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Understanding cardiac electrical phenotypes in the genomic era
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Chapter 1

Introduction
Introduction: concerning Sudden Cardiac Death

Sudden cardiac death (SCD) is defined as unexpected death due to a cardiac cause. It most often results from life-threatening ventricular fibrillation (VF) and ranks among the most common causes of death worldwide, with an incidence in the community varying between 0.6 and >1.4 per 1,000 individuals\(^1\). Because SCD mostly occurs in individuals without previously known cardiac disease, the identification of patients at risk for SCD and implementation of preventive measures could save many lives\(^2\). The etiology of SCD is complex and susceptibility to SCD is likely governed by a combination of inherited and environmental factors\(^3\).

SCD may strike at any age. In the young it mainly occurs in the setting of rare inherited arrhythmia syndromes, which typically display a Mendelian pattern of inheritance with a clear transmission from one generation to the next. They have been classically grouped into the cardiomyopathies\(^3\) and the primary electrical disorders\(^4\). In the latter category, which includes amongst others, cardiac conduction disease, sinus bradycardia and idiopathic ventricular fibrillation (studied in this thesis), arrhythmias stem from cardiac electrical abnormalities in the setting of a structurally normal heart. Genetic studies have shown that this group of disorders are primarily caused by mutations in genes encoding pore-forming subunits of cardiac ion channels or subunits that modulate ion channel expression or function (e.g. \(HCN4\) and \(DPP6\), both studied in this thesis; Figure 1). In contrast, in the cardiomyopathies, the arrhythmogenic substrate is thought to lie in the

![Cartoon of a cardiomyocyte. Some of the proteins encoded by genes studied in this thesis are presented in colour (adapted from\(^{16}\)).](image)

Figure 1

\(^1\) Source 1
\(^2\) Source 2
\(^3\) Source 3
\(^4\) Source 4
abnormal structure of the heart. The cardiomyopathies are subdivided into hypertrophic cardiomyopathy (HCM) which has been associated with mutations in genes encoding sarcomeric proteins, dilated cardiomyopathy (DCM) associated with mutations in genes encoding a broad category of proteins including amongst others cytoskeletal proteins and nuclear membrane proteins, and arrhythmogenic right ventricular cardiomyopathy (ARVC) which has been linked to genes encoding desmosomal proteins. An increasingly recognized cardiomyopathy is left ventricular noncompaction (LVNC, studied in this thesis; Figure 2) which has been mainly associated with genes encoding sarcomeric proteins.

In the older segment of the population, SCD typically arises as a consequence of coronary artery disease. While a heritable component has been demonstrated as a contributor to SCD risk in this setting, the underlying genetic factors are yet largely unexplored.

Figure 2 The HCN4 channel (top part of figure) showing the 3 mutations identified in this thesis in patients with left ventricular noncompaction cardiomyopathy (LVNC, bottom right part of figure). The morphology of the sino-atrial node action potential that is expected to result as a consequence of the HCN4 mutations is depicted in the bottom left panel of the figure. The mechanism underlying the LVNC remains unknown. We hypothesize that LVNC as a result of mutation in the HCN4 gene may arise from a congenital signalling defect or could occur as a consequence of cardiac remodelling in response to the lower heart rate.
Outline of the thesis

The aim of this thesis was to identify novel genes involved in cardiac electrical function and genetic abnormalities causing rare inherited arrhythmia syndromes.

As the ECG registers cardiac electrical activity, heart rate and ECG parameters of conduction (PR-interval, QRS-duration) and repolarization (QTc-interval), are considered ‘intermediate phenotypes’ of arrhythmia and SCD. While certainly intuitive, this is supported by the fact that abnormalities in these parameters are associated with predisposition to arrhythmia in the primary electrical disorders. Furthermore this is also supported by studies in the general population and in patients with specific cardiac diseases, that have shown that prolongation of ECG indices could be a risk factor for SCD 8,9. Therefore understanding the genetic basis of these traits can provide an increased understanding of the molecular underpinnings of cardiac electrical function and pathways that may be involved in arrhythmia.

The PR-interval represents the time between the beginning of activation of the atria and the beginning of activation of the ventricles. Prolonged PR-interval on the ECG characterizes atrioventricular (AV) conduction disease. Furthermore, a prolonged PR-interval has been established as a predictor of atrial fibrillation (AF), the most prevalent sustained arrhythmia10. While the heritability of the PR-interval duration is clearly established11, the genes regulating the PR-interval are largely unknown. Chapter 2 of this thesis focuses on dissecting a quantitative trait locus (QTL) previously linked by our group to the PR-interval in mice12. We conducted a series of studies, including studies in congenic mice and mice overexpressing TNNI3K, leading to the unequivocal identification of Tnni3k as the causal gene within the locus.

To understand the role of TNNI3K in human disease, in Chapter 3 we assessed the occurrence of TNNI3K mutations in a set of unrelated patients with cardiac conduction disease. In Chapter 4 we reviewed the current literature on the role of Tnni3k in cardiac disease and the prospects for therapy through inhibition of this cardiac specific kinase. This chapter highlights the quantitative trait locus mapping studies conducted in several mouse crosses that besides regulation of PR-interval have implicated Tnni3k in cardiomyopathy and heart failure13, most recently in ischemia-reperfusion injury 14, which occurs after restoration of blood flow after myocardial infarction.

Over the last decades the opportunities for human genetics discoveries have been broadened by the emergence of high-throughput DNA sequencing technologies, commonly referred to as next generation sequencing (NGS). Chapters 5 and 6
employ this relatively new technology to unravel the genetic basis of two rare inherited arrhythmias. **Chapter 5** entails genetic studies on four families presenting with sinus bradycardia in combination with left ventricular noncompaction cardiomyopathy (**Figure 2**). Linkage analysis followed by whole exome sequencing in the first family lead to the identification of an $HCN4$ mutation as the likely cause of the disease. The link between $HCN4$ and this overlap phenotype was corroborated by the identification of $HCN4$ mutations in three other families with the same combined phenotype. This is the first time that mutations in $HCN4$ have been associated with cardiac structural abnormalities. Although we postulate on possible mechanisms underlying the structural abnormalities as a result of mutations in $HCN4$ (**Figure 2**), the exact mechanism remains as yet unknown.

Idiopathic ventricular fibrillation (IVF), characterized by ventricular fibrillation in the setting of a structurally normal heart and normal surface ECG, is regarded as a heritable arrhythmia syndrome. In **Chapter 7** we aimed to identify the causal genetic defect within a founder haplotype that was previously identified by our group in multiple Dutch families affected by IVF\(^1\). In the absence of clinical disease features besides VF, genetic testing for this haplotype is the only diagnostic tool in affected families. The founder haplotype spanned 1.5 Mb on chromosome 7, and encompassed the upstream region of $DPP6$ (encoding dipeptidil-amino transferase 6)\(^1\). In Chapter 7, thanks to fine mapping by means of genotyping in newly identified carriers with VF, the risk haplotype was reduced to an interval of around 500kb, which still contains (part of) $DPP6$ as the only annotated gene. Through targeted NGS of the genomic region spanning the haplotype in three carriers and three non-carrier family members we identified 4 non-coding variants, among which is likely the causal variant.

**Chapter 8** entails a study aimed at investigating the contribution of rare genetic variants to the risk of SCD at the community level. In particular we focused our efforts on rare variants selected based on their recurrence (indicative of a founder effect) in DNA diagnostic testing of patients with inherited cardiac disorders at cardiogenetics clinics in the Netherlands. We screened an unbiased cohort of 1440 unselected patients presenting with out-of-hospital cardiac arrest for the presence of six Dutch founder mutations associated with either HCM, ARVC, an overlap phenotype of ARVC and DCM or IVF. Our findings support the notion that rare genetic variants contribute to sudden cardiac arrest (SCA) in the community.
Chapter 1

Summary

The research presented in this thesis aimed to contribute to the advancement of the collective understanding of the role of genetic factors in modulation of cardiac electrical function and arrhythmia susceptibility. It is hoped that this data will ultimately lead to improved patient health care and personalized medicine in patients with inherited cardiomyopathies or channelopathies by the integration of the gained genetic knowledge into clinical care.
Introduction

Reference


