Chapter 6
Summary and conclusion
Chapter 6.0

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The incidence of sudden cardiac death (SCD) is about 1 in 10,000 individuals each year (1–3), and the majority of SCD is caused by coronary artery disease (4). Patients with an myocardial infarction have up to 30% risk of dying suddenly in the acute phase of their myocardial infarction. In more than half of cases of SCD, coronary heart disease has not previously been clinically recognized and SCD occurs as its first symptom (5), i.e. ventricular fibrillation (VF) in the setting of acute ischemia without apparent evidence of structural heart disease (6,7). High risk groups have been defined, but they account for only a small absolute number of primary VF cases. Instead, the large majority of VF occurs in persons who were not identified to have an increased risk. Therefore, when we want to prevent SCD, we need a better understanding of risk factors for SCD in this apparently healthy population.

It is unmistakable that SCD is a multifactorial disease. Although coronary artery disease is the most dominant cause, a genetic susceptibility not specifically related to coronary artery disease has also been suggested. For example, previous studies uncovered that people who die suddenly had more parental sudden death than control populations. In the Paris Prospective Study a large group of men employed by the city of Paris was followed for an average of 23 years. Parental sudden death was found to be independently related to the occurrence of sudden death (8). Similar findings were derived from a case-control study by Friedlander et al. who studied a selection of out-of-hospital primary cardiac arrest cases in King County, Washington, in comparison with healthy controls (9). In these studies however it was difficult to differentiate between risk factor for ischemia and risk factors for ventricular fibrillation during ischemia, as the control group consisted of a selection of cases from the general population. Furthermore, these sudden death cases died from a variety of pathophysiologic causes. To be able to better define who actually is at risk of developing VF during ischemia, the AGNES (Arrhythmia Genetics NetherlandS) study was started in 2000 and included well defined groups of patients. In AGNES, patients with their first acute ST elevation myocardial infarction (STEMI) with VF are compared to patients with their first STEMI without VF (10). This setup enabled us for, the first time, to meticulously differentiate between risk factors for STEMI and risk factors for the development of VF in the acute phase of STEMI. The first results of the AGNES study confirmed that a family history of sudden death is a risk factor for VF during STEMI and, in addition, that also the amount of ST segment deviation on the presenting ECG is associated with the risk of VF during STEMI. In this thesis, we present further work on a better understanding of early ischemic VF, largely derived from ongoing recruitment in the AGNES study and its substudies.
Chapter 2.1
Chapter 2.1 acts as a general introduction to the understanding of the genetics of arrhythmias. The understanding of the role of genetics in the occurrence of arrhythmias is largely derived from studies into hereditary primary arrhythmia syndromes. Research in these syndromes has led to a huge advancement in our understanding of the different pathophysiologic mechanisms that can lead to life-threatening arrhythmias. Some of the genes that are involved in primary arrhythmia syndromes might play a role in SCD risk in the older population as well. However, early investigations into the genetics of SCD in the older population did not provide much insight while being performed in an era of limited possibilities. Since this review the number of genetic variants being associated with these primary arrhythmia syndromes has almost doubled. This provides a promise for the (near) future that also in the older population with ischemic heart disease we will be able to even further delineate the genetic underpinning of arrhythmias.

Chapter 2.2
In chapter 2.2 we present the first genome-wide association study for early ischemic ventricular fibrillation. This study was performed on DNA of AGNES patients (972 STEMI patients, 515 with VF and 457 without). The most significant association to ventricular fibrillation was found at 21q21 (rs2824292, odds ratio = 1.78, 95% CI 1.47–2.13, P = 3.3 × 10−10). The association of rs2824292 with ventricular fibrillation was replicated in an independent case-control set consisting of 146 out-of-hospital cardiac arrest individuals with myocardial infarction complicated by ventricular fibrillation and 391 individuals who survived a myocardial infarction (controls) (odds ratio = 1.49, 95% CI 1.14–1.95, P = 0.004). The closest gene to this SNP is CXADR, which encodes a viral receptor previously implicated in myocarditis and dilated cardiomyopathy and which has recently been identified as a modulator of cardiac conduction.

Chapter 2.3
In chapter 2.3 we extend our knowledge of the differences found in the early ischemic ECG between cases and controls. This was achieved by investigating whether SNPs known to modulate RR interval, PR interval, QRS duration or QTc in the general population also impact on the respective ECG indices during STEMI and on the risk of VF. We observed that VF cases had a shorter RR and a longer QTc interval as compared to non-VF controls. Eight SNPs showed a trend for association with the respective STEMI ECG indices. Of these, three were also suggestively associated with VF. Although the effects of the SNPs on ECG indices during an acute STEMI seemed
to be similar in magnitude and direction as those found in the general population, the effects, at least in isolation, are too small to explain the differences in ECGs between cases and controls and to determine risk of VF.

Chapter 3
In chapter 3 we investigate a singular risk factor in occurrence of SCD: kidney function. Although end-stage renal disease is known to elevate the risk of sudden cardiac death, the role of less severe renal impairment in SCD is unclear. In this study we examined the association between mild-to-moderate renal impairment and first ischemic ventricular fibrillation (VF). Cases (n = 337) of the AGNES study were compared to controls (n = 339). A decrease of the estimated glomerular filtration rate (eGFR) at the time of acute STEMI was associated with elevated odds of developing VF during STEMI. The association was essentially flat at eGFR levels greater than 105 mL/min. In contrast, the lowest eGFR quintile was associated with a greater than 6-fold increase in odds of developing VF compared to the fourth quintile. This association between eGFR and VF at the time of STEMI remained significant after adjusting for potential confounders including electrolyte levels.

Chapter 4
In chapter 4 we investigate the role of platelets in SCD. The role of platelets in the occurrence of SCD extends beyond coronary flow impairment by clot formation. Animal experiments have shown that platelet activation may increase susceptibility of ischemic myocardium to VF.

Chapter 4.1
In chapter 4.1 we review the substances released by platelets during clot formation and their arrhythmic properties.

Chapter 4.2
In chapter 4.2 we investigate the effects of activated blood platelet products (ABPPs) on electrophysiological properties and intracellular Ca\(^{2+}\) (Ca\(^{2+}\)) homeostasis of rabbit cardiomyocytes. ABPPS from activated platelets from healthy volunteers were added to superfusion solutions. Rabbit ventricular myocytes were freshly isolated, and membrane potentials and Ca\(^{2+}\) were recorded using patch-clamp methodology and indo-1 fluorescence measurements respectively. ABPPs induced changes in ICa,L and Ca\(^{2+}\), which resulted in action potential prolongation and the occurrence of early and delayed afterdepolarizations. These changes may trigger and support re-entrant arrhythmias in ischemia models of coronary thrombosis.
Chapter 4.3
In chapter 4.3 we present a case-control study in which we studied platelet function in AGNES patients. In total 26 cases and 24 control patients were included. All patients were on aspirin 100 mg OD. Baseline platelet activation was assessed with flow cytometry. Response to activation was assessed with aggregometry, flow cytometry and PFA-100 analysis. Differences in platelet contents and content release were assessed by labeling platelet-dense granules with mepacrine and by measuring serotonin and ADP/ATP content. Platelet serotonin content in cases was higher than in controls (611 ± 118 ng/10E9 platelets versus 536 ± 141 ng/109, p=0.048). These preliminary results show that even years after the event, elevations in platelet serotonin contents in VF survivors as compared to controls can be detected and may relate to their increased susceptibility for VF in the setting of acute ischemia.

Chapter 5
In chapter 5 we analyzed follow-up data of a large group of primary VF survivors to determine prognosis and risk of SCD in patients who received contemporary MI treatment. Data on mortality, cause of death, hospitalization, and implantable cardioverter-defibrillator (ICD) implantation were retrieved from national databases. In addition, data on left ventricular ejection fraction and medication use during follow-up were retrieved. Patients who survived the first month after primary VF had a similar prognosis as patients with a STEMI without VF. ICD treatment in STEMI patient with early VF and without residual ischemia or other risk factors can be safely withheld.

Conclusion and future directives
In this thesis we present research on the most prevalent form of sudden cardiac death: sudden cardiac death in patients without previous known heart disease who are confronted with acute cardiac ischemia. For this purpose we have extended the previously initiated AGNES study. In doing so, we have found specific genetic variants that predispose to the development of VF during STEMI. Also, it appears that ECG recordings in the acute phase of the STEMI differ between patients who develop VF and those who don’t. However these ECG changes are not depending on genetic variants associated with ECG changes in the general population. Aside from the genetic risk factors, even mild kidney dysfunction is an important risk factor in the development of VF during STEMI. Furthermore, platelets play an important role in the development of STEMI. Our animal studies show that platelet products can facilitate ventricular arrhythmias. And although it is very difficult to study subtle
changes in platelets in a clinical situation, our efforts in post STEMI patients showed promising preliminary results. In daily clinical practice the findings of our follow-up study are very relevant; they can relieve somewhat the psychological stress of survivors of cardiac arrest during STEMI with good clinical outcome after the first month as they have an excellent prognosis, even without an ICD.

During the course of this research many ideas about future studies have arisen. The technical possibilities and knowledge in genetic research is growing at to an unimaginable level. Full exome screening is one of the possibilities available now at a reasonable price and will reveal much more detailed knowledge than the currently used micro-array technique. There are still many remaining questions to explain the differences in ECG’s in our population. If genetics do not seem to play a major role in the ECG differences, could it be explained by differences in collateral perfusion or are subtle differences in infarct size more important? The latter cannot be assessed by the current data, where cardiac enzymes are the only available indicator. Scar quantification by cardiac MRI could possibly answer this question in the future. Also, with the increasing number of recruited AGNES patients, we also have an increasing number of ECG’s available that were made long before the STEMI occurred, e.g. for an occupational physical exam. New ECG markers of sudden death in the general population, such as early repolarization, can now be assessed in this database. In the platelet studies we are using our animal model to focus whether a specific substance can be isolated that is responsible for the arrhythmogenic properties of platelet products. Finally, the follow-up study of the whole AGNES population is intensified and additional data is being gathered to further profile the prognosis and risks of our patients in their post STEMI period, focusing on a genetic role in the development of heart failure.

References