Arrhythmogenic mechanisms in inherited and acquired cardiac diseases

Offerhaus, J.A.

Publication date
2023

1

GENERAL INTRODUCTION AND SCOPE OF THE THESIS

GENERAL INTRODUCTION

Normal cardiac function critically depends on the coordinated generation and propagation of cardiac electrical impulses. Dysregulation of cardiac electrical activity leads to arrhythmias, which can arise from different regions of the heart. Cardiac arrhythmias are associated with significant morbidity and mortality. For example, atrial fibrillation which has a lifetime risk of 1 in 3 individuals in Europeans, leads to a 3.5-fold increased risk of mortality.[1] Similarly, ventricular fibrillation is a major cause of death in the general population.[2]

A multitude of factors contribute to the genesis of cardiac arrhythmias, with acquired conditions playing a major role. These include hypertension, coronary artery disease, valvular disease and inflammatory disease.[1,3] The latter often results from infectious agents, such as viruses.[4] In a minority of cases, arrhythmias occur in the setting of inherited cardiac conditions, categorized as the cardiomyopathies and the primary electrical disorders. The cardiomyopathies are classified as dilated cardiomyopathy, hypertrophic cardiomyopathy and arrhythmogenic cardiomyopathy, which are all characterized by structural abnormalities and are in many cases caused by rare large-effect genetic variants in genes encoding structural proteins such as sarcomeric proteins (hypertrophic cardiomyopathy) and desmosomal proteins (arrhythmogenic cardiomyopathy). In contrast, primary electrical disorders present with arrhythmia, often in the absence of structural abnormalities, and are caused by genetic defects in genes encoding ion channel subunits or regulatory proteins. These disorders include short- and long QT syndrome, catecholaminergic polymorphic ventricular tachycardia and the so-called J-wave syndromes, consisting of the Brugada syndrome (BrS) and the early repolarization syndrome.

Although arrhythmic diseases are often categorized into acquired and inherited, in most cases, genetic and acquired risk factors both contribute to arrhythmic risk. For instance, while hypertension and valvular heart disease are well-recognized risk factors for atrial fibrillation, recent large-scale genome-wide association studies have uncovered an important genetic component in the determination of risk for this arrhythmia.[5] Similarly, although the identification of the exact genetic factors has lagged behind, inherited factors are also considered to play a relevant role in predisposition to ventricular fibrillation in the setting of myocardial infarction.[6,7] Conversely, in the rare inherited cardiac disorders, acquired risk factors also contribute to arrhythmic risk. For instance, in patients with *SCN5A* overlap syndrome caused by the *SCN5A*-1795insD founder mutation, hypertension is thought to increase risk of ventricular arrhythmia,[8] while exercise may exacerbate disease in patients with arrhythmogenic cardiomyopathy.[9] As such, to determine the arrhythmic risk of an individual, acquired and inherited risk factors should be taken into account. Likewise, both factors are equally important for the discovery of mechanistic pathways, which are crucial for the development of novel therapies.

Genetic complexity underlying arrhythmic disorders

Genetic variants that contribute to risk of arrhythmia occur across the entire spectrum of variant frequency and effect-size in the general population. In the rare inherited cardiac conditions such as the cardiomyopathies and the primary electrical disorders, gene discovery efforts conducted over

the last 25 years have primarily focused on the identification of rare large-effect genetic variants, leading to the notion that these disorders are monogenic. Recent work, however, has made it increasingly clear that the perception of monogenic disorders may be too simplistic and that for these disorders there is a (varying) contribution of common small-effect variants to disease susceptibility and severity. For instance, for BrS, a primary electrical disorder initially considered monogenic, complex inheritance is now widely recognized. In this disorder, loss-of-function variants in *SCN5A* are identified in ~20% of probands. The possibility of a more complex genetic basis became apparent over the years when studies in families harboring rare *SCN5A* pathogenic variants uncovered very low disease penetrance among variant-carriers as well as absence of the familial *SCN5A* pathogenic variant in affected individuals in some families.[10,11] Moreover, gene discovery efforts based on the monogenic paradigm were unsuccessful in this disorder. A revolutionary contribution to this paradigm shift has been the implementation of genome-wide association studies (GWAS). In GWAS, common variants, also known as single nucleotide polymorphisms (SNPs), are analyzed for their association to a disease phenotype or a trait. For BrS for example, a total of 21 single nucleotide polymorphisms (SNPs) spread over 12 loci have so far been associated with risk for the disorder.[12] These loci highlighted the importance of ion channel regulation by transcription factors, as well as ion channel trafficking through trafficking proteins.

Late-onset arrhythmia phenotypes such as atrial fibrillation, have been long considered to have a complex inheritance. GWAS on atrial fibrillation has been remarkably successful, due to recruitment of large number of affected individuals, identifying over 100 loci.[5,13] In such complex disorders genetic contribution results from an aggregate of multiple genetic variants of different frequencies and different associated effect sizes.

An alternative approach for the identification of common disease variants for arrhythmic disorders involves GWAS on individual ECG parameters (such as heart rate, QT-interval, PR-interval, QRS duration) in large samples of individuals from the general population. This approach rests on the notion that common SNPs that modulate ECG parameters represent high priority candidates for involvement in arrhythmia susceptibility. Besides being intuitively bona fide intermediate phenotypes of disease, as they capture relevant electrophysiological processes, population studies have demonstrated association between duration of these parameters and risk of sudden death.[14,15]

Identified common susceptibility variants by GWAS can be utilized in two manners, namely for risk prediction and for discovery of novel disease mechanisms. Regarding risk prediction, an associated common SNP has a small effect size and on an individual basis will have limited contribution to disease risk. However, the accumulative effect of combinations of SNPs is currently being tested in the form of polygenic risk scores for clinical relevance for risk stratification and early diagnosis. Notwithstanding their small effect, these SNPs have the potential to point us to novel disease-associated genes and new disease mechanisms. Elucidating the gene and its accompanying mechanism through which these variants associate with a trait, will allow for the potential identification of novel therapeutic targets. Since drug targets that are supported by genetic evidence are known to have a higher probability of making it to the market, GWAS loci harbor a great potential in this aspect.[16] Nevertheless, so far GWAS have resulted in very few novel therapeutics. Rather than changing the protein coding DNA and thereby changing the amino

acid composition of a protein, GWAS variants are generally found in the non-coding DNA. This makes it challenging to identify which genes are being regulated by associated variants. As such, these variants are assumed to modulate the function of regulatory elements, thereby affecting gene transcription in an epigenetic matter.[17] Thus, although hundreds of genomic loci have been associated with traits important for arrhythmogenesis, for the far majority the causal gene and its accompanying mechanism remain to be elucidated.

To prove gene causality at GWAS loci, experimental studies employing genetic models are necessary. Given that these are expensive and time consuming, it is important that genes are prioritized for a given locus, in order to define the best candidate gene with certain accuracy. For this, several methods such as expression quantitative trait loci analysis, which tests association of common variants to gene expression, and chromosome conformation capture, which can detect chromatin contacts in the 3D architecture of the genome, are useful. Although such data can provide strong evidence, functional laboratory studies, where phenotypic changes as a consequence to *in vivo* deletion of a gene or GWAS locus can be studied, remain crucial as an ultimate confirmation.

Arrhythmic risk in COVID-19

In the midst of the preparation of the current thesis, the world was struck by an unusual event, spreading around the world rapidly. In December 2019, the coronavirus disease 2019 (COVID-19) pandemic arose from Wuhan, China, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Although most COVID-19 patients remain asymptomatic or experience mild symptoms, a substantial proportion of patients develop acute respiratory distress syndrome, a life-threatening consequence of a systemic inflammatory response.[18] To date, over 6 million deaths have been reported worldwide.[19]

Together with the pandemic, concerns arose that SARS-CoV-2 could also affect the heart, thereby potentially resulting in an increase of arrhythmic risk. These concerns were based on various early findings during the pandemic.

First, the receptor that SARS-CoV-2 uses to infect a cell was shown to be the protein angiotensin-converting enzyme 2 (ACE2).[20] The human heart is one of the main organs in which ACE2 is highly expressed.[21] Among other cell types, ACE2 is specifically expressed on the membrane of cardiomyocytes.[22] In fact, autopsy reports of COVID-19 patients showed that SARS-CoV-2 was detected in the heart in the majority of cases.[23]

Second, in a pressing surge for data on COVID-19 that could be used for risk stratification as well as patient treatment during the early phase of the pandemic, a large number of studies on COVID-19 were published. Among these were observational studies, involving relatively small number of COVID-19 patients. With regard to the potential arrhythmic risk, these studies had two concerning findings. The first was that COVID-19 patients had signs of increased myocardial injury, as shown by high plasma levels of Troponin T, and that this was associated with increased risk of in-hospital mortality.[24] The second concern was that these studies observed a worrying number of arrhythmias in COVID-19 patients,[25–27] with percentages reported as high as 44% in severely affected subgroups.[25] However, these studies contained small numbers of COVID-19 patients and often did not specify the types of arrhythmias.

Third, given the high necessity for therapeutic options to treat patients, a worldwide debate was ongoing whether or not to treat patients with chloroquine and hydroxychloroquine, drugs used to treat patients with malaria and systemic lupus erythematosus.[28] This idea emerged from *in vitro* studies showing that these agents may prevent SARS-CoV-2 viral infection of cells. However, chloroquine and hydroxychloroquine both have well-known side-effects. In particular, both drugs may block the human ether-a-go-go-related potassium channel (hERG) leading to action potential duration- and QTc interval prolongation, and increasing the risk of ventricular arrhythmias, typically torsade-de-pointes. This could be of particular relevance in severely ill COVID-19 patients, where concomitant QTc-prolonging factors can be present, such as electrolyte-disturbances, increased cytokine levels,[29] hypoxia-induced increase of late sodium current,[30] and the potential use of other QTc-prolonging drugs.

These concerns increased the need for large population studies investigating the arrhythmic risk in COVID-19, identifying possible risk factors and investigating the effect of arrhythmias on mortality.

OUTLINE OF THE THESIS

As detailed above, arrhythmias can occur in the setting of inherited as well as acquired disease. Independent of the underlying disease, genetic- and acquired risk factors are not mutually exclusive and conspire together to establish arrhythmic susceptibility. As such, both must be addressed for risk stratification as well as antiarrhythmic therapy. This thesis aimed to discover arrhythmic mechanistic pathways in inherited disease and define arrhythmic risk in the setting of COVID-19.

Part I – Arrhythmogenesis in inherited cardiac diseases

Part I of this thesis is dedicated to inherited cardiac disorders, which comprise two major categories of disorders, respectively the primary electrical disorders, and the cardiomyopathies.

In **chapter 2** we focus on the primary electrical disorders and review current knowledge concerning their epidemiology. We provide an overview of the prevalence of these disorders and, where possible, we establish the worldwide prevalence. Given that the prevalence is much higher in geographical regions containing founder populations, we also present an overview of known founder populations worldwide. Furthermore, we highlight the current status of Mendelian gene discovery in these disorders and the undisputed causal role of certain genes, the natural history of the disease and proarrhythmic risk factors, including non-genetic risk factors.

In **chapter 3** we focus on the cardiomyopathies, reviewing the complex contribution of different classes of genetic variants in non-sarcomeric genes to the different types of cardiomyopathies. Genetic variants in non-sarcomeric genes are causal in only a minority of hypertrophic cardiomyopathy cases. However, common, as well as rare Mendelian variants in these non-sarcomeric genes contribute to both hypertrophic- and dilated cardiomyopathy, often with opposite effects. As such, these genes harbor information on common pathways involved in different types of cardiomyopathies. In this chapter, we provide insights into the genetic basis of cardiomyopathies by reviewing the contribution of the full range of cardiomyopathy-associated variants in these non-sarcomeric genes.

Chapters 4 and **5** are dedicated to GWAS and are aimed at identifying the causal gene at ECG parameter-associated GWAS loci as well as a BrS-associated GWAS locus. Many GWAS have been conducted in the general population on different ECG parameters, identifying hundreds of associated genomic loci, in an attempt to gain biological insights into cardiac electrophysiology. A limiting factor of GWAS, however, is that these loci do not inform us on the gene as well as the underlying mechanism, by which the association is driven. In **chapter 4** we set out to identify candidate causal genes involved in cardiac electrophysiology. Given that GWAS variants are thought to associate with a trait by modulating regulatory elements such as enhancers, we predict target genes of putative enhancers through a genome-wide correlative framework. We then prioritize candidate genes at loci associated with ECG GWAS, by incorporating this dataset in a computational model with other chromatin conformation-, genetic- and transcriptomic datasets. We validate our approach by *in vivo* studies of the gene *Tmem182*, a prioritized gene for two PR-associated loci, with use of a knock-out mouse model. In **chapter 5** we home in on BrS. GWAS studies conducted in patients with BrS previously identified a total of 12 loci harboring common susceptibility variants,

associated with the disease. Scrutinizing these loci through functional studies holds the potential of uncovering novel pathophysiological mechanisms involved in the disorder. As such, we aimed to gain further insight into BrS disease mechanisms by studying the chromosome 11 BrS associated locus, spanning the gene Wilms' tumor 1 (*WT1*). Through computational and bioinformatic analyses, we provide evidence that *WT1* is the most likely causal gene at that locus. Using a *Wt1* haploinsufficient mouse model, we subsequently study the gene in the setting of reduced conduction reserve and provide further evidence for *WT1* as a potential novel BrS susceptibility gene.

In **chapter 6** we dive into potential mechanisms underlying the J-waves in the ECG. J-waves are found in various pathological settings, which can be acquired, such as during hypothermia, as well as genetic, as is the case for BrS and early repolarization syndrome, which are often referred to as J-wave syndromes. In small rodents such as mice, a large transient voltage-gated outward potassium current (I_{to}), resulting from rapid activation of I_{to} channels causes early repolarization at a cellular level. In the ECG this is reflected by a J-wave. We hypothesize that early repolarization is an evolutionary aspect in small endothermic animals, enabling high heart rates, which is needed to maintain the endothermic state. In this chapter, we investigate this by comparing electrophysiological studies of the isolated mouse heart with electrophysiological studies of the isolated heart of the zebra finch, an endothermic bird with high heart rates.

Part II – Arrhythmogenesis in COVID-19

In **part II** of the thesis we shift our focus from inherited arrhythmia disorders to arrhythmogenesis in the setting of COVID-19 infection.

During the beginning of the pandemic the scientific world was in search of a therapy to treat COVID-19 patients. This led to the repurposing of chloroquine and hydroxychloroquine, drugs known from treating patients with malaria and systemic lupus erythematosus. However, during this period, little was known about the true efficacy of these drugs and concerns were raised on potential pro-arrhythmic side-effects. In **chapter 7** we review the current evidence on- and discuss different potential strategies for chloroquine and hydroxychloroquine as a therapy for COVID-19. We review potential arrhythmic side-effects and provide recommendations for which preventive measures to take when administering these drugs to patients. **Chapters 8** and **9** are dedicated to COVID-19 related arrhythmias. In **chapter 8**, we comment on a study investigating the prevalence and effect on in-hospital mortality from atrial fibrillation in a COVID-19 population. In **chapter 9** we set out to identify the prevalence of different arrhythmias in a large European COVID-19 cohort. For the most prevalent arrhythmia, atrial fibrillation/atrial flutter, we investigate its impact on in-hospital mortality, with specific attention for sex- and age-related differences.

REFERENCES

1. Hindricks G, Potpara T, Dagres N, et al.: 2020 ESC Guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS). Eur Heart J, 2021; 42:373–498.
2. Huikuri H V., Castellanos A, Myerburg RJ: Sudden Death Due to Cardiac Arrhythmias. N Engl J Med, 2001; 345:1473–1482.
3. Zeppenfeld K, Tfelt-Hansen J, de Riva M, et al.: 2022 ESC Guidelines for the management of patients with ventricular arrhythmias and the prevention of sudden cardiac death. Eur Heart J, 2022; 43:3997–4126.
4. Kindermann I, Barth C, Mahfoud F, et al.: Update on Myocarditis. J Am Coll Cardiol, 2012; 59:779–792.
5. Roselli C, Chaffin MD, Weng L-C, et al.: Multi-ethnic genome-wide association study for atrial fibrillation. Nat Genet, 2018; 50:1225–1233.
6. Jabbari R, Engstrøm T, Glinge C, et al.: Incidence and Risk Factors of Ventricular Fibrillation Before Primary Angioplasty in Patients With First ST-Elevation Myocardial Infarction: A Nationwide Study in Denmark. J Am Heart Assoc, 2015; 4:e001399.
7. Dekker LRC, Bezzina CR, Henriques JPS, et al.: Familial Sudden Death Is an Important Risk Factor for Primary Ventricular Fibrillation. Circulation, 2006; 114:1140–1145.
8. Rivaud MR, Jansen JA, Postema PG, et al.: A common co-morbidity modulates disease expression and treatment efficacy in inherited cardiac sodium channelopathy. Eur Heart J, 2018; 39:2898–2907
9. James CA, Bhonsale A, Tichnell C, et al.: Exercise Increases Age-Related Penetrance and Arrhythmic Risk in Arrhythmogenic Right Ventricular Dysplasia/ Cardiomyopathy–Associated Desmosomal Mutation Carriers. J Am Coll Cardiol, 2013; 62:1290–1297.
10. Priori SG, Napolitano C, Gasparini M, et al.: Clinical and genetic heterogeneity of right bundle branch block and ST-segment elevation syndrome: A prospective evaluation of 52 families. Circulation, 2000; 102:2509–2515.
11. Probst V, Wilde AAM, Barc J, et al.: SCN5A Mutations and the role of genetic background in the pathophysiology of brugada syndrome. Circ Cardiovasc Genet, 2009; 2:552–557.
12. Barc J, Tadros R, Glinge C, et al.: Genome-wide association analyses identify new Brugada syndrome risk loci and highlight a new mechanism of sodium channel regulation in disease susceptibility. Nat Genet, 2022; 54:232–239.
13. Nielsen JB, Thorolfsdottir RB, Fritsche LG, et al.: Biobank-driven genomic discovery yields new insight into atrial fibrillation biology. Nat Genet, Springer US, 2018; 50:1234–1239.
14. Schnabel RB, Sullivan LM, Levy D, et al.: Development of a risk score for atrial fibrillation (Framingham Heart Study): a community-based cohort study. Lancet, 2009; 373:739–745.
15. Straus SMJM, Kors JA, De Bruin ML, et al.: Prolonged QTc Interval and Risk of Sudden Cardiac Death in a Population of Older Adults. J Am Coll Cardiol, 2006; 47:362–367.
16. Nelson MR, Tipney H, Painter JL, et al.: The support of human genetic evidence for approved drug indications. Nat Genet, Nature Publishing Group, 2015; 47:856–860.
17. Maurano MT, Humbert R, Rynes E, et al.: Systematic Localization of Common Disease-Associated Variation in Regulatory DNA. Science, 2012; 337:1190–1195.
18. Yang X, Yu Y, Xu J, et al.: Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med, 2020; 2600:1–7.
19. WHO Coronavirus (COVID-19) Dashboard, https://covid19.who.int/
20. Hoffmann M, Kleine-Weber H, Schroeder S, et al.: SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell, 2020; :1–10
21. Donoghue M, Hsieh F, Baronas E, et al.: A Novel Angiotensin-Converting Enzyme–Related Carboxypeptidase (ACE2) Converts Angiotensin I to Angiotensin 1-9. Circ Res, 2000; 87:e1–e9.
22. Nicin L, Abplanalp WT, Mellentin H, et al.: Cell type-specific expression of the putative SARS-

CoV-2 receptor ACE2 in human hearts. Eur Heart J, England, 2020; 41:1804–1806.

23. Lindner D, Fitzek A, Bräuninger H, et al.: Association of Cardiac Infection With SARS-CoV-2 in Confirmed COVID-19 Autopsy Cases. JAMA Cardiol, 2020; 5:1281.

24. Shi S, Qin M, Shen B, et al.: Association of Cardiac Injury With Mortality in Hospitalized Patients With COVID-19 in Wuhan, China. JAMA Cardiol, 2020; 5:802–810.

25. Wang D, Hu B, Hu C, et al.: Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus–Infected Pneumonia in Wuhan, China. JAMA, 2020; 323:1061–1069.

26. Guo T, Fan Y, Chen M, et al.: Cardiovascular Implications of Fatal Outcomes of Patients With Coronavirus Disease 2019 (COVID-19). JAMA Cardiol, 2020; 5:811–818.

27. Du Y, Tu L, Zhu P, et al.: Clinical Features of 85 Fatal Cases of COVID-19 from Wuhan. A Retrospective Observational Study. Am J Respir Crit Care Med, United States, United States: NLM (Medline), 2020; 201:1372–1379.

28. Wilde AAM, Offerhaus JA: The 'president's drug.' Netherlands Hear J, 2020;28:363-365

29. Lazzerini PE, Boutjdir M, Capecchi PL: COVID-19, Arrhythmic Risk and Inflammation: Mind the Gap! Circulation, 2020; 142:7-9.

30. Belardinelli L, Giles WR, Rajamani S, Karagueuzian HS, Shryock JC: Cardiac late Na+ current: Proarrhythmic effects, roles in long QT syndromes, and pathological relationship to CaMKII and oxidative stress. Hear Rhythm, 2015; 12:440–448.