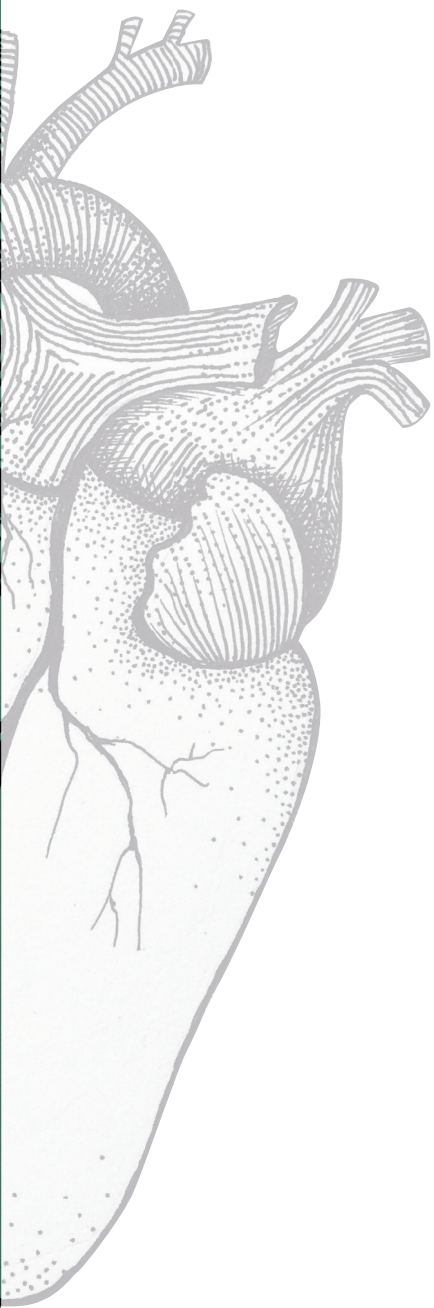

# General discussion

CHAPTER 13

Since the early 1990s, extraordinary progress has been made in understanding the genetic basis of inherited cardiomyopathies enabling clinicians to perform genetic screening of the index patients and cascade screening in their families. More recently, the clinical implementation of next-generation sequencing techniques brought both the advantages and challenges of massive parallel sequencing of DNA to everyday practice. Indeed, the interpretation and implications of the sequencing results towards the patient and family poses a huge challenge to attending physicians. In this light, it is ever more crucial to exactly phenotype and study in depth the morphological and clinical aspects of the disease.

## Morphological aspects

For ACM, the first detailed pathological description dates back to 18th century when René Laennec published his book 'De l'auscultation me 'diate ou traite ' du diagnostic des maladies des poumons et du coeur' in 1819.[1] In chapter XV on the accumulation of fat in the heart, he wrote 'In medical writing we find many examples of the heart being overloaded with fat [. . .] and even the sudden death [. . .] The fatter the heart is, the thinner [. . .] are its walls. Sometimes these are extremely thin, being reduced almost to nothing, especially at the apex of the heart and the posterior side of the right ventricle. [. . .] On examining ventricles [. . .] the scalpel seems to reach the cavity without encountering almost any muscular substance [. . .]'. The first systematic description of morphologic abnormalities of ACM was by Thiene et al.[2] investigating a series of young sudden deaths victims in the Veneto-region of Italy, where they recognized the disease as a major cause of cardiac arrest in the young with a particularly high occurrence in athletes.

In this thesis, we studied autopsy and explant heart specimen of PLN p.Arg14del mutation carriers with an ACM phenotype, in a similar way. We hypothesized, based on previous electrocardiographical and experimental findings, that extensive myocardial fibrosis is present in both ventricles of mutation carriers and fibrosis to be a substrate for the ventricular arrhythmia and the progressive development of abnormalities in cardiac function. Macro- and microscopically we observed a biventricular phenotype with fibrofatty replacement, mainly in the RV wall, and the presence of fibrosis, mainly in the LV left posterior wall. The observed fibrofatty replacement did not differ from that described in ACM due to mutations in desmosomal genes.[3,4] Even in hearts in which the fibrofatty replacement was not obvious to the naked eye and only focally detectable by microscopy, we observed the distinct morphologic characteristics of ACM. In trichrome-stained tissue sections, there were foci with atrophic and hypertrophic cardiomyocytes, embedded in collagen and surrounded by fat cells.

## Distinct molecular signature

We found phospholamban p.Arg14del cardiomyopathy to have a distinct molecular signature in comparison to desmosomal ACM. Plakoglobin redistribution, reduced SAP97 and abnormal GSK3β immunoreactive signal at myocyte intercalated disks, findings consistently seen in desmosomal ACM,[5-7] were observed only in the minority of PLN p.Arg14del cardiomyopathy cases. This is in line with previous observations in a similar biventricular ACM phenotype, caused by a desmoplakin truncating variant, where also no plakoglobin redistribution was observed. This suggests that different signalling pathways are involved and therefore a different molecular

signature is observed in classical right-dominant ACM and biventricular forms.

The precise pathophysiological mechanism of how the PLN p.Arg14del mutation leads to cardiac fibrosis and adiposis still needs to be elucidated. At the molecular level, coexpression of the normal and mutant PLN in HEK-293 cells results in reduced SERCA2a activity, an effect that leads to disturbed calcium metabolism of myocytes and cardiac dysfunction.[8] In addition, overexpression of the PLN p.Arg14del mutation in transgenic mice causes extensive myocardial fibrosis[8] and a dramatic increase in size of mouse hearts relative to PLN- wildtype controls.[9] A tentative mechanism for the observed fibrofatty replacement and the subsequent clinical phenotype is the mechanical and electrical uncoupling of ventricular cardiomyocytes because of desmosomal instability. Desmosomes are proteins in the intercalated disk that connect adjacent cardiomyocytes, thereby providing mechanical integrity and electrical stability. Calcium homeostasis plays a critical role in maintaining desmosomal integrity.[10] It is thus conceivable that the PLN p.Arg14del mutation causes cardiac desmosomal disintegration due to disturbed calcium handling. This is supported by the finding that plakoglobin is absent or diminished at intercalated disks in the majority of PLN p.Arg14del cardiomyopathy cases fulfilling ARVC criteria.[11]

Furthermore, PLN p.Arg14del cardiomyopathy is characterized by large perinuclear PLN aggregates, aggresomes and autophagic degradation. These are detectable in complete heart specimens and myocardial tissue samples obtained from the apex of the left ventricle (LV) harvested during left ventricular assist device (LVAD) implantation but rarely in right ventricular endomyocardial biopsy samples. In clinical practice, PLN IHC analysis of LVAD specimens can be of incremental value in the diagnostic workup of this cardiomyopathy, even more so if genetic analysis is not readily available. We plan to further analyze the composition of the observed pathological protein aggregates. These results will hopefully contribute to a better understanding of the pathophysiological mechanisms involved and possibly lead to the development of therapeutic targets.

### Fibrosis localisation: clinical utility roadmap

The presence of myocardial fibrosis was found to be most pronounced subepicardially, as is seen more often in other nonischemic cardiomyopathies. This pattern contrasts with ischemic heart disease, where there is coronary artery-related distribution and where the subendocardial layer is usually involved with or without transmural extension.[12,13] In human ACM, it is well known that cardiomyocytolysis and fibrofatty replacement progress from subepicardial and midmyocardial layers towards the endocardium.[2,14] Experimental animal models have also shown that the disease process in ACM starts on the epicardial side and extends as a wave-front from the epicardium towards the endocardium.[15] There is as yet no clear explanation for this apparent predilection of cardiomyopathic changes in the subepicardium but in PLN p.Arg14del-related cardiomyopathy these histopathological changes may well be related to the disturbed interaction between mutated PLN and SERCA2a and differences in the regional expression of these proteins in the human heart. SERCA2a expression is higher in epicardial myocytes than in endocardial myocytes, whereas there appears to be no regional differences in PLN expression.[16,17] The transmural heterogeneity of SERCA2a expression and function is considered to be one of the factors underlying the variations in excitation-contraction coupling across the ventricular

wall, factors responsible for the 20-30 milliseconds delay in the onset of contraction of epicardial myocytes during every normal single heartbeat.

With regard to regional differences in the presence of fibrosis, the inferolateral wall of the LV was found to be mostly affected. Indeed, also in our cardiac magnetic resonance (CMR) imaging study we observed that segments 5 and 11, corresponding with the LV inferolateral wall, were most profoundly affected. Most probably the higher vulnerability of the inferolateral LV free wall, and also the RV, to mechanical wall stress plays a role in this distribution[18,19], in combination with regional molecular changes caused by the mutation. In a recent pathology study, the observed fibrosis pattern was found to be distinctive for PLN p.Arg14del cardiomyopathy in comparison with other hereditary cardiomyopathies.[20] In a new CMR imaging study comprising a broader group of genotyped inherited cardiomyopathies we, together with the Academic Medical Center in Amsterdam, will further investigate these differences in fibrosis patterns. Furthermore, we plan to further investigate the correlation between radiology (CMR) and histology findings in ACM subtypes.

### Early myocardial fibrosis: canary in a coalmine?

Beside the distribution, the timing of occurrence of myocardial fibrosis in PLN p.Arg14del mutation carriers also seems to be distinctive: in previous studies low voltage and repolarization changes on the surface ECG, including the left lateral leads, were shown to be early hallmarks of PLN p.Arg14del cardiomyopathy.[11,21] It was postulated that these features are a reflection of fibrosis but this remained to be proven.

In our multicenter CMR imaging cohort, consisting of mainly presymptomatic mutation carriers, we indeed found myocardial fibrosis to be present in a large subgroup. Index patients showed more extensive structural and functional evidence of disease but fibrosis was also seen in many subjects with a preserved LV systolic function, which provides more evidence that the development of fibrosis occurs as an early phenomenon in PLN p.Arg14del mutation carriers.

Moreover, we found in a subgroup of presymptomatic PLN p.Arg14del mutation carriers early biventricular abnormalities using echocardiography: subtle but significant structural remodeling as well as loss of LV diastolic early identification of these features, and therefore of disease development and progression sometimes even before the onset of symptoms, may guide early therapeutic intervention and lifestyle adjustments (i.e. refraining from strenuous sports activity). This subsequently might prevent sudden cardiac death as a result of malignant ventricular arrhythmias and/or slow down progression of heart failure.

In a previous study by our group, it was shown that the occurrence of (non-)sustained VA and an LVEF of less than 45% (rather than 35%)[22,23] is an independent risk factor for VA.[24] In this thesis, we refined this finding by showing that LV-LGE on CMR is an even stronger risk factor than LVEF. In fact, even in the setting of preserved LVEF, the mere presence of LV-LGE is associated with a higher risk of VA in PLN p.Arg14del mutation carriers. This is in line with previous studies showing LV-LGE on CMR imaging is an extra independent risk factor in selected groups of DCM-patients.[25-29] Evenmore, we observed a clear trend towards incremental value of LV-LGE in prognostication of PLN p.Arg14del mutation carriers in a follow-up study of the CMR cohort. The predictive value of LV-LGE is of particular clinical interest in the subgroup with a preserved LVEF.

We strongly believe LV-LGE will be prove to be an important independent risk factor that can be used in daily clinical practice for treatment decisions. But to show this a longer follow-up period is required. These combined data support the early use of CMR with contrast-enhancement and echocardiography in this patient group, and should include the presymptomatic carriers. We will also implement these findings in the analysis of the iPHORECAST.

### Preventive treatment: iPHORECAST

The iPHORECAST (intervention in PHOspholamban RElated CArdiomyopathy Study) is based on the observation of early presence of myocardial fibrosis in presymptomatic p.Arg14del mutation carriers. We hope to show that eplerenone, with its established antifibrotic effects, can reduce disease progression and postpone the onset of overt disease, comparable to a previous non-randomized trial with diltiazem in hypertrophic cardiomyopathy.[30] The inclusion of the first participant was May 13, 2014. A total of 82 participants were included in the 4 participating centers. Because of slow recruitment it was decided on April 1, 2017, to stop further inclusion of participants but to continue and complete the study with the included cohort. The follow-up, and collection of the follow-up data, is ongoing and will be finished around January 2021. By then we can evaluate the efficacy of pre- emptive eplerenone treatment in presymptomatic carriers. In the meantime we will evaluate baseline parameters and biomarkers, including biomarkers of fibrosis (e.g. N-terminal propeptide of procollagen type I (PINP), and N-terminal propeptide of collagen type III (PIIINP), to analyze their incremental value for the prediction of disease progression and cardiac events.

### A distinct biventricular disease entity within the ACM spectrum

More knowledge regarding the distinct features of PLN p.Arg14del cardiomyopathy may help us with the diagnosis, optimization of treatment and the understanding of the underlying pathophysiological mechanisms and natural course of this disease. The combination of (histo) pathological, immunohistochemical, clinical and genetic findings in this thesis provide evidence for this disease to be a distinct biventricular disease entity within the ACM spectrum. Left ventricular involvement is typical in PLN p.Arg14del cardiomyopathy.[20,31,32] Many symptomatic PLN p.Arg14del carriers have overlapping phenotypes and may fulfil international DCM criteria[33] and/or ARVC revised task force criteria.[34] Task force criteria are only available for classical right-dominant ACM[34], but are less suitable for left-dominant or biventricular subtypes.[35] Morphologically, heart specimens of PLN p.Arg14del mutation carriers show features of both ACM and DCM. This illustrates the overlap between different cardiomyopathy subtypes, i.e. ACM and DCM, and challenges the strict distinction made between them as two separate entities. Even more, this underscores the importance of a molecular diagnosis in patients with inherited cardiomyopathy. The distinct features of PLN p.Arg14del cardiomyopathy support the use of a descriptive classification system, i.e. MOGE(S) classification[36], while waiting for further knowledge that may eventually support a genetic classification of cardiomyopathies, i.e. the ultimate intent of the AHA- and ESC classifications.

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