Inherited Cardiomyopathies

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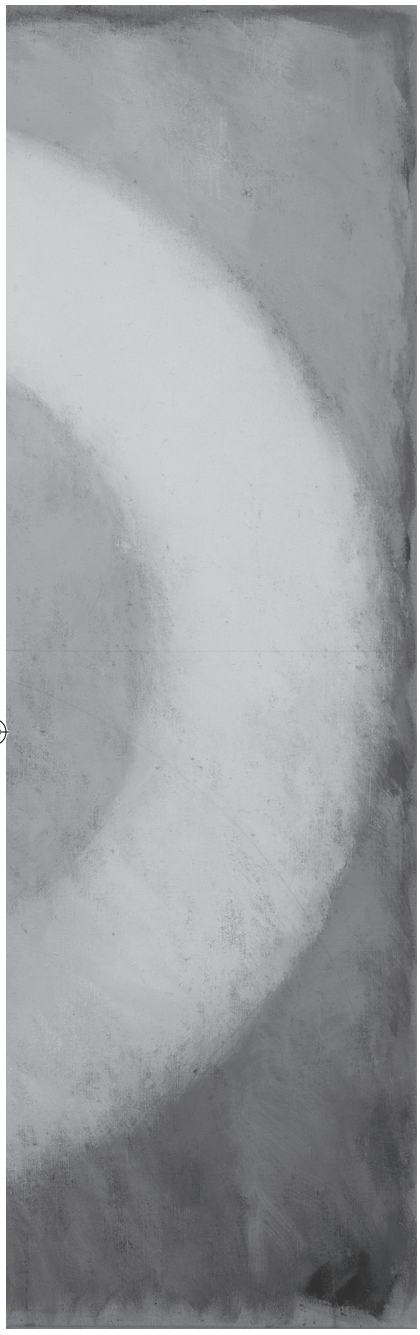

11

Summary and future perspectives

SUMMARY

In the last two decades there have been important breakthroughs in unraveling the genetic factors underlying inherited cardiomyopathies, enabling genetic cascade screening in families and providing insight into the pathomechanisms. Inherited cardiomyopathies are genetically highly heterogeneous. This thesis focuses mainly on dilated cardiomyopathy (DCM). However, in several chapters, we report cases with genetic and clinical overlap between various cardiomyopathy subtypes.

The first part of this thesis focuses on genetic causes and phenotypic characterization of inherited cardiomyopathies, mainly DCM, with associated neuromuscular disease in a subset of cases. Several Dutch founder mutations are discussed.

Chapter 2 presents an overview of 10 years' experience with genetic analysis in index patients with idiopathic DCM. Cardiological and neurological evaluations, family screenings, and genetic analyses for 418 index patients with idiopathic DCM were collected. Thirty-five (putative) pathogenic mutations were identified in 82 index patients (20%). The type of DCM influenced the yield: mutations were found in 25% of familial DCM cases, compared to 8% of sporadic DCM cases, and 62% of cases where DCM was accompanied by neuromuscular disease. Thus, clinical clues, like associated neuromuscular disease or signs of familial occurrence, can strongly increase the yield of genetic analysis in DCM. A *PLN* founder mutation and *LMNA* mutations were most prevalent and often demonstrated a specific phenotype.

Chapters 3 and **4** are about desmin-related myopathy, an inherited skeletal and cardiac myopathy mainly caused by *DES* mutations. **Chapter 3** describes a meta-analysis of all reported *DES* mutation carriers up to April 2010 (n=159 with 40 different mutations). The majority of *DES* mutation carriers demonstrate cardiological signs (74%), particularly cardiac conduction disease and DCM. Although neurological signs were frequently present (74%), nearly a quarter of carriers exhibited an isolated cardiological phenotype. More than half of the mutations were located in the 2B domain. These 2B mutations were predominant in patients with an isolated neurological phenotype, whereas head and tail domain mutations were predominant in patients with an isolated cardiological phenotype. **Chapter 4** describes the cardiac phenotype of two Dutch founder mutations in the *DES* gene (p.S13F and p.N342D). We summarized the clinical details of 39 p.S13F carriers (eight index patients) and of 21 p.N342D carriers (three index patients). The cardiac phenotype of p.S13F carriers is fully penetrant and severe, characterized by cardiac conduction disease and cardiomyopathy, often with right ventricular involvement. Although muscular weakness is a prominent and presenting symptom in p.N342D carriers, their cardiac phenotype is similar to that of p.S13F carriers. Recognition of the *DES*-related phenotype can be difficult due to clinical variability, but it is important to recognize it, because early detection of the cardiac phenotype and subsequent treatment can save lives.

Chapter 5 describes the discovery of the genetic cause of infantile type I muscle fiber disease

and cardiomyopathy, an autosomal recessive lethal condition. Multipoint parametric linkage analysis of six Dutch patients and sequence analysis of the entire linkage region led to the identification of a founder mutation: a homozygous splice site mutation in *MYL2*. Whole exome sequencing of an Italian patient with the same clinical picture showed compound heterozygosity for two other mutations affecting the same exon of *MYL2*. We currently know eight Dutch families with one or more patients caused by the identified *MYL2* founder mutation. All the patients died during their first year of life, of heart failure due to cardiomyopathy, mainly DCM, although some patients had features of other cardiomyopathy subtypes (hypertrophic, restrictive or non-compaction). Several heterozygous missense mutations in *MYL2* are known to cause dominant hypertrophic cardiomyopathy. However, none of the patients' parents who carried the identified founder mutation showed signs of cardiomyopathy at the time of evaluation (ages 30-69 years).

The second part of this thesis presents two examples of hitherto unknown gene-environment interactions in inherited cardiomyopathies: pregnancy and chemotherapy.
Chapters 6 and **7** show that peripartum cardiomyopathy (PPCM) can be part of familial DCM. Although PPCM was classified as a non-familial, non-genetic form of DCM, we hypothesized that some cases of PPCM are part of the spectrum of familial DCM presenting in the peripartum period. **Chapter 6** describes the identification of a substantial number (5/90, 6%) of DCM families with PPCM patients. Secondly, cardiological screening of first-degree relatives of three PPCM patients who did not show full recovery revealed undiagnosed cases of DCM in all three families. Finally, genetic analyses revealed a mutation in *TNNC1* segregating with disease in a DCM family with a member with PPCM, supporting the genetic nature of disease in this case. These findings strongly suggest that a subset of PPCM is indeed an initial manifestation of familial DCM. Hence, cardiological screening for covert DCM in first-degree relatives of PPCM patients is advisable. In addition, cardiological screening during pregnancy and puerperium should be considered for first-degree relatives (or relatives carrying an underlying mutation) of familial DCM patients. **Chapter 7** supports the finding of chapter 6. Targeted next-generation sequencing demonstrated that mutations in cardiomyopathy-related genes, especially in *TTN*, are common in families with both PPCM and DCM. In 10/18 tested families, potential underlying mutations (4 pathogenic mutations (22%) and 6 variants of unknown clinical significance that are likely to be pathogenic (33%)) were identified: 7 in *TTN* (39%), 1 in *BAG3*, 1 in *TNNC1*, and 1 in *MYH7*. Measurements of passive force in single cardiomyocytes and titin isoform composition potentially support an upgrade of one of the variants of unknown clinical significance in *TTN* to a pathogenic mutation. Only 2/20 PPCM cases in these families showed recovery of left ventricular function. This is low compared to reported recovery rates of around 25 to 50% in PPCM groups not selected for familial cases. Thus, presence of an underlying mutation or positive family history for cardiomyopathy in a PPCM patient may be a prognostic factor for low recovery rate.

Chapter 8 presents a review of the literature about pregnancy in women with inherited cardiomyopathies. Pregnancy is generally well tolerated in asymptomatic patients with inherited cardiomyopathies. However, worsening of the clinical condition can occur during pregnancy, despite intensive medical treatment. If prior cardiac events, poor functional class (New York Heart Association class III or IV), or advanced left ventricular systolic dysfunction are present, the risk of maternal cardiac complications during pregnancy is markedly increased. The postpartum condition is generally no worse than the antepartum condition, but no long-term follow-up studies have been reported. Preconception evaluation and (genetic) counseling are important aspects of managing women with inherited cardiomyopathies.

Chapters 9 and **10** describe that a genetic/familial predisposition for DCM can be a risk factor for anthracycline-associated cardiomyopathy (AACM). Five DCM families with one AACM patient, and one AACM patient with a family member with a previously unrecognized, possible early sign of mild DCM were identified. Moreover, in two of these six families we confirmed the genetic character of the disease by identifying pathogenic *MYH7* mutations. Prior to treatment of a cancer patient with anthracyclines, careful evaluation of his/her familial history for cardiomyopathy is advisable. In those cases with a family history of DCM/ heart failure, one should be vigilant for increased susceptibility to AACM and one may consider more intensive cardiovascular monitoring before and during cancer treatment or alternative non-cardiotoxic cancer therapy.

FUTURE PERSPECTIVES

Remarkable progress has been made in understanding the genetic basis of cardiomyopathies. Since the 1990s, many genes involved in the development of cardiomyopathies have been identified with the classical candidate gene approach. Inherited cardiomyopathies were presented as traditional Mendelian single-gene diseases, but the clinical variability and incomplete disease penetrance point towards more complex scenarios. Genomic variation (i.e. genetic modifiers), epigenetic, and environmental factors may also play a role in the clinical variability.
New genomic techniques, like next-generation sequencing, now make it possible to analyze larger numbers of genes, even the entire exome or genome, quickly and at reasonable cost. This will not only lead to a rapid increase in knowledge about the frequency of mutations in known cardiomyopathy-related genes, but also provide the opportunity to identify novel cardiomyopathy-related genes, and to unravel the complex genetics in cardiomyopathies, like digenic/oligogenic inheritance or genetic modifiers. However, the accurate interpretation of the results poses an enormous challenge for the future. It is crucial to determine the clinical and functional effect of sequence variants identified. For this purpose, it remains critical to study co-segregation in families with carriers of such variations. But clinical genetics studies are insufficient to provide answers in the majority of cases, especially due to the rarity of most variants involved in cardiomyopathies, and due to the lack of relatives available for co-segregation. Various mutation characteristics can help to establish the likelihood of pathogenicity, but no method based on these characteristics is completely reliable. Functional expression studies can be helpful, but are impractical and have huge limitations. They are costly and time-consuming, which makes it impossible to study all the genetic variation. Even when studying a certain variant, it is not necessarily possible to extrapolate the knowledge of functional studies in cells or animals to a complex human being. Moreover, heart tissue is only available for a small subset of mutation carriers (from endomyocardial biopsies, heart transplantations or post-mortem surveys). An alternative strategy, without this limitation, is to study cardiomyocytes derived from induced-pluripotent stem (IPS) cells, generated from fibroblasts of cardiomyopathy patients. This may lead to prognostic information for these particular patients.
More extensive genomic studies in much larger cohorts of rigorously phenotyped probands and family members are needed to improve our understanding of the genomic basis of cardiomyopathies. Further understanding of the pathomechanisms, and a better understanding of the etiological factors that play a role in individual patients, may lead to better treatment modalities and personalized medicine in the next few decennia. This work will be complicated by the heterogenic etiology of inherited cardiomyopathies, and by the complex interactions suspected between genetic and environmental factors. Special focus on targets for preventive strategies for asymptomatic mutation carriers is important. The Netherlands is exceptionally well-suited for these types of extensive cardiogenetic studies. In the Netherlands, the level and organization of patient care in cardiogenetics is outstanding. There are multiple multidisci-

plinary cardiogenetic outpatient clinics with a good geographic spread, where integrated teams of cardiologists and clinical geneticists and molecular geneticists consult patients and their families from the whole country. By paying special attention to the collection and sharing of information we create the opportunity to obtain a nationwide cohort of rigorously phenotyped probands and family members. This also helps in leveraging the knowledge of the discovered founder mutations to further study the phenotypes related to these mutations and the subsequent elucidation of modifying factors underlying the clinical variability.

Increasing our knowledge on gene-environment interactions is critically important for clinical practice. This thesis describes two examples of highly penetrant genetic factors (i.e. causing primary genetic cardiomyopathies) influenced by environmental factors (pregnancy and chemotherapy). If we also consider less penetrant genetic factors, we can speculate that these play a role in pathomechanisms of numerous, higher prevalent cardiovascular diseases. For example, less penetrant genetic factors may modify cardiac remodeling after myocardial infarction, or right ventricular failure in primary arterial hypertension, or development of tachycardiomyopathy in case of tachycardia, or development of heart failure with preserved ejection fraction in case of hypertension.

So the question remains: to what extent will the fast expanding genetic knowledge influence prevention and patient care in cardiomyopathies? A clearly pathogenic mutation has high diagnostic value and can subsequently be used for genetic cascade screening in relatives, but the prognostic utility is low and there are no preventive modalities for mutation carriers who have not yet developed a phenotype. The interpretation of genetic results from novel genomic techniques is an enormous challenge for the near future and the full benefits of genetic testing remain to be realized.