cardiomyopathies are characterized by marked locus and allelic heterogeneity (i.e. the phenotype can result from variants in different genes and different variants in the same

gene, respectively), reduced penetrance and variable expressivity. Both HCM and DCM are usually non-syndromic and inherited in an autosomal dominant fashion. HCM is predominantly caused by variants in genes that encode for sarcomeric proteins. The majority (70-80%) of disease-causing variants identified reside in only two genes, *MYH7* and *MYBPC3*, encoding beta-myosin heavy chain and cardiac myosin-binding protein, respectively. DCM shows more diverse ontology, affecting nearly every compartment in the cell, including the sarcomere, cytoskeleton, nuclear envelope, and sarcoplasmic reticulum [69]. Rare truncating variants in *TTN*, encoding the giant protein titin, can be found in 25% of familial DCM and 18% of sporadic DCM [70]. However, these variants have also been identified in 2-3% of the general population, complicating variant interpretation. Rare variants in *LMNA*, encoding lamin A/C, account for approximately 6% of DCM, which is often associated with conduction defects and arrhythmias [71]. In the Netherlands, a founder variant in the phospholamban (*PLN*) gene accounts for an additional 15% of DCM [72].

The majority of genes described in adults also contribute to cardiomyopathy in children [73]. Children with early-onset and severe disease presentation are more likely to carry *de novo*, biallelic or multiple gene variants in classical autosomal dominant disease genes [74-76]. Pediatric cardiomyopathy can also be part of numerous syndromes (e.g. Noonan syndrome), neuromuscular disorders (e.g. Duchenne muscular dystrophy) and metabolic conditions, warranting detailed investigation by a genetic specialist.

In this thesis, we discuss the pathogenicity of a previous cardiomyopathy-associated gene and describe two newly identified genes involved in early-onset cardiomyopathy with autosomal recessive inheritance. In **Chapter 4.1**, we investigate the role of *CALR3* variants in monogenic cardiomyopathy. In **Chapter 4.2** and **4.3**, we describe the first families with cardiomyopathy due to biallelic variants in *ALPK3*, encoding a transcriptional regulator involved in early cardiomyocyte differentiation. Finally, in **Chapter 4.4**, we describe a family with rapidly progressive cardiomyopathy due to biallelic variants in *ASNA1*, providing the first evidence for a link between the tail-anchored protein insertion pathway and CVD in humans.

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