Genetic modifiers in familial cardiac rhythm disorders
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Summary
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Sudden cardiac death (SCD) is one of the most prevalent causes of death in Western societies. It underlies 20% of total mortality, and 50% of cardiovascular mortality. In young individuals (below 40 years of age) SCD often occurs in the setting of disorders displaying Mendelian inheritance, with the cardiomyopathies (chapter 3) and primary electrical disorders (chapters 4 - 6) being the most prevalent. Here, the inheritance of very rare genetic variants with large effects potentially increases risk for SCD substantially. The primary electrical disorders have been linked primarily to mutations in genes encoding ion channel subunits or their interacting proteins (Figure 1 chapter 1). On the other hand, the cardiomyopathies are caused by mutations affecting genes coding for the contractile apparatus and structural components of the cardiomyocyte such as the sarcomere and desmosomes.

Genotype-phenotype studies in these disorders have clearly established that they are not spared from the phenomena of reduced penetrance and variable expression typical of Mendelian diseases. For instance, in the primary arrhythmia syndromes, extensive variability in clinical manifestations is often observed among family members carrying an identical ion channel gene mutation, with some individuals exhibiting overt abnormalities on the electrocardiogram (ECG) and suffering potentially fatal arrhythmias, whereas others do not display any ECG changes and do not develop rhythm disturbances throughout life. Probands and families with these Mendelian disorders, harboring known disease-causing mutations, likely provide a permissive, genetically sensitized setting for the identification of novel genes and pathways modulating cardiac (electrical) function.

In this thesis we employ the phenotypic variability evidenced among probands and their relatives with Mendelian cardiac disorders to identify genetic modifiers of disease expression. We focused on two distinct groups of disorders associated with increased risk of SCD, namely the primary electrical disorders (Long QT Syndrome, Brugada Syndrome, Conduction Disease) and hypertrophic cardiomyopathy (HCM). The aim of this thesis was to identify such genetic modifiers using both linkage and (family based) association analyses. A candidate SNP / gene approach as well as a genome-wide unbiased approach were used in the study of common genetic variants as possible modifiers of disease severity.

In chapter 2, we reviewed the available literature on the genetic and allelic architecture of SCD. In this review we focused on the common genetic variation that has been recently identified through genome-wide association studies to modulate risk of SCD and to modulate heart rate and ECG indices of conduction (PR-interval, QRS-duration).
and repolarization (QTc-interval) as intermediate phenotypes of SCD. Several studies reported that a family history of SCD increases an individual’s risk for SCD giving evidence for a heritable component. In the general population however, the genetic and allelic architecture remains largely unknown.

In patients with hypertrophic cardiomyopathy (HCM) the occurrence of phenotypic variability even in the presence of an identical pathogenic mutations suggests a role for genetic modifiers (chapter 3). The renin-angiotensin-aldosterone system (RAAS) plays a regulatory role in cardiac function, blood pressure and electrolyte homeostasis making it an interesting candidate system that could modify phenotypic expression in HCM patients. Five Single nucleotide polymorphisms (SNPs) in this system were previously suggested to modify the extent of hypertrophy in HCM patients. In order to investigate the effect of these SNPs, we selected a large cohort of carriers of one of 3 functionally-equivalent truncating mutations in the MYBPC3 gene. We used family based association to analyze the effect of these five RAAS polymorphisms (ACE, rs4646994; AGTR1, rs5186; CMA, rs1800875; AGT, rs699; CYP11B2, rs1799998) on interventricular septum (IVS) thickness and the Wigle score. We detected two modest associations. Carriers of the CC genotype in the AGT gene had less pronounced IVS thickness compared to CT and TT genotype carriers. The DD polymorphism in the ACE gene was associated with a high Wigle score (p=0.01). In our large study population of HCM patients with functionally-equivalent mutations in the MYBPC3 gene we did not find major effects of genetic variation within genes of the RAAS system on phenotypic expression of HCM or the previously described association between the pro-LVH score and IVS thickness/Wigle score.

In chapter 4 we studied a large set of individuals (probands and, their family-members where available) carrying a mutation in the KCNH2 gene and presenting clinically with Long QT syndrome type 2 (LQT2). LQT2 is a cardiac repolarization disorder that is caused by mutations in the KCNH2 gene which encodes kv11.1 (HERG) underlying the I_{kr} repolarizing K^+ current. Although mutation type and location are known to impact on the severity of clinical manifestations, the occurrence of phenotypic variability among patients harboring the same mutation points to the presence of additional modulatory genetic factors. Here we comprehensively investigated the effect of 1201 haplotype-tagging SNPs in and around 18 candidate genes on the QTc-interval in 438 patients with LQT2. We performed family based association analysis, taking the effect of KCNH2 mutation type and location into account in our analysis for modifiers of QTc-interval. Two SNPs passed the Bonferroni-corrected significance threshold for association (p<4.16×10^{-5}). rs16847548 located immediately 5’upstream of the NOS1AP gene, and rs956642, located in the vicinity of KCNH2, were associated with the QTc-interval. Of these, rs956642 was also found to be associated with cardiac events (p=0.02). Two other SNPs in NOS1AP, rs10494366 and
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rs12567211, identified previously by GWA studies, were also significantly associated with QTc in the LQT2 patients studied (p-values, 9.96×10^{-3} and 2.24×10^{-4}, respectively). We provide the first evidence that common genetic variants at the KCNH2 locus modulate severity of clinical manifestations in the Long QT Syndrome type 2. Furthermore, we extend previous observations that common genetic variants in NOS1AP modulate the extent of QTc-prolongation in this disorder.

In the last two chapters we studied a large Dutch kindred with the SCN5A mutation 1795insD. An extensive genealogical search allowed us to trace this family back to the eighteenth century, enabling the construction of a highly extended pedigree. Individuals in this kindred present with manifestations of Long QT syndrome, Brugada syndrome and progressive conduction disease occurring either in isolation or in combinations thereof.

In chapter 5, we performed linkage and association analysis with heart rate and ECG indices of conduction and repolarization using 1308 haplotype-tagging SNPs in and around 18 candidate genes in 215 family members (100 carriers) of the 1795insD mutation. Both significant linkage (LOD=3.7) and association (p=9.8e-08) with PR-interval was found at the region of chromosome 21 harboring the KCNE1 and KCNE2 candidate genes. The SNP displaying the most significant association within this region (rs2834506, p=9.8e-08), was observed within intron 3 of the Rcan1 (Regulator of Calcineurin 1) gene. This association was subsequently validated in an independent set of patients harboring different mutations in SCN5A, also indicating a significant stronger effect of rs2834506 on PR-interval in the SCN5A mutation carriers compared to non-carriers. The KCNE1 and KCNE2 genes were considered unlikely modulators of PR-interval, so we sought additional evidence for a role of Rcan1 in mediating the observed effect. In Scn5a^{1798insD/+} F2 progeny of FVB/N and 129P2 mice displaying variable conduction disease severity, a significant correlation was found between ventricular Rcan1 mRNA transcript levels and PR-interval (n=56 mice; r=-0.333, p=0.012). Because Rcan1 is a regulator of the pro-hypertrophic calcineurin/Nfat-pathway, we hypothesized that the Scn5a^{1798insD/+} mutation disrupts intracellular Ca^{2+}-homeostasis, thereby setting the stage for calcineurin-activation which subsequently impacts on PR-interval. In cardiomyocytes of Scn5a^{1798insD/+} mice elevated intracellular Na^+ and diastolic Ca^{2+} levels were observed. Additionally, chronic activation of the calcineurin/Nfat pathway through application of Transverse Aortic Constriction (TAC) elicited extreme AV-dysfunction, AV-block and sudden death in Scn5a^{1798insD/+} mice, which was prevented by treatment with the Nfat-inhibitor cyclosporine-A. This evidence all pointed us to the calcineurin/Nfat pathway as a possible modifier of the PR-interval in the setting of sodium channelopathy, making it a very interesting pathways for further future studies.
Additionally in this family we performed a genome-wide association study (GWAs) (chapter 6). DNA and ECG data were available for 276 family members (120 carriers) of the SCN5A-1795insD family. We performed family based genome wide association analysis. One SNP (rs2631864) in the region of GFRα2 passed the genome-wide significance threshold with PR-interval (p-value=3.1 \times 10^{-9}). This SNP is located in an intergenic region and the closest characterized gene is GFRα2 encoding GDNF (glial cell line-derived neurotrophic factor) family receptor alpha 2. Of note, mice that are knock-out for this gene [Gfra2(-/-)] showed reduced cholinergic innervation by 40% in the ventricles and by 60% in the ventricular conduction system. This would support a role for this gene at this locus in mediation of the effect observed on PR-interval. In addition, two suggestive associated SNPs were found in LMCD1. Both SNPs are located within the LMCD1 gene encoding LIM and cysteine-rich domains protein 1. This gene, like RCAN1 plays a role in the calcineurin signaling pathway, providing further evidence in support of this pathway in modulation of cardiac conduction. Further studies into the possible role of LMCD1 in mediating the effect on the PR-interval at this locus are therefore highly warranted and are expected to shed more light on the relevance of the calcineurin/Nfat pathway in modulation of atrio-ventricular conduction in the setting of cardiac sodium channelopathy. Along this line, it will be pertinent to determine in future studies whether the effect of the LMCD1 locus is restricted to carriers of an SCN5A mutation as demonstrated for the RCAN1 locus. Several other SNPs found to be associated with heart rate and the ECG indices and multiple interaction effects were also detected, but all at a suggestive significance level. Future mechanistic work on the candidate genes found in this study will likely provide further insight into the molecular underpinnings of cardiac electrophysiological function.

In summary, in this thesis, we have focused on identifying genetic modifiers of disease in families with Mendelian cardiac disorders associated with a high risk of SCD. Our aim was to identify genetic modifiers that could explain, at least in part, the phenomena of reduced penetrance and variable disease expression observed in these families, features commonly encountered in Mendelian disorders in general. We uncovered several interesting new candidate genes and pathways associated with heart rate and ECG indices of conduction (PR-interval, QRS-duration) and repolarization (QTc-interval) that form prime candidates for functional studies in the future.