The Heart of the Matter: Discovery of new genetic loci for heart rate variability and its relationship with blood pressure and mortality

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Chapter 1

General introduction

The cardiac autonomic nervous system

Through its parasympathetic and sympathetic branches, the autonomic nervous system (ANS) is a major regulator of cardiovascular functioning that includes the regulation of heart rate and blood pressure (BP). The parasympathetic nervous system (PNS) is best known for controlling the body's "rest and digest" functions. In contrast, the sympathetic nervous system (SNS) plays a crucial role in the "fight-or-flight" response[1,2]. PNS stimulation-induced effects on the heart (mainly on the sinoatrial (SA) and atrioventricular (AV) nodes and some atrial muscles) result from acetylcholine secretion that binds to cholinergic receptors. Stimulation of the SNS releases norepinephrine from postganglionic fibers that activate adrenergic receptors in the heart, notably on SA and AV nodes, His-Purkinje conductive tissue, and atrial and ventricular contractile tissue. The SNS and PNS exert different effects on the heart (Table 1). Sympathetic stimulation increases myocardial contractility, heart rate and velocity of the conduction. In contrast, activation of the PNS decreases these functions[1–3].

Table 1: Sympathetic (SNS) and parasympathetic (PNS) nervous system receptors and their effect on function of the heart

Receptor		Effect on function		
		Myocardial contraction	Heart rate	Conduction velocity
SNS	Adrenergic	+	+	+
PSN	Cholinergic	-	-	-

During exercise, the regulation of the cardiovascular system involves several adaptations which includes increased heart rate, myocardial contractility, and venous return. The increase of heart rate during exercise is for a major part attributable to the decrease in vagal tone followed by an increase in sympathetic outflow and an increase in levels of circulating catecholamines[4]. On the other hand, following cessation of exercise, the heart rate decreases (heart rate recovery), reflecting the balance of reactivation of the PNS and withdrawal of the SNS, and possibly circulating catecholamines. The heart rate response to exercise, heart rate increase during and heart rate recovery after exercise, offers unique insights into cardiac physiology compared to heart rate at rest and can therefore be exploited to obtain additional information on cardiac function[5].

The cardiac ANS plays a crucial role not only in normal physiology but also in the pathogenesis and clinical course of different diseases. Autonomic imbalance, often characterized by increased sympathetic activity and reduced vagal tone, has been strongly implicated in the risk for cardiovascular disease[6,7]. De Geus and colleagues summarized different measures of ANS activity currently in use[2]. Among the various available techniques for assessing cardiac autonomic function, heart rate variability (HRV) has emerged as a simple and non-invasive method to evaluate the sympathovagal balance at the sinoatrial level and is the main focus of this thesis. Another tool for the assessment of cardiac autonomic regulation is the baroreflex sensitivity (BRS)[8]. The baroreflex loop is an important cardiovascular control mechanism for short-term BP regulation. BRS is clinically relevant for prediction of hypertension and cardiovascular mortality[9].

Heart rate variability

HRV reflects the beat-to-beat fluctuations in heart rate frequency over time[7,10]. This variation is under the control of the ANS, which is responsible for adjusting the heart rate in response to external or internal physical or emotional stimuli. The heart normally beats in a specific synchronized pattern between the atria and the ventricles. This rhythmic contraction and relaxation of the cardiac muscle are preceded by electrical activity called the action potential (the depolarization and repolarization of the cardiac muscle). Typically, the action potential originates in the SA node known as "cardiac pacemaker", which propagates a specific sequence in the atria first and then in the ventricles through specialized conducting tissues (Figure 1).

The heart's electrical currents spread through the tissues surrounding the heart up to the skin's surface. By placing electrodes on the skin, electrical impulses generated in the heart can be recorded by an electrocardiogram (ECG)[1]. Normal ECG tracing of a single heartbeat consists of a P-wave, QRS-complex (which includes an R-peak), and T-wave, as illustrated in Figure 2. The P-wave indicates the electrical activity of the SA node that triggers the beat (depolarization of the atria).

The QRS-complex is related to the ventricular contraction (depolarization of the ventricles) and determines the inter-beat interval (IBI, in milliseconds). This interval is also called the RR-interval because it is the time interval between two R-peaks. The T-wave is related to the relaxation of the heart muscle, and within this refractory period, it is not possible for the heart to contract again (repolarization of the ventricles). The RR-intervals can be easily detected from a continuous ECG recording. Consecutive RR-intervals can be used to calculate HRV[7,11,12].

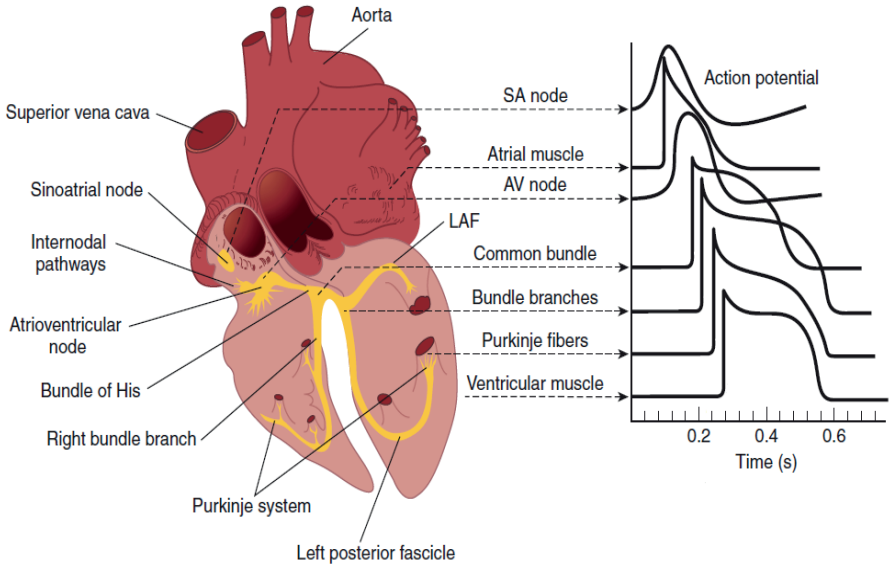

Figure 1: Conduction system of the heart. Anatomical depiction of the human heart with additional focus on areas of the conduction system (left). Typical transmembrane action potentials for the sinoatrial and atrioventricular nodes, other parts of the conduction system, and the atrial and ventricular muscles are shown. The action potentials are plotted on the same time axis but with different zero points on the vertical scale (right). Abbreviations: LAF, left anterior fascicle. (Reprinted with a permission from Barrett K, Barman S, Boitano S, Brooks H. Ganong's Review of Medical Physiology, 25e. New York, NY McGraw-Hill. 2016).

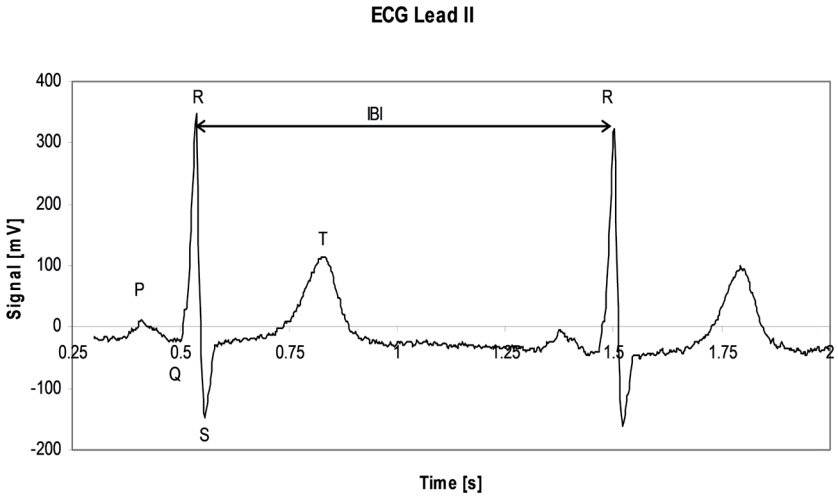

Figure 2: Typical ECG signal of lead II demonstrating the interval between R peaks. Abbreviation: ECG, Electrocardiogram; mV, millivolt, IBI, Inter-beat interval.

In the early eighteenth century, the increased availability of more accurate measurement of time allowed to evaluate periodic fluctuations in arterial pulse. Stephen Hales, in 1733, was the first to report that the beat-to-beat interval between individual arterial pulsations varied in horses[11]. Later in 1847, Carl Ludwig noted that pulse in dogs regularly increased during inspiration and slowed during expiration, thereby providing the first documented report of what is referred to as the respiratory sinus arrhythmia (RSA) which is still one of the established HRV measures.

With the development of the ECG by Willem Einthoven in 1895 and the later advent of modern signal processing it became possible to evaluate beat-to-beat changes in the cardiac rhythm. In 1961, Norman Holter developed a small portable ECG recorder that could record over long periods, such as 24 hours, which further sparked the interest in understanding the relationship between beat-to-beat variation in the heart interval and disease[11]. In 1965, the first clinically relevant observation was documented by Hon and Lee[13] when they noted acute alterations in the HRV as a marker of fetal distress in humans. Since then, the importance of HRV as a tool for assessing cardiac ANS activity in many different diseases and conditions has steadily increased.

Estimating HRV from R-peak detection derived IBI time-series can be performed by several statistical techniques, although the two main approaches are the time domain and frequency domain based analyses[7,10,12,14]. Time domain measures use statistical analysis techniques based on IBIs directly or on differences between successive IBIs. Only beats that result from the SA node depolarization (i.e., normal-to-normal [NN]-intervals) should be included; any artifacts (premature atrial and/or ventricular beats) must be removed during the preprocessing of the IBI data[15]. The most straightforward and most commonly used HRV measures in the time domain are the standard deviation of the NN-intervals (SDNN), and the root mean square of the successive differences of NN-intervals (RMSSD). The SDNN reflects an estimate of all the cyclic components responsible for variability in the period of recording (i.e., overall assessment of HRV). The RMSSD captures beat-to-beat variance in heart rate which is a reflection of high frequency HRV components only. The time domain approaches do not provide a means to discriminate between sympathetic and parasympathetic contributions of HRV[10]. This led researchers to apply power spectral density analysis, which yielded a frequency domain HRV measure that provides the basic information of how power (i.e., variance) distributes as a function of frequency[7].

Frequency domain analysis add insight into heart rate fluctuations' nature by separating the overall variability into its frequency components[16]. In short, in ECG recordings (2 to 5 min) three main spectral components are recognized: very low frequency (VLF; <=0.04 Hz), low frequency (LF; 0.04 - 0.14 Hz), and high frequency (HF; 0.15 - 0.40 Hz) components. HF is parasympathetically mediated and represents respiratory variation, LF is modulated by both SNS and PNS and is affected by baroreflex feedback loops, and VLF may represent the influence of thermoregulatory, peripheral vasomotor or renin-angiotensin systems. The distribution of the power and the central frequency of LF and HF are not fixed but may vary in relation to changes in autonomic modulations of the heart period[7]. An overview of commonly used time and frequency domain measures are given in Table 2.

Many technical factors can significantly affect HRV measures[7,17]. One of them is the length of the ECG recording. HRV is typically measured using short-term (~5-minutes) or long-term (typically up to 24-hours) ECG recordings. More recently, using HRV from ultra-short recordings of 10-seconds has become more common as it is practical and easier to apply in clinical and research settings. Studies have shown that RMSSD and SDNN measured from ultra-short recordings are reliable and good proxies for those measured from longer recording lengths[18–20]. Today growing evidence shows the role of reduced HRV in a wide range of diseases and mortality.

Table 2: Most common time and frequency domain HRV measures. (Table adapted from Malik et al, 1996[7]).

HRV measure	Description	Unit
Time-domain		
SDNN	The standard deviation of all NN intervals	ms
SDANN	Standard deviation of the averages of NN intervals in all 5 min segments of the entire recording	ms
SDNN index	Mean of the standard deviations of all NN intervals for all 5 min segments of the entire recording	ms
SDSD	Standard deviation of differences between adjacent NN intervals	ms
RMSSD	The square root of the mean of the sum of the squares of differences between adjacent NN intervals.	ms
Frequency-domain		
TP	Total power in the whole range (approximately <= 0.4 Hz)	ms^2
VLF	Power of very low frequency range (<= 0.04 Hz)	ms^2
LF	Power of the low frequency range (0.04 - 0.14 Hz)	ms^2
HF	Power of the high frequency range (0.15 - 0.4 Hz)	ms^2

Note: HRV, heart rate variability; NN, normal-to-normal intervals (i.e., all intervals between adjacent QRS complexes resulting from sinus node depolarizations); ms, milliseconds; Hz, Hertz. HRV indices used in this thesis are printed in bold face.

Reduced HRV is associated with cardiovascular diseases[21,22], diabetes[23], mental stress[24,25] and mortality in patients with cardiovascular disease[22]. Also some studies that were performed using 10-second ECG recordings showed the association of low HRV with cardiovascular events[26], cognitive function[27], and mortality[28]. Although the above associations may partly reflect impaired cardiac vagal (or parasympathetic) control caused by these diseases, reduced HRV does not simply indicate disease severity but it also predicts all-cause mortality[29] and cardiac morbidity and mortality[21] in apparently healthy individuals.

Factors influencing heart rate variability

Thus, individual differences in the cardiac ANS activity as indexed by HRV likely play a vital role in the risk for cardiovascular disease and mortality[29]. Therefore, unraveling the factors determining HRV to better understand its impact is of major importance. Inter-individual differences in HRV have been observed in connection with non-modifiable demographic factors such as age and sex[30], but numerous lifestyle and psychosocial factors have also been reported to influence HRV[31]. However, the role of these factors has not been thoroughly studied in a comprehensive way among the general population yet.

Besides being influenced by the established demographic and lifestyle factors, HRV is partly genetically determined. Family and twin studies have uniformly confirmed a genetic contribution, with heritability estimates between 11 and 71%[2,32] for different HRV time-domain and frequency domain indices. However, very few studies have tried to identify the genetic variants responsible for this heritability. Candidate gene studies based on current parasympathetic nervous system biology have not yielded results that could be replicated[33].

Increasingly, gene finding attempts in large-scale samples have used the more agnostic strategy of the genome-wide association study (GWAS) that makes no a priori assumptions on the genes or biological pathways involved[34]. A GWAS offers the opportunity to test effects of common genetic variation in the genome using high-throughput genotyping arrays[35]. So far, three GWASs on HRV have been performed[36–38]. From these studies only a few loci were established, with the identified common variants explaining only about 0.9-2.6% of the phenotypic variability of HRV, leaving a large proportion of heritability unexplained.

Higher resolution single nucleotide polymorphism arrays, the increasing availability of novel statistical approaches and larger imputation reference datasets will likely aid in fine-mapping of known loci and discovering novel

loci for HRV[39,40]. Furthermore, recent availability of GWAS data from diverse populations and large cohorts such as the UK Biobank[41], in which HRV can reliably be determined from ultra-short ECG recordings as mentioned before, offer an exciting opportunity to increase the power to detect novel loci since large sample sizes are needed to detect minor effects of genetic variants[19].

Likewise, although the association between reduced HRV and health-related outcomes has been shown in many observational studies, the actual proof of causality beyond a reasonable doubt is still missing. Identifying more genes and their functional variants is an essential next step to elucidate the biological pathways through which HRV contributes to cardiovascular disease risk and mortality and facilitates investigation of its causal role.

Main aims of my thesis

This thesis has three main aims, that are graphically displayed in Figure 3. First, to accurately quantify and estimate the influence of demographic and clinical factors on HRV and BRS in the general population. The second aim is to investigate the genetics of HRV and heart rate response to exercise. Third, this thesis aimed to gain a better understanding of the potentially causal link of HRV with BP and mortality.

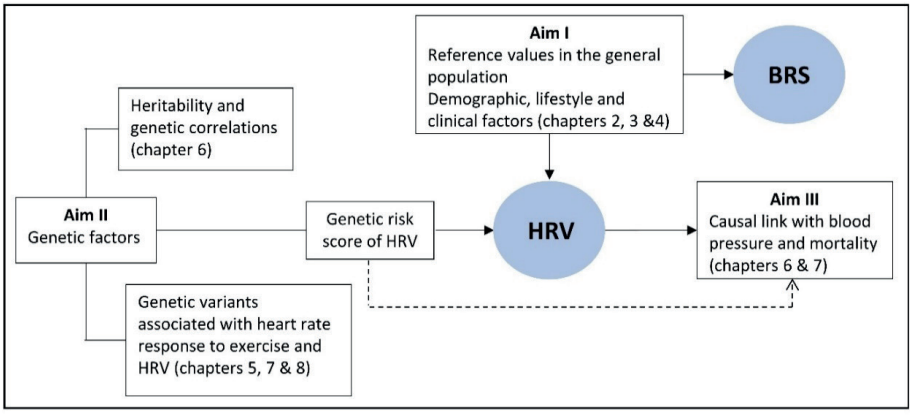

Figure 3: Depiction of the research questions examined in the different chapters of this thesis. Arrows indicate the direction of the association. HRV: Heart rate variability; BRS: Baroreflex sensitivity.

Thesis outline

In the first part of this thesis, I assessed a wide range of factors influencing cardiac ANS indices. In the second part, the genetics of HRV and heart rate response to exercise was investigated. Finally, in the third part I provide an in-depth discussion of the main findings and suggest opportunities for future research.

Part I: Cardiac autonomic function indices: Heart rate variability and baroreflex sensitivity

In **chapter 2,** I focus on generating population-based HRV reference values using 10-second resting ECG recordings. The age and sex-specific reference values provided constitute benchmarks for application in both research and clinical settings.

In **chapter 3**, I describe the demographic, lifestyle, and psychosocial determinants of HRV in the general population using the baseline data from the Lifelines Cohort Study and Biobank, a large prospective study of more than 167,000 participants[42]. The most important determinants of HRV derived from this chapter will be used to control for potential confounding effects in subsequent chapters of the thesis.

In **chapter 4**, I investigate demographic, lifestyle, and clinical factors that influence BRS in a population-based study. BRS is a marker for sympathetic and parasympathetic cardiac ANS innervation affecting short-term BP regulation.

Part II: Genetics of heart rate response to exercise, heart rate variability and the relationship with blood pressure and mortality

In **chapter 5**, I review and summarize current knowledge of the heart rate increase during and heart rate recovery after exercise, with a focus on the genetic contributions. Candidate gene, linkage, and GWASs investigating the heart rate response to exercise are comprehensively discussed.

In **chapter 6**, I estimate the family-based heritability of HRV and BP from the Lifelines Cohort Study, which is the most extensive family study to date. Moreover, this chapter entails two independent approaches (familial relationships in pedigree data and linkage disequilibrium score regression models using GWAS data) to determine the genetic correlation between HRV and BP.

In **chapter 7**, I perform a GWAS in more than 46 000 European individuals of the UK Biobank to identify loci associated with HRV[41]. The identified genetic variants were subsequently used to construct genetic risk scores in the remainder of

European UK Biobank participants to assess the association of the genetic risk scores with all-cause and cardiovascular mortality. I also explore the prospective relationship between HRV and mortality using Cox-regression analyses.

In **chapter 8**, I meta-analyze GWAS results from 22 cohorts, encompassing more than 164 000 individuals of European ancestry to identify novel loci associated with HRV.

Part III: General discussion and suggestions for future research

In **chapter 9**, I summarize and discuss the main findings of the thesis, list some methodological considerations and make suggestions for future research.

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