Further insights into inheritable arrhythmia syndromes: Focus on electrocardiograms
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Citation for published version (APA):
Postema, P. G. (2010). Further insights into inheritable arrhythmia syndromes: Focus on electrocardiograms

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Summary

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INTRODUCTION

Each year, inheritable arrhythmia syndromes prematurely end the lives of thousands of individuals worldwide. While atherosclerotic disease is the major denominator of sudden cardiac deaths after the age of 45 years, inheritable arrhythmia syndromes mostly affect younger individuals and, importantly, also endanger the lives of their next of kin. In addition, cardiovascular disease is closely linked to lifestyle, while the risk of a premature death due to an inheritable arrhythmia syndrome is largely determined by one’s genetic footprint.

In the past two decades an enormous amount of the genetic and electrophysiological basis of inheritable arrhythmia syndromes has become uncovered. It is now clear that these syndromes result from mutations affecting essential cardiac proteins. Due to these mutations normal cardiac development, structure and/or behaviour is disrupted and, ultimately, may result in lethal cardiac arrhythmias. Often, the changes induced by these mutations are mirrored in changes in the electrocardiogram (ECG), providing a means to recognise, study and stratify the arrhythmic nature of these syndromes.

In this thesis we describe further insights into inheritable arrhythmia syndromes. In particular, we studied ECGs in several of these syndromes and compared these to syndrome-specific genetic changes. Hopefully, this work may prove to be of value for earlier and better recognition, understanding and treatment of inheritable arrhythmia syndromes. Meanwhile, we point to the limitations that are an integral part of our research and suggest directions for future study.

The heart beat is driven by cardiac electric activity. In the introductory Chapter 1 we review the basic biophysical properties of cardiac ion channels which form the basis of the cardiac action potential which in turn results in the heart’s electrical activation and recovery. In addition we discuss various associated syndromes (also known as cardiac channelopathies) and their clinical relevance. These include the QT-U syndromes, Brugada syndrome and idiopathic ventricular fibrillation, on which we focussed in the remaining of this thesis.

PART I: QT-U SYNDROMES

In Chapter 2 we performed a proof-of-principle study in response to a study performed by Viskin et al. in 2005. Viskin and co-workers demonstrated that large numbers of physicians could not recognize one of the most prevalent inheritable arrhythmia syndromes we know; Long-QT syndrome (LQTS). Importantly, treatment of LQTS is very effective, whereas unrecognised and thus untreated LQTS may have fatal consequences. They asked 877 physicians, cardiologists and non-cardiologists, to study 4 ECGs, two of LQTS patients and two of healthy individuals, and tell who of these patients had LQTS. The results from the cardiologists who were not specialised in arrhythmias and the non-cardiologists were clear;
less then 25% of these physicians gave 4 correct answers while cardiologists specialised in arrhythmias yielded 62% success. In a search for a better recognition of LQTS we taught 151 2nd year medical students a QT measuring method which appeared to be easy to learn, reproducible and rather accurate. Correct answers were given by more than 70% of the students. If the presented method would be used by physicians, a better stratification of their patients’ risk for sudden death due to LQTS should be possible. Naturally, there are several limitations to this study. For example, we only taught our students one method, whereas there are also other methods. Also, we did not test this method on physicians yet and we did not study whether this method indeed lowers the number of LQTS deaths. These issues need to be addressed in the future.

Because the existence of LQTS may sometimes be hidden on a patient’s ECG, we sought to obtain another way to increase the sensitivity of the ECG to more accurately uncover LQTS. Therefore, as shown in CHAPTER 3, we collaborated with Viskin et al. to study the response to a sudden increase in heart rate. The basis for this study was formed by the long known fact that the heart adapts to changes in heart rate. When the heart rate increases, the recovery phase of the heart (repolarization) hastens too. However, this adaptation mechanism is often impaired in LQTS, which, importantly, forms the basis of arrhythmias associated with LQTS. Therefore we studied the ECGs of LQTS patients and control individuals while lying supine and shortly after brisk standing. A low-tech manoeuvre like standing propelled the whereabouts of LQTS in these groups. While on the baseline supine ECGs we had a 90% sensitivity for LQTS accompanied by only 61% specificity, measuring the QT at maximal heart rate after standing resulted in a 90% sensitivity accompanied by 86% specificity. Importantly, in this exercise we excluded obvious LQTS and obvious non-LQTS ECGs. So we concluded that the evaluation of the response of the QT-interval to the brisk increase in heart rate induced by standing provides important information that aids in the diagnosis of LQTS. However, its additive value still needs to be confirmed in prospective studies. Also the exact method on how to measure the QT when performing supine-standing ECG studies to gain best result needs further evaluation, as discussed in chapter 2.

In CHAPTER 4 we continued with studying the part of cardiac repolarization after the T-wave, the U-wave. Although the U-wave was already recognised by Einthoven in 1906, its genesis has been debated ever since. In this study we took advantage of the occurrence of specific ion channel mutations in several patients to study the U-wave with designated techniques. When the U-wave would be actuated by changes in the final part of the cardiac action potential, changes in this part of the action potential should result in changes of the U-wave. As the final part of the cardiac action is dominated by \( I_{K1} \) we studied patients with either a gain- or a loss-of-function in the \( I_{K1} \) encoding gene: KCNJ2. Because short QT-intervals augment recognition of the U-wave we also studied patients with a KCNQ1 and KCNH2 gain-of-function mutation which results from an increase of \( I_{Ks} \) and \( I_{Kr} \), respectively,
and short QT-intervals. We found that patients with an increase in $I_{K1}$ show small U-waves and patients with a decrease of $I_{K1}$ show large U-waves. Increase of $I_{Kr}$ and $I_{Ks}$ does not alter the U-wave. However, it became clear from comparison with earlier echocardiographic studies in the latter patients with an increase of $I_{Kr}$, that the U-wave starts before closure of the aortic valve. This contradicts that the U-wave is primarily actuated by electromechanical feedback, a mechanism earlier presumed. Subsequently we concluded that the U-wave is caused by intrinsic potential differences in the terminal part of the cardiac action potential. However, we did not study the influence of changes of ventricular filling in relation to the U-wave. Also, the studied patients form an extremely unique group and this limited our ability to study a larger number of patients.

As opposed to the previous study, we were able to describe a very large number of individuals with a unique mutation (SCN5A 1795insD) in Chapter 5. Carriers of this mutation in the cardiac sodium channel gene SCN5A come from a family that has been at risk for sudden cardiac death in the past centuries. Already in the 1950s, long before this gene was uncovered and treatment possible, the family came to our attention. Quickly it was observed that many family members had abnormal ECGs with impaired conduction and prolonged repolarisation, especially at slow heart rates. It was not until the 1970s that treatment with pacemakers became available, which prevented slow heart rates and thereby prevented premature deaths. Late in the 1990s we uncovered the responsible mutation in SCN5A. In addition we found that this family harboured three separate arrhythmia syndromes: LQTS, Brugada syndrome and cardiac conduction disease. Such a combination of arrhythmia syndromes had not been recognised before, which revealed the unique changes that had occurred in this family. In the following years we discovered from experimental studies that this combination was possible due to several effects of the mutation: prolongation of repolarisation, initiation of structural changes and slowing of conduction. However, there remained two major issues in this family. The first being the unexpected deaths of several family members while carrying a pacemaker and the second being the vast amount of variation in the clinical expression when carrying the mutation.

In Chapter 6 we studied these two remaining issues. Because the described family is so large we were able to perform both linkage and association analysis in a search for reasons for the variable phenotypic expression which could possibly be related to the occurrence of sudden death while slow heart rates were prevented by a pacemaker. We used gene assays to label 1329 ‘single nucleotide polymorphism’ (SNPs) located in and around candidate genes encoding cardiac ion channels. These SNPs are normal variants in the human genome but they may point to an association with detrimental effects when combined with specific mutations. Indeed, we found that SNPs on chromosome 21 in the region of the KCNE1 and KCNE2 genes altered cardiac conduction indices. A closer examination of the top SNP associated with PQ-interval identified that it was actually located within intron 3 of the RCAN1 gene,
located just a few kilo base-pairs upstream of KCNE1. The RCAN1 gene encodes ‘Regulator of Calcineurin 1’ which is highly expressed in heart and regulates calcineurin, a calcium-activated phosphatase that promotes hypertrophic growth of the heart. Transgenic mice overexpressing constitutively active calcineurin display premature sudden death and profoundly prolonged PQ-intervals, and cells isolated from these mice before the development of hypertrophy display reduced $I_{Na}$. In fact, three of the four mutation carriers who died suddenly while having a pacemaker were homozygous for this SNP. Hence, we identified a locus on chromosome 21 as a modulator of cardiac conduction, providing highly relevant candidate target genes for future studies aimed at deciphering the molecular basis of cardiac conduction. In future studies we will need to relate variations in RCAN1 to cardiac conduction in families with other (SCN5A) mutations and we will study anatomic, histological and electrophysiological changes related to RCAN1 in the family, and in experimental studies in cardiomyocytes and in mice carrying the equivalent mutation.

As can be appreciated from the previous chapters our understanding of inheritable arrhythmia syndromes is increasing. However, surely we can still be puzzled by the result of genetic testing as described in Chapter 7.10 In this report we describe a family with two SCN5A mutations, a gain-of-function mutation and a loss-of-function mutation. Unexpectedly, one of the sons displayed a phenotype which was rather typical for the mutation he did not carry while he did not have the phenotype of the mutation he did carry. Once again this shows that we still have a lot to learn about the influence of mutations and variations in the human genome and their relevance for changes in the cardiac electrical system.

PART II: BRUGADA SYNDROME

In Chapter 8 we continue with a focus on Brugada syndrome.11 As reviewed in this chapter the contemporary concept of Brugada syndrome is a disorder characterised by specific ECG changes in the right precordial ECG leads known as the ‘coved type’ or ‘type 1’ Brugada syndrome ECG and an association with sudden cardiac deaths at a relatively young age. This specific ECG hallmark typically fluctuates over time, and in some patients it may only be elicited after provocation with sodium channel blocking drugs or by fever. Several cardiac ion channels have been associated to Brugada syndrome; the sodium channel through the SCN5A/SCN1B/SCN3B and GPD1L genes, the calcium channel through CACNA1C and CACNB2 and also one of the potassium channels involved in $I_{to}$ through KCNE3. Brugada syndrome is generally regarded a right ventricular disorder, although the underlying pathophysiological mechanism remains unclear. There are separating views among experts; some considering it a primary repolarisation disorder with transmural dispersion of action potentials and others consider it to be a depolarisation disorder, or more precise, a conduction disorder. These separating views on the pathophysiology are even exaggerated because of disagreement on how
to perform accurate stratification of risk for sudden cardiac death in asymptomatic patients. The latter holds very important consequences for prophylactic treatment, i.e., whether or not to advice an implantable cardioverter defibrillator which may save one's live when ventricular fibrillation would occur but is also potentially associated with severe complications.

In Chapter 9 we discuss another important part of treatment of Brugada syndrome patients without the necessity of adequate risk stratification, that is the avoidance of certain drugs. As mentioned earlier, the typical Brugada syndrome ECG can be elicited by drugs. Of importance, (fatal) arrhythmic events may occur because of the use of certain drugs even in a patient otherwise not at high risk. Therefore every Brugada syndrome patient should be aware of the potential troublesome effects that these drugs may have on them. Unfortunately, associations between Brugada syndrome, drugs and arrhythmias are scattered throughout the literature and there was no group of experts reviewing their value as is in fact the case in long QT syndrome. Moreover, this information on long QT syndrome is readily available on the internet: www. qtdrugs.org. Therefore, we performed an extensive review of the literature on the association between drugs and Brugada syndrome, formed an international expert panel to produce a consensus recommendation on each drug, and initiated a website: www. brugada-drugs.org. By this we ensured world-wide and up to date availability of this knowledge base for physicians and patients alike. However, this exercise remains importantly limited by the intrinsic limitations of the reports of drugs associated with Brugada syndrome. Large studies are not performed and most information is anecdotal. Although supporting evidence can often be derived from experimental studies. Another complicating issue is the disparate effects that drugs can have in different patients. Still, from the official launch of the website in September 2009 to July 2010 there were already 16,428 visitors from 128 countries worldwide. Many of these visitors joined the mailing list to receive updates, downloaded a letter listing all the drugs to (preferably) avoid to provide to their health care providers (available in 13 languages) and received our opinion on their questions.

In Chapter 10 and Chapter 11 we investigated the pathophysiological mechanisms underlying Brugada syndrome. This with the aim to differentiate between repolarisation and conduction abnormalities. For this purpose we first used three-dimensional right ventricular endocardial mapping in Brugada syndrome patients and in control subjects followed by pacing protocols to further study probable arrhythmogenic properties. By this study we found that in Brugada syndrome patients there is prominent impairment of right ventricular impulse propagation. This was evidenced by slowed activation of the right ventricle in Brugada syndrome patients combined with fragmented and widened electrograms, and exaggerated slowed conduction upon premature pacing. We did not find primary repolarisation abnormalities to be involved. These findings suggest that there is both impairment of conduction and coupling in the right ventricle in Brugada syndrome patients, which will result in slowed and discontinuous conduction and may form its arrhythmic substrate. In the second study we
extended our research to provocation tests. These tests are used to uncover Brugada syndrome in patients who did not earlier have its characteristic ECG documented. By using the synergy of three different non-invasive electrocardiographic assays during the tests we were able to study, in detail, both depolarisation and repolarisation changes simultaneously. Again we found right ventricular conduction abnormalities to be strongly associated with the typical ECG of Brugada syndrome. Moreover, the extensive number of repolarisation parameters studied did, again, not show support for primary repolarisation abnormalities to be involved. These data add to previous studies which demonstrated conduction abnormalities and suggest that there may also be ultrastructural changes, such as fibrosis, involved. Unfortunately, we can not directly measure action potentials. Further it is difficult and complication sensitive to obtain cardiac tissue during life. Therefore we can not draw final conclusions from these studies still as we can not rule out whether transmural dispersion of action potentials, as advocated in the repolarisation hypothesis, plays a significant role.

Despite the two studies discussed above, there remains a large number of studies which did find primary repolarisation abnormalities to be implicated in Brugada syndrome. In Chapter 12 we discussed our views on Brugada syndrome, supporting it to be a disorder associated with conduction abnormalities, with colleagues who consider it to be a disorder associated with repolarisation abnormalities. We both put forward our points and counterpoints but we did not come to an agreement, however. Hence, only future research will provide better insights on the cause of Brugada syndrome. Possibly, this knowledge will provide new means for treatment.

PART III: IDIOPATHIC VENTRICULAR FIBRILLATION

In Chapter 13 we shift our focus to idiopathic ventricular fibrillation. As opposed to long QT syndrome and Brugada syndrome, in this disorder there are no signs of the risk for a cardiac arrest that precede the arrhythmias. This makes idiopathic ventricular fibrillation a notoriously difficult disorder to study. Moreover, it is clear that there are hereditary forms of idiopathic ventricular fibrillation which implicates that whole families may be predisposed to sudden death. Risk stratification for cardiac arrest has not been possible until recently when an association with the DPP6 gene was uncovered. Secondary prevention of cardiac arrest may be achieved with implantable cardioverter defibrillator, ablation therapy and/or quinidine treatment. Primary prevention of cardiac arrest in relatives is seriously hampered by its idiopathic nature, but may still be indicated in selected cases.

In Chapter 14 we describe our research into several cases of familial idiopathic ventricular fibrillation which resulted in the discovery of an association with the DPP6 gene and a large founder family. A genome-wide haplotype sharing analysis was performed for identification of the responsible gene in three distantly related families in which multiple
individuals died suddenly or were successfully resuscitated at young age. We identified a haplotype on chromosome 7 that was conserved in these three families. In addition it was also shared by several other families with familial idiopathic fibrillation which appeared to be related, confirming a founder effect. The shared chromosomal segment harbors part of the DPP6 gene, which encodes a putative component of the transient outward current (Ito) in the heart. We demonstrated a 20-fold increase in DPP6 mRNA levels in myocardium of carriers compared to controls. Clinical evaluation of risk-haplotype carriers and non-carriers revealed no ECG or structural parameters indicative of cardiac disease. Penetrance of idiopathic ventricular fibrillation was high; 50% of risk-haplotype carriers experienced (aborted) sudden cardiac death before the age of 58 years. Hence, we proposed DPP6 as a gene for idiopathic ventricular fibrillation with increased DPP6 expression as the likely pathogenetic mechanism. As there have not been other reports (yet) on DPP6 and idiopathic ventricular fibrillation, data remains limited to this report. Obviously, this study initiates many future investigations into the relevance of DPP6 in idiopathic ventricular arrhythmia syndromes and inheritable arrhythmia syndromes in general, into the pathophysiological mechanisms determining a pro-arrhythmic state associated with increased DPP6 expression and into the possibilities of treatment with ablation, quinidine or other drugs.

In Chapter 15 we describe our further experiences in the family with idiopathic ventricular fibrillation related to DPP6. By this time we had clinical data on 255 family members of whom 117 carry the risk haplotype. Notably, ventricular fibrillation in this family was provoked by very short coupled monomorphic extrasystoles from the right ventricular apex/lower free wall. Our future focus on this family and their vulnerability to ventricular fibrillation has at the moment three directions. First, further clinical studies are deployed. Partly this involves further characterisation of the affected versus the non-affected family members by detailed ECG and imaging studies. When available we also plan to perform detailed histological studies. Second, further characterisation of the exact derangement in DPP6 is pursued. And third, we are working on in-vitro studies to recapitulate the effect of DPP6 derangements on the cardiac action potential to be able to better understand its arrhythmogenic potential.

PART IV: SUMMARY AND FINAL REMARKS

In conclusion, in this thesis we described various forms of inheritable arrhythmia syndromes, with a focus on electrocardiograms, to promote recognition and better treatment, and to study pathophysiological mechanisms and their genetical underpinning. It is clear that although there has been an enormous increase in the understanding of inheritable arrhythmia syndromes in the past and recent years, there also remains an incredible amount of work to be done. Which, of course, is intrinsic to research.
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