Subtle killers and sudden death: Genetic variants modulating ventricular fibrillation in the setting of myocardial infarction

Pazoki, R.

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Chapter 8

Summary and conclusions
Sudden cardiac death (SCD) is a catastrophic cardiovascular event most commonly occurring as a consequence of ventricular fibrillation (VF) in adults suffering from acute myocardial infarction (MI) or its late consequences. Several studies have demonstrated the role of family history of SCD in the determination of risk of SCD and VF\textsuperscript{1-4}; however, the genetic underpinnings of SCD and VF remain largely unknown. In this thesis, we investigated genetic determinants of VF using multiple genomic approaches including genome-wide association studies (GWAS), candidate gene approaches, pathway analysis as well as integration with expression quantitative loci (eQTL) data. These approaches were undertaken in the Arrhythmia Genetics in the NEtherlands (AGNES) study. In this final chapter, we summarize the main findings of this thesis and discuss future perspectives.

Main Findings

In the first two chapters of the thesis (Chapters 2 and 3)\textsuperscript{5,6}, we provide an overview of SCD and the current knowledge on clinical and genetic risk factors of VF/SCD. In Chapters 4-7, we provide original information about the genetic architecture of SCD in the context of the AGNES study\textsuperscript{7,8}. GWAS analyses are presented in Chapters 4 and 5. In Chapter 5, next to GWAS, we additionally present results from the complementary eQTL investigations, pathway analysis and data from a candidate gene approach assessing single nucleotide polymorphism (SNPs) previously implicated in published studies. Chapters 6 and 7 describe results from two further candidate gene approaches, including the assessment of the effects of genetic variants in aggregate by means of genetic risk score analysis, focusing on genes involved in biological pathways that may constitute intermediate phenotypes of VF.

1. Genetic predisposition to SCD: Overview

In Chapters 2 and 3, we reviewed existing literature that investigated genetic risk factors of SCD including biological mechanisms of SCD during MI, and recent knowledge about genetic predisposition to SCD. We concluded that while significant progress has been made concerning gene discovery for inherited cardiac disorders associated with SCD in the young (e.g. in the primary electrical disorders and the cardiomyopathies), progress in dissecting the genetic underpinnings of SCD risk in complex cardiac pathologies has been slow. Disorders associated with SCD in the young usually display monogenic / Mendelian inheritance, which favors gene discovery. In contrast, the genetic architecture of SCD occurring in the setting of complex cardiac pathologies such as MI is complex and multiple genetic factors are expected to conspire to determine risk. These genetic factors may occur at different frequencies in the general popula-
tion and may be frequent or rare. They are also expected to carry different effect sizes on risk of VF. Such complex genetic architecture hinders gene discovery. Moreover, genetic studies on VF in the setting of these complex cardiac pathologies are hindered by difficulties that are inherent to the ascertainment of patients experiencing VF/SCD.

2. **GWAS approach for identification of genetic loci predisposing to VF in MI**

Chapters 4 and 5 present the results of two GWAS we conducted in the AGNES study. The initial GWAS (Chapter 4) included 515 cases and 457 controls, whereas the second (extended) GWAS (Chapter 5) included 672 cases and 761 controls. The GWAS presented in Chapter 4 was the first GWAS investigating the genetic architecture of VF during MI. We identified a locus on chr.21q21 (rs2824292) at which the G-allele increases the risk of VF. This effect was replicated in an independent Dutch case-control sample (Chapter 4), while it did not replicate in small case-controls sets from Italy and Germany (Chapter 5). The closest genes to this locus are the **CXADR** gene encoding the Coxsackie and Adenovirus Receptor and the **BTG3** gene encoding B-cell translocation gene 3. The protein encoded by **CXADR** was previously implicated in virus uptake and in cardiac conduction. Our group showed that the VF risk allele at rs2824292 is associated with a decreased **CXADR** mRNA abundance in human heart. We also demonstrated that mice haplo-insufficient for **CXADR** have slowed conduction in the ventricles and greater susceptibility to arrhythmia in the setting of myocardial ischemia. This data supports a biologically plausible role of **CXADR** in predisposition to VF in the setting of a first acute MI, in spite of the fact that the locus is not uniformly associating across studies. In our view, this warrants further investigation in additional cohorts and an effort to determine the factors underlying the observed heterogeneity across studies.

The locus at rs2824292 remained associated with VF during MI in the second GWAS in the extended set of AGNES (Chapter 5). The effect of this locus and nine other single nucleotide polymorphisms (SNPs) identified in this second GWAS were tested for replication in the PREDESTINATION study, an independent case-control sample of patients with a first MI from Italy (n = 641) with inclusion criteria similar to AGNES. One locus (rs1750041) was replicated with a nominally significant P-value. After meta-analysis, two of the SNPs displaying the strongest association with VF were taken forward for replication and meta-analysis in an additional similar case-control study from Denmark (GEVAMI, n = 783). A review of online eQTL databases (Chapter 5) showed that rs1750041 was associated with the mRNA abundance of multiple genes (including the **SYNJ2** gene in which it occurs) in human heart tissue and whole blood. Our multiple independent genomic approaches in Chapter 5 converged at another promising gene: **XKR6** (encoding XK, Kell blood group complex subunit-related family, member 6). SNP rs10096381 located in **XKR6** showed the second strongest
association with VF in GWAS after the SNP at chr.21q21 and it was associated with increased levels of XKR6 mRNA. In addition, a SNP (rs6982308) located in the methionine sulfoxide reductase (MSRA) gene (identified through its eQTL effect on XKR6) was also associated with VF (Chapter 5). In spite of non-replication of these SNPs in the PREDESTINATION case-control set, this convergence on XKR6 is potentially interesting and deserves further investigation.

3. Candidate gene approach to VF in MI

Previously, Lemmert et al. demonstrated that PR interval and QRS duration are associated with risk of VF during MI\(^1\). In Chapter 6 of this thesis we investigated AGNES patients for such correlations between ECG parameters during STEMI and occurrence of VF, uncovering shorter mean RR interval and longer mean QTc-interval among patients who developed VF compared to patients who did not develop VF\(^8\). In this chapter we also investigated whether 65 candidate SNPs previously associated with heart rate and/or ECG indices of conduction and repolarization in GWAS in the general population also play a role in variation of these traits during MI. We identified eight SNPs displaying a nominal association with either heart rate, PR interval, QRS duration or QTc interval during MI. We subsequently investigated these eight SNPs for association with VF during MI and demonstrated that three (located in SCN10A, KCNQ1 and near MYH7) displayed a nominal association with VF. The effect of the MYH7 (Myosin heavy chain 7, cardiac muscle, beta) locus was modified by the culprit artery showing an association only in patients with either an occlusion in the left circumflex or right coronary artery (i.e. non-anterior wall MI). The SNP rs6795970 in the SCN10A gene, which encodes a sodium channel isoform primarily expressed in neurons, turned out to be especially interesting. This SNP was associated with PR interval and with VF in the initial AGNES set (Chapter 6), as well as with VF in the extended AGNES set (Chapter 5), and with VF after meta-analysis of AGNES with the Italian cases and controls from the PREDESTINATION study (Chapter 5). Quite intriguingly, the PR-prolonging allele of rs6795970 is protective against VF. Similarly, the PR-prolonging allele of this SNP has been demonstrated to be protective against atrial fibrillation\(^11,12\). Thus, in spite of the fact that the protective effect of the PR-prolonging allele appears counter-intuitive, consistent observations appear to be emerging relating to the modulatory effects of this SNP on cardiac arrhythmia susceptibility. SCN10A is located near the SCN5A gene, which encodes the major sodium channel isoform in heart and underlies depolarization in cardiomyocytes. Experimental studies have shown that a common variant in SCN10A, which is in high LD with rs6795970, regulates expression of the SCN5A gene in heart through disruption of a T-box (TBX5/TBX3) binding element\(^13-15\).

Another candidate SNP approach that we undertook involved pro-inflammatory SNPs. Inflammatory response increases dramatically during MI and several lines of
evidence support a role of inflammation in susceptibility to VF (Chapter 7). Therefore, in chapter 7, we selected SNPs from previous GWAS of inflammatory-biomarkers and tested their individual and aggregate effects on VF through construction of a genetic risk score (GRS). We showed that rs6901250 located in GPRC6A (G protein-coupled receptor, family C, group 6, member A), and rs4420638 located in the vicinity of the apolipoprotein C-I (APOC1) gene were associated with VF. While the GRS was associated with VF at the nominal statistical significance level, it only explained a marginal part of the variation in VF risk. This data nevertheless, points to a possible role of genetic variation in the inflammation system on predisposition to VF.

Future Perspective

Using various approaches, this thesis uncovered a number of common genetic variants that may be involved in risk of VF during MI. While this data is interesting and has already instigated functional studies aimed at understanding molecular pathways involved in the predisposition to VF, these findings await further validation in additional patients. Furthermore, the number of variants identified and the variance in VF susceptibility that is explained by them is very limited, precluding their imminent clinical utility in VF prediction. The identification of additional common genetic variants will require larger patient sets, justifying ongoing efforts to enlarge the AGNES patient set and similar sets.

The AGNES study was designed to specifically include patients presenting with VF in the setting of a first ST-segment elevation MI. The rationale behind this study design was that including one specific (homogeneous) phenotype would increase statistical power favoring gene discovery. However, one should realize that sub-groups can still be distinguished within AGNES. For instance analysis could be conducted in sub-groups defined by culprit artery or time-to-VF, as one could hypothesize those VF mechanisms may differ among these subgroups. This again argues in favor of expanding the patient sets for such studies. Efforts should, therefore, be made to increase the sample size through world-wide collaborations between studies to achieve sufficiently large and homogeneous patient (sub) groups. Variants identified could, subsequently, be tested in populations with SCD occurring in the setting of other cardiac pathologies to investigate the generalizability of the effects outside the specific cardiac pathology of first MI.

Larger patient sets will additionally provide in the future the opportunity to start investigating the role of rare genetic variants that presumably carry a larger effect on risk. By virtue of their rarity the investigation of these variants will also necessitate large sets of uniformly phenotyped patients.
In conclusion, this thesis has set the first steps towards the dissection of the genetic underpinnings of VF risk in the context of MI, providing the first insights and highlighting the challenges involved.
References


