Summary

Cardiovascular disease (CVD) is the leading cause of death and disability in the world. Lifestyle and environmental factors greatly influence the risk for CVD. However, over the years it has become clear that genetic factors also substantially contribute to its pathogenesis. The majority of CVD are thought to result from multifactorial inheritance, involving a complex interplay between multiple susceptibility genes and environmental factors. Despite extensive research efforts, the molecular mechanisms underlying these complex forms of CVD remain poorly understood.

In this thesis, we focus on monogenic forms of CVD in an effort to identify new disease genes and pathways, and to further delineate the phenotypes in families with thoracic aortic aneurysms and dissections, left-sided congenital heart disease, and cardiomyopathies. Lessons learned from studying monogenic forms of CVD may also improve our understanding of molecular processes resulting in more common and complex forms of the disease.

Chapter 1 highlights the most important forms of inherited CVD, including thoracic aortic disease, congenital heart disease and cardiomyopathy, and describes the main aims of this thesis.

Chapter 2 focuses on thoracic aortic aneurysms (TAA) and dissections. In **Chapter 2.1**, we investigate which factors increase the chance of finding a disease-causing variant, what genetic tests should be offered for efficient and cost-effective detection of disease-causing variants, which relatives should undergo cardiovascular screening, and how this screening should be conducted. The results were used as starting point for developing a national multidisciplinary consensus statement, available on the website of the Dutch societies for Clinical Genetics (VKGN) and Cardiology (NVVC), and published in an international journal. In **Chapter 2.2**, we discuss the key points that should be addressed during genetic counseling of families with Loeys-Dietz syndrome type 3 (also known as aneurysms-osteoarthritis syndrome). This is an autosomal dominant condition primarily characterized by a combination of arterial aneurysms and tortuosity, and early-onset osteoarthritis. Extensive clinical studies have revealed an age-dependent penetrance and variable expressivity. In this chapter, we pay special attention to genetic testing in minors and reproductive decision making. In **Chapter 2.3**, we describe a new autosomal dominant syndromic form of TAAD caused by variants in the *TGFB3* gene, which encodes the ligand transforming growth factor beta-3. Studies in 43 patients from 11 families showed extensive cardiovascular involvement, including aneurysms and dissections of the thoracic and abdominal aorta and mitral valve disease, and craniofacial, skeletal and cutaneous anomalies. The clinical features show considerable overlap with other TGF-β pathway-related vasculopathies. The condition is now referred to as Loeys-Dietz syndrome type 5. In **Chapter 2.4**, we apply RNA sequencing in aortic wall tissue to provide further insight

in the pathogenesis of aneurysm formation. We discuss the gene expression signature of Marfan syndrome, and report important pathways involved in the pathogenesis of TAA.

Chapter 3 focuses on genetic factors involved in left-sided congenital heart disease (CHD), including bicuspid aortic valve, aortic valve stenosis, coarctation of the aorta, and hypoplastic left heart syndrome. Previous studies have established familial clustering and high heritability of left-sided CHD. Despite strong evidence for a genetic basis, only a few genes have been associated with CHD, and the contribution of each individual gene is generally small. In **Chapter 3.1**, we discuss the results from *NOTCH1* mutation analysis and family screening in 428 patients with left-sided CHD. We report that, in familial CHD, the probability of finding a protein-truncating variant in the *NOTCH1* gene is approximately 7%, whereas this is only 1% in sporadic patients. In contrast to previous reports, the phenotypes observed in our families included a wide variety of congenital heart disease, including right-sided and conotruncal defects. In addition, 1 in 10 carriers of a pathogenic variant had TAA. In **Chapter 3.2**, we describe a consanguineous family with an autosomal recessive form of hypoplastic left heart syndrome due to a homozygous truncating variant in the *PKP2* gene. This genes codes for the plakophilin-2 protein, a major component of highly specialized junctions between cardiomyocytes called desmosomes. Heterozygous variants in *PKP2* have previously been associated with autosomal dominant arrhythmogenic cardiomyopathy. Our findings suggest that complete loss of plakophilin-2, on the other hand, leads to abnormal myocardial development, resulting in severe congenital heart malformations. These observations are in line with previous studies in mice and zebrafish.

Chapter 4 discusses the pathogenicity of a gene previously associated with cardiomyopathy, and reports on the search for new genes involved in pediatric cardiomyopathy. In **Chapter 4.1**, we investigate the association between cardiomyopathy and variants in the *CALR3* gene. The corresponding protein, calreticulin-3, belongs to a family of calcium-binding proteins in the endoplasmic reticulum. More recent studies, however, have indicated that the calcium-binding capacity of calreticulin-3 is absent or very low. Since the initial publication, more than a decade ago, no other studies have confirmed the link between *CALR3* and cardiomyopathy. We performed an extensive study in a large Dutch cohort of over 6000 cardiomyopathy patients. No statistically significant difference in allele frequency between these patients and controls was detected. In 1 in 5 patients, the *CALR3* variant was accompanied by a disease-causing variant in another, well-known cardiomyopathy gene. Furthermore, we could not detect calreticulin-3 protein expression in myocardial tissue. Based on these findings, we highly question the impact of CALR3 genetic variation on cardiomyopathy. **Chapter 4.2** describes the identification of a new autosomal recessive form of pediatric cardiomyopathy caused by biallelic variants in the *ALPK3* gene. This gene codes for a nuclear kinase that is essential in early stages of cardiomyocyte differentiation. Studies in 5 patients from 3 families indicated

severe biventricular hypertrophic and/or dilated cardiomyopathy from an early age. Three patients died from heart failure during pregnancy or shortly after birth. In addition, two adult relatives displayed atypical hypertrophic cardiomyopathy, which might be related to their heterozygous carrier state. The latter suggests that *ALPK3* may also be implicated in adult-onset cardiomyopathy. In **Chapter 4.3**, we further explored the clinical and genetic characteristics in 18 patients with *ALPK3*-related cardiomyopathy. The most striking observation is the progression of primary dilated cardiomyopathy to hypertrophic cardiomyopathy after birth. To our knowledge, this phenomenon has not been described in other cardiomyopathies thus far. Furthermore, several patients displayed extracardiac features, including short stature, contractures and scoliosis. We found no correlation between the type or locations of *ALPK3* variants and the clinical presentation. In **Chapter 4.4** we describe the identification of another new autosomal recessive form of pediatric cardiomyopathy, due to biallelic variants in the *ASNA1* gene. Both patients died within few weeks after birth from rapidly progressive heart failure. Knockout of the corresponding gene in zebrafish resulted in a similar cardiac phenotype. The *ASNA1* gene encodes a cytoplasmic chaperone protein that mediates membrane insertion of newly formed tail-anchored proteins. Several of these tail-anchored proteins have previously been linked to cardiomyopathy. The early lethal phenotype observed in our family is probably the result of mislocalization of multiple substrates.

Finally, in **Chapter 5**, we discuss how the results of the studies described in this thesis have contributed to our understanding of the underlying mechanisms in heritable CVD. Furthermore, we discuss the implications and suggestions for future research.