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

Tegegne, Balewgizie

DOI:
10.33612/diss.193633004

Chapter 8

Meta-analysis of genome-wide association studies for heart rate variability

Balewgizie S Tegegne, Alireza Ani, Eco J C Geus, Ilja M Nolte, Harold Snieder, on behalf of the VgHRV Consortium

In preparation

Abstract

Introduction: Heart rate variability (HRV) reflects the beat-to-beat fluctuations in heart rate over time, which is under the control of the cardiac autonomic nervous system. To better understand the genetic basis and etiology of HRV, we conducted a large meta-analysis of genome-wide association studies (GWASs).

Methods: Fixed-effect meta-analyses of GWASs were performed for HRV traits comprising of up to 143,269 individuals of European ancestry across 20 cohorts participating in the Genetic Variance of HRV (VgHRV) consortium. In-silico sequencing was done to annotate significant GWAS variants.

Results: We found 59 independent single nuclear polymorphisms (SNPs) in 27 loci to be associated with at least one of the HRV traits at a genome-wide significance level ($P < 5\times10^{-8}$). Eleven of the 27 loci have not been previously identified to be associated with HRV. A meta-analysis of African-American and Hispanic/Latino GWASs comprising 12,130 and 5,695 subjects confirmed SNPs in 6 and 12 of the European GWAS loci, respectively. Five of the associated SNPs (in *KCNJ5*, *CCDC141*, *MYH6* and *NDUFA11*) were non-synonymous themselves or were in high linkage disequilibrium with non-synonymous SNPs. Thirteen of the 27 lead SNPs were previously reported to be associated with cardiovascular traits such as heart rate, heart rate response to exercise, atrial fibrillation, HRV, diastolic and systolic blood pressure, PR interval, and ECG morphology. Likely causal genes in the novel loci are mainly involved in cardiomyocyte function, cell communication and cell-cell signaling in cardiac conduction, and neural development.

Conclusion: We conducted the largest meta-analysis of HRV GWASs to date and report eleven new loci associated with HRV. Many of the genes that were newly identified in this study have plausible functions that broaden our mechanistic understanding of biological pathways involved in HRV. Future follow-up experiments, ideally in vivo, should be conducted to elucidate the exact functional role of these genes in HRV.

Introduction

Heart rate variability (HRV) reflects the beat-to-beat fluctuations in heart rate over time, which is under the control of the cardiac autonomic nervous system (ANS)[1]. This physiological variability between heart beats can be quantified non-invasively using an electrocardiogram (ECG), making HRV a useful tool to assess the role of autonomic imbalance in morbidity and mortality[2,3]. Several studies have reported that lower HRV is associated with cardiac disease and death[2,4–6], as well as with psychiatric disorders[7,8].

Individual differences in the activity of the cardiac ANS as indexed by HRV play a key role in the risk for negative health outcomes. Many previous studies, including our own, have shown that HRV has a substantial genetic component[9,10]. However, despite heritability estimates of HRV in twin and family studies ranging from 14 to 71%, the molecular mechanisms influencing HRV remain poorly understood.

To date three genome-wide association studies (GWASs) were conducted to identify genetic variants associated with HRV among individuals of European[11,12] and Hispanic/Latino[13] ancestry. The first attempt, based on a very small sample (n=548) from the offspring sub-study of the Framingham Heart Study and a limited number of genotyped common variants (n=70,987), did not find any genome-wide significant single nucleotide polymorphisms (SNPs)[11]. The Hispanic/Latino GWAS reported two genome-wide significant SNPs at two loci[13]. In 2017, The Genetic Variance of Heart rate variability (VgHRV) consortium reported the first meta-analysis of GWASs and identified 17 SNPs at eight loci associated with HRV[12] based on considerable sample sizes ranging from 24,342 to 28,112. Since then, GWAS data from more cohorts including large cohorts such as the Lifelines Cohort Study and Biobank[14] and the UK Biobank[15] became available. Furthermore, individual GWASs included in the consortium used the HapMap phase 2 reference panel for imputation implying that only ~2.5M common genetic variants were tested. A fourth (unpublished) GWAS was recently conducted by our group on 46,075 individuals participating in the UK biobank and identified nine additional new loci associated with HRV (chapter 7 of this thesis). In this study, we hypothesized that increasing the power by adding large cohorts to the VgHRV consortium together with more accurate imputation reference panels[16] would provide an opportunity for discovery of new genetic variants involved in HRV. Therefore, we performed a large-scale meta-analysis of GWASs of HRV in round II of the VgHRV consortium in up to 143,269 individuals of European ancestry. Identification of more genetic loci of the HRV will expand our understanding of the genetic basis and etiology of

HRV. Furthermore, the improved genetic risk score and prediction could be used to test a potential causal role of HRV in cardiometabolic and psychiatric outcomes.

Methods

Study design and samples

As part of the VgHRV consortium round II analyses, we conducted a single stage meta-analysis of GWASs performed using participants of European ancestry for three HRV traits: the standard deviation of normal-to-normal intervals (SDNN), the root mean square of successive differences (RMSSD), and the high frequency power or peak valley respiratory sinus arrhythmia (HF/pvRSA). Next to the raw HRV traits, additional analyses were conducted for HRV traits corrected for heart rate, that is the SDNNc, RMSSDc and HF/pvRSAc, using coefficients of variations[17]. The total number of participating cohorts ranged from 14 for HF/pvRSA to 20 (with a total of 29 distinct datasets) for SDNN, and the maximum sample size for each trait was 143,269, 139,586, and 32,263 for SDNN, RMSSD, and HF/pvRSA, respectively (Supplementary Table 1 and 2).

HRV traits

In this study, the HRV measures were determined based on electrocardiogram (ECG) recordings lasting 10 seconds to 10 minutes in a standardized setting at rest in sitting or supine position. For ambulatory records, cohorts were advised to extract a ~10-minute period of quiet sitting in the evening and compute the HRV measures in this period. Two of the HRV indices, SDNN and RMSSD, were calculated from the inter-beat interval (IBI) time series obtained from the R waves in the ECG. SDNN is the beat-to-beat standard deviation in heart rate and reflects an estimate of all the cyclic components responsible for variability in the period of recording (overall HRV variability). RMSSD also captures the beat-to-beat variance in heart rate but more specifically reflects the vagally mediated changes in HRV[1]. HF, which is parasympathetically mediated and represents primarily respiratory variation, was calculated from Wavelet or Fourier decomposition with power obtained from a high frequency band of 0.15–0.40Hz. RSA, the difference in heart period during the inspiratory and expiratory phases of the respiratory cycle, was derived by peak–valley estimation using the time series of IBIs in combination with the respiration signal. Estimates of pvRSA were obtained by subtracting the shortest IBI during heart rate acceleration in the inspiration phase from the longest IBI during heart rate deceleration in the expiration phase. Previous studies show that the time- and frequency-domain measures of RSA are highly correlated and stable

across time, ambulatory conditions, and a wide range of resting heart rate and RR values[18,19]. Thus, the GWASs of HF and pvRSA were meta-analyzed together as one HRV trait.

Because HRV has a strong relationship with heart rate, and it has been recommended to correct its dependency on the mean IBI of consecutive R-peaks using coefficients of variation[17]. Each cohort applied this method to additionally calculate HRV values that were corrected for the influence of mean IBI, that is the SDNNc, RMSSDc, and HF/pvRSAc.

Genotyping and imputation

All cohorts used high-density genotype arrays and performed study-specific sample quality control (QC) such as excluding individuals with low call rate, extreme heterozygosity, sex mismatch, duplicates, family relatedness, and/or non-European ancestry (Supplementary Table 3). Variant QC included exclusion due to low call rate, low minor allele frequency, Hardy-Weinberg disequilibrium, and/or Mendelian errors. Following the QC, studies performed imputation using a 1000 Genomes (Phase 3 v5)[20] or Haplotype Reference Consortium[21] global reference panel.

Study-level genome-wide association analyses

Each participating cohort performed genome-wide association analyses of HRV traits available in their cohort following a standard analysis plan and uploaded summary statistic results to the central analysis site. Because HRV measures tend to be skewed, all HRV measures (including the heart rate corrected values) were transformed using a natural logarithm transformation before running the GWAS analyses. Associations of each genetic variant with log-transformed HRV traits were tested using linear regression models assuming an additive model. Participants with the following diseases and medications that have been reported to influence HRV were excluded: angina, myocardial infarction, heart failure, use of antidepressants and vagal modulating agents (digoxin, atropine and acetylcholinesterase inhibitors). All studies included age, age^2, sex, and principal components as covariates, and cohorts included study-specific covariates such as study center or genotyping platform batches, when deemed necessary. The study-level GWAS analyses were run using SNPTEST[22], ProbAbel[23], GCTA[24], R[25], EMPACTS (https://genome.sph.umich.edu/wiki/EPACTS), GRIMP[26], and in case of family data SAIGEgds[27] or BOLT-LMM[28] (Supplementary Table 3).

Centralized quality control

The GWASinspector package[29] in R was used to check the quality of all study-level results prior to meta-analysis and to harmonize variant IDs and alleles across studies. Correlations of reported allele frequencies were calculated with those of the 1000 Genomes reference panel[20] and reported effect sizes were correlated with the GWAS summary statistics of the VgHRV round I consortium[30] to identify potential strand issues. Quantile-quantile (QQ) plots were examined, and the genomic inflation factor (λ_{GC}) was calculated to check for study-level genomic inflation. Manhattan plots were inspected to identify spurious associations. For GWAS results that did not pass the QC, the data analyst from the respective study was contacted to fix the problems and upload a new file until the results passed QC. Before meta-analysis, filters for minor allele frequency (>= 0.01) and imputation quality (>0.3) were applied to include only high-quality variants. For a few studies more strict criteria were used to ensure the quality of their data (Supplementary Table 4).

Meta-analysis

We performed fixed-effects inverse-variance weighted meta-analyses for SDNN and RMSSD GWAS results implemented in METAL[31]. For HF/pvRSA sample size weighted meta-analysis of z-values was performed because results of two related HRV traits (HF and pvRSA) were combined which had different units and ranges of values. Effect sizes and standard errors of the SNPs on HF/pvRSA were obtained from an additional fixed-effects inverse-variance weighted meta-analysis on the summary results of only the cohorts that reported HF. We conservatively applied double genomic control[32] to the meta-analyses result to control for potential inflation as a result of population stratification within and between cohorts. After the meta-analyses, variants were removed if they were present in less than half of the studies or half of the total sample size and if they had a minor allele frequency in the meta-analysis of <0.01. The final dataset consisted of 8,412,133, 8,469,246, 8,376,330, 8,412,133, 7,513,834, and 7,516,047 autosomal SNPs for SDNN, SDNNc, RMSSD, RMSSDc, HF/pvRSA, and HF/pvRSAc, respectively. To identify SNPs independently associated with each HRV trait, we analyzed meta-analyses results using the SNP2GENE function implemented in FUMA v1.3.6a[33]. Variants were filtered using a P-value < 5×10^{-8} and independent genomic loci were linkage disequilibrium pruned based on a 250 kb window and an $r^2 < 0.1$ using 1000Genomes phase 3 European population as the LD reference panel. The lead SNP was defined as the strongest associated variant within a locus.

Internal replication

We looked up the identified lead SNPs for SDNN, SDNNc, RMSSD, and RMSSDc in the UK Biobank (n=46,075) and Lifelines-UGLI (n=30,578) dataset, which were the two cohorts with the largest sample size included in the meta-analyses. We considered the SNP internally replicated if the direction of effect was consistent and the replication p-value was < 0.05. HF/pvRSA was not measured in these cohorts, thus for this parameter and the heart rate corrected one no internal replication was performed.

Look-up in other ethnicities

Loci identified by the meta-analyses in European individuals were looked up in meta-analyzed GWAS data from Hispanic and African-American individuals. For these ethnicities GWAS data were available for 12,130 and 5,695 individuals, respectively (Supplementary Tables 1-3). These cohorts analyzed their data in the same way as for the Europeans and QC and meta-analysis was done in a similar fashion as well.

Look-up of previously reported hits

We looked up the 17 SNPs identified in an earlier meta-analysis of GWASs for HRV[12] to see if they replicated in our study. A SNP was considered replicated if the direction of effect was consistent and the one-sided replication p-value was < 0.05.

Heritability estimation

Using meta-analyses summary statistics, SNP-based heritability explained by common variants for each trait were estimated using LD Score Regression[34] with the default LD Scores computed from 1000 Genomes European data.

Functional variants and candidate causal genes

The pipeline suggested by Vaez et al.[35] was implemented to look for functional characteristics of significant GWAS SNPs (gSNP) and linked SNPs in their vicinity within 1 Mb at either side of each gSNP. We used the available data from the 1000 Genomes Project (Phase 3)[20] and tabix[36] software package for generating the appropriate Variant Call Format files. Subsequently, VCFtools[37] was used for filtering the SNPs and keeping only subjects of European ancestry. Linkage disequilibrium between the gSNP and SNPs residing within the corresponding 2 Mb area was then calculated using PLINK[38]. Linked SNPs in moderate to high (r^2>0.50) LD with the corresponding gSNP were selected for further analysis. Annotation of the significant genetic variants and their linked SNPs was carried

out using the ANNOVAR software[39]. This included finding the nearest genes and distinguishing exonic variants from other variant types (intronic, intergenic) as well as synonymous from non-synonymous SNPs. The Sorting Intolerant From Tolerant (SIFT)[40] scoring tool was used to predict the possible damaging effects of non-synonymous SNPs on protein structure and function.

Association of HRV loci with other phenotypes

We queried pleiotropic effects of HRV associated genetic variants and their linked variants with $r^2>0.80$ with any trait reported in previous GWAS studies available in the GWAS Catalog of the National Human Genome Research Institute[41] (accessed on 2021-07-08).

Network and functional enrichment analyses

The GeneMANIA algorithm[42] was used to perform gene network and enrichment analysis. GeneMANIA searches many large, publicly available biological datasets to find related genes. These include protein-protein, protein-DNA and genetic interactions, pathways, reactions, gene and protein expression data. Annotated genes to HRV SNPs and genes mapped to linked SNPs ($r^2>0.80$) were included as input to build a functional interaction network. Networks were weighted based on gene ontology (GO) terms. Subsequently, functional enrichment analysis of all genes of the constructed interaction network against GO terms was performed to find the most enriched GO terms. The GO terms and q-values from a false discovery rate corrected test for enrichment were reported. GO terms with q-value < 0.10 were reported.

Tissue enrichment analysis

A tissue enrichment analysis of HRV associated genes using gene expression data for 54 tissues from the Genotype-Tissue Expression (GTEx) project was performed using MAGMA[43], implemented in FUMA v1.3.2[33]. Tissue enrichments were considered significant after Bonferroni correction for the number of tissues ($p < 0.00093$).

Results

Fixed-effect meta-analyses of GWAS results were conducted for the six HRV traits comprising up to 143,269 individuals of European ancestry across 20 cohorts participating in the VgHRV consortium. The ECG duration for HRV assessment was 10-15 seconds in six studies, 2-5 minutes in nine studies, 10-20 minutes in three

studies, and 90-120 minutes in two studies (Supplementary Table 2). In 13 of the cohorts, genotype data was imputed using the Haplotype Reference Consortium reference panel and the rest used the 1000 Genomes Project (Supplementary Table 3).

New genomic loci associated with HRV

We identified 100 SNPs, of which 59 are independent, in 27 loci to be associated with at least one of the HRV traits at a genome-wide significance level ($P < 5\times10^{-8}$) (Figure 1, Table 1). Ten of these loci were associated with at least four HRV traits. Eleven of the 27 loci have not previously been found to be associated with HRV. In total 52 lead SNPs at 25 loci were associated with RMSSD, 46 SNPs at 19 loci with RMSSDc, 37 SNPs at 16 loci with SDNN, 25 SNPs at 10 loci with SDNN, 10 SNPs with HF/pvRSA at 7 loci, and 8 SNPs at 5 loci with HF/pvRSAc. The quantile–quantile plots for each HRV traits are shown in Supplementary Figure 1.

The top signal with the smallest p-value, rs34568271 (p-value=1.54×10^{-129}), was located on chromosome 19. This variant was associated with four of the HRV traits (RMSSD, RMSSDc, SDNN, and SDNNc) with effect sizes between -0.067 and -0.092 for allele T. Another SNP (rs55928223) in the same locus that was in high LD with rs34568271 (r^2=0.944) was significantly associated with HF/pvRSA (β for allele C=-0.274; p-value=9.22×10^{-56}).

Internal replication in the UK biobank revealed that all identified SNP-trait associations were nominally significant (p-value<0.05) and had the same direction of effect sizes (Supplementary Table 5). Similarly, most of the SNP-trait associations were also replicated in Lifelines (143 out of 160). Look-ups in the two meta-analyses of African-American and Hispanic/Latino GWASs showed that SNPs in 6 and 12 loci, respectively were nominally significant (Supplementary Table 5). All previously reported genome-wide significant associations for HRV in European ancestry[12] were replicated in this study (Supplementary Table 7). The SNP heritabilities (h^2_{SNP}) based on LD Score Regression estimation were 5.12%, 6.35%, and 11.11% for SDNN, RMSSD, and HF/pvRSA respectively.

Functional variants and candidate causal genes

Annotation of the lead SNPs and their linked SNPs (r^2 >0.5) detected five non-synonymous SNPs (Supplementary Table 8) including the lead SNP (rs7102584) in gene *KCNJ5*. This SNP was characterized as tolerated, with a SIFT score of 1, indicating no possible damaging effect. We also identified non-synonymous variants in *CCDC141* (rs17362588, in perfect LD [r^2 =1] with lead SNP rs151041685),

in *MYH6* (rs365990, r^2 =0.767 with lead SNP rs376439), and two in *NDUFA11* (rs12980262, r^2=0.946 with rs34568271 and in perfect LD [r^2 =1] with lead SNP rs55928223; rs1678868, r^2 =0.513 with lead SNP rs11085154). The two missense variants in *NDUFA11* and one on *CCDC141* had a SIFT score less than 0.05 suggesting deleterious effects (Supplementary Table 9). A total of 57 genes nearest to the lead SNPs were identified by ANNOVAR at the 27 loci. An additional 11 genes were found to be closest to variants in high LD (r^2 > 0.8) with sentinel SNPs (Supplementary Table 8).

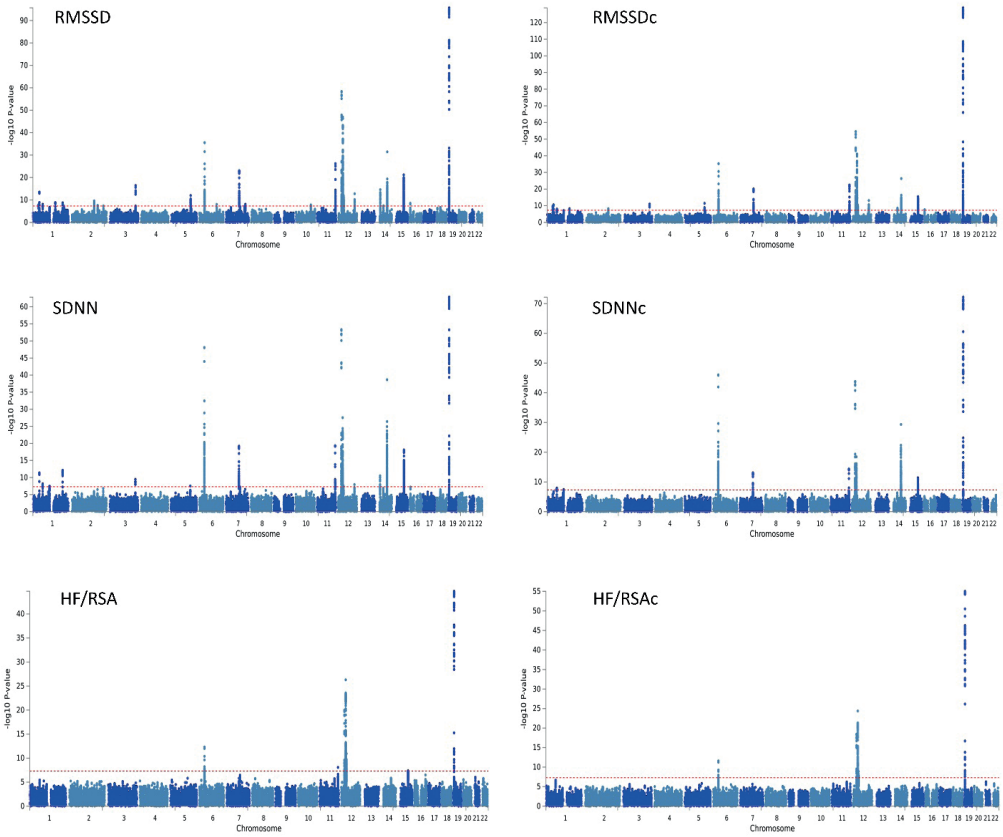

Figure 1. Manhattan plots of genome-wide HRV associations in up to 143,269 individuals of European ancestry. The x-axis represents the genome in physical order; the y-axis shows -log10 (P-values), genome-wide significance at P < 5×10-8 is indicated by the red line. RMSSD, root mean square of successive differences; RMSSDc, heart rate corrected RMSSD; SDNN, SD of normal-to-normal intervals; SDNNc, heart rate corrected SDNN; HF/pvRSA, high frequency power or peak-valley respiratory sinus arrhythmia; HF/pvRSAc: heart rate corrected HF/pvRSA.

Association of HRV-associated variants with other phenotypes

We looked up association between the identified loci and other traits to gain more insight. Thirteen of the lead SNPs associated with HRV in this study were previously reported for heart rate (five SNPs)[44], heart rate response to exercise[45,46] (five SNPs), atrial fibrillation[47] (two SNPs) and diastolic and systolic blood pressure[48], PR interval[49], ECG morphology[50], morning person and chronotype[51] (one SNP) (Supplementary Table 8). Moreover, a wider search for SNPs in high LD (r^2>0.8) showed even more variants that have been previously identified for heart rate[44].

Tissue enrichment analysis

Tissue specificity analysis across 54 tissue types from the GTEx project identified the atrial appendage and left ventricle as the highest ranked significant tissues ($p < 0.05$) for RMSSD, SDNN, and HF/pvRSA based on the gene expression enrichment (Supplementary Figure 2).

Gene network and functional enrichment analyses

From the 68 nearest genes mapped to the identified HRV SNPs or their linked SNPs (r^2>0.80), 11 genes could not be found by GeneMANIA. In total 57 query genes were used as input to build a functional interaction network (Supplementary Figure 3). The functional network and enrichment analysis on these genes resulted in 34 significantly enriched gene ontology (GO) terms (Supplementary Table 10) of which GTPase complex and extrinsic component of cytoplasmic side of plasma membrane had the smallest false discovery rate (4.62×10^{-11}). Out of the 33 genes belonging to this gene ontology in the genome nine genes in our network are related to these functions. Cell-cell signaling and cell communication involved in cardiac conduction, (cardiac) muscle contraction, heart contraction and regulation of heart contraction were also among the top GO terms.

***Table 1**: Genome-wide significant variants associated with heart rate variability. Only SNPs that were independently associated (that is, lead SNPs) to the traits are shown. At some loci lead SNPs were the same for the different traits, at other loci there were different lead SNPs for the different traits. Sub-clusters of SNPs in a locus that are independent of each other are shown with letters.*

Locus	SNP	chr	Position*	Nearest gene	Function	trait	EA	OA	N	EAF	Effect	SE	*P*-value
1	rs55837266	1	39090095	*LINC01685, RRAGC*	intergenic	RMSSDc	T	C	135223	0.1411	0.0187	0.0029	1.79E-10
	rs16825320	1	39122694	*LINC01685, RRAGC*	intergenic	RMSSD	G	A	134451	0.1459	0.0185	0.0033	1.32E-08
2	rs156653	1	45003255	*RNF220*	intronic	SDNN	C	T	134407	0.7276	0.0165	0.0024	4.24E-12
						RMSSDc	C	T	130068	0.7261	0.0153	0.0023	2.60E-11
	rs272564	1	45012273	*RNF220*	intronic	RMSSD	C	A	133022	0.2771	-0.0196	0.0026	3.04E-14
3	rs1276314	1	68267670	*GNG12*	intronic	SDNNc	A	G	137283	0.3383	0.0118	0.0021	1.24E-08
	rs12563734	1	68301147	*GNG12-AS1*	ncRNA_intronic	SDNN	A	G	137278	0.3401	0.0129	0.0022	7.26E-09
						RMSSD	T	G	125192	0.3363	0.0154	0.0027	9.00E-09
	rs113128021	1	68365554	*GNG12-AS1*	ncRNA_intronic	RMSSDc	A	G	132939	0.3403	0.0123	0.0021	1.05E-08
4	rs4839484	1	116325537	*CASQ2*	intergenic	SDNN	A	G	135377	0.5523	-0.0117	0.0021	3.37E-08
						SDNNc	A	G	135353	0.5525	-0.0108	0.002	3.72E-08
5	rs2274316	1	156446242	*MEF2D*	intronic	RMSSD	A	C	129599	0.6572	-0.0146	0.0024	1.53E-09
						RMSSDc	A	C	130314	0.6577	-0.0124	0.0021	6.17E-09
6	rs6701735	1	208030923	*MIR29B2CHG, CD34*	ncRNA_intronic	SDNN	T	C	139930	0.115	-0.0238	0.0033	7.71E-13
	rs36074690	1	208032537	*MIR29B2CHG, CD34*	ncRNA_intronic	RMSSD	T	C	134875	0.1131	-0.0217	0.0036	2.10E-09
7	rs6728625	2	155482937	*LOC100144595, KCNJ3*	intergenic	RMSSDc	G	T	130504	0.6351	-0.0123	0.0021	5.82E-09
	rs1965085	2	155510468	LOC100144595, KCNJ3	intergenic	RMSSD	G	T	129794	0.6354	-0.015	0.0024	2.65E-10
8	rs151041685	2	179725237	*CCDC141*	intronic	RMSSD	T	G	135368	0.086	-0.0228	0.0041	2.73E-08
9	rs7611674	3	179169230	*GNB4*	UTR5	RMSSD	G	T	133847	0.1941	0.0247	0.0029	3.84E-17
						RMSSDc	G	T	134562	0.1931	0.0178	0.0026	1.01E-11
						SDNN	G	T	138901	0.1944	0.017	0.0027	3.44E-10
10	rs2040862	5	137419989	WNT8A	intronic	RMSSD	T	C	134126	0.1747	0.02	0.003	3.55E-11
						RMSSDc	T	C	134841	0.1748	0.0162	0.0027	1.31E-09
11a	rs77394127	6	36702387	*RAB44, CPNE5*	intergenic	SDNNc	T	C	140113	0.1059	0.0181	0.0032	1.14E-08

	rs41272176	6	36709515	*CPNE5*	UTR3	SDNN	T	G	140116	0.1078	0.019	0.0034	2.36E-08
11b	rs13193217	6	36719728	*CPNE5*	intronic	RMSSDc	A	G	130256	0.675	0.0135	0.0022	5.21E-10
	rs13203743	6	36727258	*CPNE5*	intronic	SDNN	A	C	134676	0.6553	0.0163	0.0022	2.59E-13
						SDNNc	A	C	134652	0.6549	0.015	0.002	2.67E-13
						RMSSD	A	C	129622	0.656	0.0151	0.0024	4.35E-10
11c[#]	rs7745099	6	36780142	*CPNE5*	intronic	SDNN	C	A	138611	0.2228	-0.0197	0.0025	7.96E-15
						SDNNc	C	A	138587	0.2228	-0.0165	0.0023	1.89E-12
	rs72856461	6	36782580	CPNE5	intronic	RMSSD	C	A	133660	0.2131	-0.0207	0.0028	1.53E-13
	rs236360	6	36798868	*CPNE5*	intronic	RMSSDc	C	T	131564	0.4926	0.0147	0.002	5.11E-13
	rs236352	6	36817113	*CPNE5, PPIL1*	intergenic	RMSSD	G	A	129708	0.6535	-0.0303	0.0024	2.83E-36
	rs236349	6	36820565	*CPNE5, PPIL1*	intergenic	SDNN	G	A	134762	0.653	-0.0326	0.0022	8.01E-49
						SDNNc	G	A	134738	0.6523	-0.0294	0.002	9.76E-47
						RMSSDc	G	A	130423	0.653	-0.0268	0.0021	5.01E-36
						HF/pvRSA	G	A	29048	0.6442	-0.0593	0.0099	4.87E-13
						HF/pvRSAc	G	A	29036	0.6441	-0.0593	0.0099	2.60E-12
	rs79809702	6	36841110	*PPIL1, C6orf89*	intronic	SDNNc	G	A	140048	0.1039	-0.0191	0.0033	4.68E-09
	rs9368971	6	36907694	*PPIL1, C6orf89*	downstream	RMSSDc	T	C	133767	0.2749	-0.0144	0.0024	1.04E-09
	rs60875770	6	36915662	PPIL1, C6orf89	intergenic	SDNN	C	T	139084	0.1943	-0.0148	0.0027	3.48E-08
						SDNNc	C	T	140072	0.1038	-0.0194	0.0035	4.26E-08
11d	rs457624	6	36806492	*CPNE5*	intronic	RMSSD	T	C	132203	0.0577	0.0415	0.0053	3.67E-15
						RMSSDc	T	C	134655	0.0578	0.035	0.0047	5.32E-14
						SDNN	T	C	140176	0.0584	0.0376	0.0048	7.90E-15
						SDNNc	T	C	138711	0.059	0.0308	0.0045	4.73E-12
11e	rs236348	6	36826897	*PPIL1*	intronic	RMSSDc	C	T	134531	0.1043	-0.0262	0.0034	1.00E-14
						SDNN	C	T	140052	0.105	-0.0268	0.0035	1.96E-14
						SDNNc	C	T	140028	0.1051	-0.0233	0.0032	4.27E-13

						RMSSD	C	T	134998	0.1047	-0.0274	0.0038	4.74E-13
12	rs7759673	6	121771621	*GJA1*	downstream	RMSSD	T	A	130491	0.5505	-0.0133	0.0023	9.98E-09
13	rs180274	7	93537158	*GNGT1*	intronic	RMSSDc	T	C	130322	0.6503	0.0201	0.0021	8.44E-21
						SDNNc	T	C	134637	0.6513	0.0154	0.0021	8.66E-14
	rs180269	7	93539527	*GNGT1*	UTR3	RMSSD	T	A	129602	0.6525	0.0243	0.0024	8.89E-24
	rs180249	7	93547512	*GNG11*	intergenic	SDNN	A	G	134631	0.6599	0.0205	0.0022	6.61E-20
14a	rs1424569	7	136569416	*CHRM2*	ncRNA_intronic	RMSSD	C	T	130404	0.5344	0.0134	0.0023	9.04E-09
14b	rs58394792	7	136626027	*LOC349160*	ncRNA_intronic	RMSSD	A	G	134287	0.1626	-0.0171	0.0031	3.72E-08
15	rs10883928	10	105615074	*SH3PXD2A*	UTR5	RMSSD	T	C	131949	0.3548	0.0135	0.0024	1.64E-08
16a	rs76097649	11	128764570	*KCNJ5*	intronic	RMSSD	A	G	133255	0.0881	0.046	0.0043	6.28E-27
						RMSSDc	A	G	134922	0.0896	0.0373	0.0038	3.71E-23
	rs74399915	11	128769010	*KCNJ5*	intronic	SDNN	T	C	140416	0.0902	0.0353	0.0038	4.70E-20
						SDNNc	T	C	140392	0.0907	0.0277	0.0035	4.52E-15
16b	rs2846700	11	128768938	*KCNJ5*	intronic	RMSSD	T	C	126873	0.8139	0.019	0.003	2.22E-10
						RMSSDc	T	C	128540	0.8137	0.0155	0.0026	5.04E-09
						SDNN	T	C	134061	0.8141	0.0151	0.0028	4.15E-08
16c	rs7102584	11	128782012	*KCNJ5*	exonic	RMSSD	G	C	127187	0.978	0.064	0.0082	4.02E-15
						SDNN	G	C	131928	0.978	0.0474	0.0076	3.58E-10
						HF/pvRSA	G	C	27484	0.9771	0.1553	0.0337	8.75E-09
	rs117311861	11	128833545	*ARHGAP32*	intergenic	RMSSDc	T	C	136761	0.0253	-0.0485	0.0067	3.28E-13
17a	rs4963772	12	24758480	*LINC00477, BCAT1*	intergenic	RMSSD	A	G	134443	0.1484	0.0522	0.0032	4.36E-59
						RMSSDc	A	G	135158	0.1484	0.0449	0.0029	2.99E-55
						HF/pvRSA	A	G	29048	0.1455	0.1151	0.0133	1.03E-20
	rs34219605	12	24760456	*LINC00477, BCAT1*	intergenic	SDNN	G	A	139500	0.1479	0.0461	0.003	4.59E-54
						SDNNc	G	A	139476	0.1474	0.0385	0.0028	1.55E-44
	rs12829213	12	24773562	*LINC00477, BCAT1*	intergenic	HF/pvRSAc	A	G	29036	0.1445	0.1124	0.0132	3.60E-19
17b	rs79557668	12	24801362	*LINC00477, BCAT1*	intergenic	RMSSDc	A	G	135548	0.016	0.0511	0.0087	3.80E-09
17c	rs75958639	12	24848135	*LINC00477, BCAT1*	intergenic	RMSSD	T	C	124628	0.0273	0.0438	0.0078	1.63E-08

18a	rs3850973	12	32952784	*PKP2, SYT10*	intronic	RMSSD	C	T	134519	0.1549	-0.019	0.0032	1.84E-09
18b	rs11609975	12	33062818	*PKP2, SYT10*	intergenic	RMSSD	A	G	134727	0.1273	-0.0194	0.0034	1.71E-08
18c	rs73305609	12	33075811	*PKP2, SYT10*	intergenic	RMSSD	G	C	134120	0.1784	0.0214	0.0031	3.50E-12
						RMSSDc	G	C	134835	0.1786	0.0183	0.0027	2.17E-11
18d	rs1392339	12	33252154	*PKP2, SYT10*	intergenic	SDNN	C	T	135889	0.4902	0.0138	0.0021	6.12E-11
	rs10844500	12	33367883	*PKP2, SYT10*	intergenic	RMSSD	A	G	130525	0.5287	0.0185	0.0023	6.77E-16
	rs1457681	12	33369289	*PKP2, SYT10*	intergenic	RMSSDc	C	G	131229	0.5284	0.0153	0.002	9.00E-14
18e	rs1343676	12	33537387	*SYT10*	intronic	RMSSD	C	T	130408	0.5275	0.0332	0.0023	1.12E-47
						RMSSDc	C	T	131123	0.5275	0.0275	0.002	7.98E-42
	rs6488162	12	33593127	*SYT10*	upstream	SDNN	C	T	135080	0.5903	0.0238	0.0022	3.00E-28
						HF/pvRSA	C	T	29048	0.6059	0.0893	0.0097	5.03E-27
						HF/pvRSAc	C	T	29036	0.6058	0.0893	0.0097	4.15E-25
						SDNNc	C	T	135056	0.5915	0.0178	0.002	3.51E-19
18f	rs80309378	12	33546780	*SYT10*	intronic	RMSSD	A	G	133504	0.0691	-0.0328	0.0048	6.34E-12
						RMSSDc	A	G	136353	0.069	-0.0265	0.0042	2.85E-10
18g	rs116988526	12	33592746	*SYT10*	UTR5	RMSSD	T	G	135708	0.0633	-0.0342	0.0048	1.22E-12
						RMSSDc	T	G	136423	0.0631	-0.0281	0.0043	6.17E-11
						SDNN	T	G	140762	0.0629	-0.0254	0.0045	1.40E-08
18h	rs12317346	12	33660716	*SYT10, ALG10*	intergenic	RMSSD	A	C	134927	0.1087	0.0208	0.0037	1.95E-08
19	rs11526064	12	34683675	*ALG10*	intergenic	RMSSD	A	T	127131	0.4929	0.019	0.0024	1.53E-15
						RMSSDc	A	T	125634	0.4919	0.0161	0.0021	3.11E-14
	rs9669582	12	34693428	*ALG10*	intergenic	HF/pvRSA	G	C	29048	0.3748	-0.0454	0.0099	8.17E-09
						HF/pvRSAc	G	C	29036	0.3748	-0.0454	0.0099	1.49E-08
20a	rs4123926	12	37894987	*ALG10B*	intergenic	RMSSD	A	G	112667	0.0397	-0.0439	0.0074	2.76E-09
	rs7958361	12	37962229	*ALG10B*	intergenic	HF/pvRSA	A	G	26559	0.3713	-0.0453	0.0107	2.20E-08
	rs11514395	12	38381045	*ALG10B*	intergenic	RMSSDc	G	A	114480	0.0388	-0.0354	0.0065	4.74E-08
20b	rs4002300	12	38635105	*ALG10B*	intergenic	RMSSD	G	T	131255	0.4298	-0.0167	0.0023	1.20E-12

						RMSSDc	G	T	131970	0.4293	-0.0138	0.0021	3.68E-11
	rs12230414	12	39015336	*CPNE8*	intergenic	SDNN	G	A	70021	0.4481	0.0163	0.0029	2.54E-08
	rs35749392	12	39170874	*CPNE8*	intronic	HF/pvRSA	T	G	29048	0.3955	0.0533	0.0098	1.17E-11
						HF/pvRSAc	T	G	26818	0.3944	0.0533	0.0098	1.31E-09
20c	rs80089930	12	38767793	*ALG10B*	intergenic	HF/pvRSA	T	C	19725	0.0639	-0.1666	0.0287	7.13E-09
						HF/pvRSAc	T	C	19712	0.0639	-0.1666	0.0287	5.66E-09
21	rs802122	12	116247694	*TBX3,MED13L*	downstream	RMSSDc	A	G	132450	0.3748	0.0157	0.0021	7.50E-14
						RMSSD	A	G	131735	0.3746	0.0174	0.0024	1.71E-13
						SDNN	A	G	136789	0.3767	0.0124	0.0022	1.14E-08
22	rs376439	14	23869029	*MYH6*	intronic	RMSSD	G	A	129628	0.3926	-0.0189	0.0024	2.52E-15
						SDNN	G	A	136816	0.3913	-0.0145	0.0022	3.13E-11
23	rs12587770	14	46255716	*LINC02303, LINC00871*	intergenic	RMSSDc	C	G	134480	0.1945	0.0153	0.0026	2.75E-09
						RMSSD	C	G	131631	0.1941	0.0161	0.0029	4.32E-08
24a	rs77840046	14	71473307	*PCNX*	intronic	SDNN	G	A	140048	0.0569	-0.0304	0.0049	5.20E-10
						SDNNc	G	A	138717	0.057	-0.0267	0.0045	4.17E-09
24b	rs36418	14	72353731	*SIPA1L1, RGS6*	ncRNA_intronic	SDNN	G	T	133927	0.8685	0.0328	0.0031	1.21E-25
						SDNNc	G	T	133903	0.8685	0.0285	0.0029	4.60E-23
						RMSSDc	G	T	129588	0.8697	0.0239	0.003	2.68E-15
	rs36415	14	72355675	*SIPA1L1, RGS6*	ncRNA_intronic	RMSSD	G	A	126787	0.8542	0.0251	0.0033	1.49E-14
24c	rs72735952	14	72460289	*SIPA1L1, RGS6*	intronic	RMSSD	G	A	132022	0.1645	-0.024	0.0031	9.12E-15
	rs11623535	14	72462381	*RGS6*	intronic	SDNNc	A	G	134375	0.737	0.0154	0.0022	3.47E-12
	rs4902966	14	72464278	*RGS6*	intronic	SDNN	T	A	134396	0.7376	0.0186	0.0024	7.41E-15
	rs8013467	14	72465579	*RGS6*	intronic	RMSSDc	T	C	130056	0.7378	0.0139	0.0023	1.79E-09
24d	rs764624	14	72460574	*RGS6*	intronic	SDNN	G	A	140994	0.0481	0.0326	0.005	5.79E-11
						SDNNc	G	A	140970	0.0484	0.0266	0.0046	6.93E-09
24e	rs72737858	14	72538769	*RGS6*	intronic	SDNN	T	C	138513	0.2276	-0.0139	0.0025	3.83E-08
24f	rs10138152	14	72846658	*RGS6*	intronic	SDNN	A	G	136828	0.3868	-0.0201	0.0022	3.19E-20

						RMSSD	A	G	129640	0.3873	-0.0209	0.0024	1.56E-18
						SDNNc	A	G	136804	0.3871	-0.0162	0.002	8.98E-16
						RMSSDc	A	G	132489	0.3875	-0.0168	0.0021	1.11E-15
24g	rs17180489	14	72885471	*RGS6*	intronic	SDNN	C	G	137503	0.1404	0.0437	0.0033	2.29E-39
						RMSSD	C	G	129664	0.1411	0.0429	0.0036	3.54E-32
						SDNNc	C	G	137613	0.1406	0.0347	0.003	4.54E-30
						RMSSDc	C	G	125746	0.1425	0.0349	0.0032	4.87E-27
24h	rs17115503	14	72887897	*RGS6*	intronic	SDNN	G	C	133571	0.0364	-0.0356	0.0064	2.29E-08
						SDNNc	G	C	135425	0.0363	-0.0319	0.0058	3.35E-08
	rs79419996	14	72886267	*RGS6*	intronic	RMSSDc	G	A	122208	0.0208	-0.0453	0.0082	3.33E-08
25a	rs1979409	15	73465477	*NEO1*	intronic	RMSSD	G	A	128492	0.5296	0.0222	0.0023	6.55E-22
						RMSSDc	G	A	131341	0.5294	0.0165	0.002	3.73E-16
						SDNN	G	A	135680	0.5299	0.0187	0.0021	8.21E-19
						SDNNc	G	A	135656	0.5294	0.0135	0.0019	4.13E-12
	rs62016858	15	73471810	*NEO1*	intronic	HF/pvRSA	A	G	29048	0.4031	-0.0444	0.0096	4.23E-08
25b	rs7172796	15	73661687	*HCN4*	upstream	RMSSD	G	T	132296	0.1592	0.0258	0.0031	1.77E-16
						RMSSDc	G	T	135145	0.159	0.0187	0.0028	1.14E-11
	rs11630367	15	73662855	*HCN4, REC114*	intergenic	SDNN	G	A	139483	0.159	0.022	0.0029	1.37E-14
						SDNNc	G	A	139459	0.1591	0.0149	0.0026	1.82E-08
26	rs17671597	16	7381106	*RBFOX1*	intronic	RMSSD	G	C	128546	0.4939	0.0138	0.0023	3.05E-09
						RMSSDc	G	C	131395	0.4946	0.0115	0.002	2.22E-08
27a[#]	rs12982373	19	5782487	*PRR22*	downstream	RMSSDc	G	C	116826	0.0731	-0.0539	0.0047	6.40E-31
						RMSSD	T	C	124402	0.0722	-0.0509	0.0052	7.67E-23
						SDNN	T	C	130934	0.0715	-0.0362	0.0048	7.27E-14
	rs36055559	19	5799433	*DUS3L*	intergenic	SDNNc	A	G	132887	0.1669	-0.0235	0.003	2.13E-15
						HF/pvRSAc	A	G	22124	0.1609	-0.0946	0.016	5.66E-09

	rs8106995	19	5827620	*NRTN*	intronic	RMSSDc	T	C	128941	0.5562	0.0137	0.0022	1.87E-10
	rs1678849	19	5836964	*FUT6*	intronic	RMSSD	A	G	125055	0.7024	0.0175	0.0027	4.45E-11
27b	rs4807817	19	5853008	*LOC101928844*	ncRNA_intronic	RMSSDc	C	A	121902	0.5298	0.0169	0.0023	9.30E-14
	rs55669463	19	5855329	*LOC101928844*	ncRNA_intronic	RMSSD	T	G	124976	0.2753	-0.0183	0.0029	1.24E-10
						RMSSDc	T	G	119821	0.277	-0.0175	0.0026	8.26E-12
27b	rs16993239	19	5862467	*FUT5*	intergenic	RMSSDc	T	C	123704	0.3675	-0.0177	0.0022	2.51E-15
						RMSSD	T	C	126642	0.3683	-0.0175	0.0025	2.76E-12
						SDNNc	T	C	133150	0.3635	-0.0118	0.0021	4.11E-08
27c	rs34568271	19	5879711	*FUT5, NDUFA11*	intronic	RMSSD	T	C	132647	0.0837	-0.0892	0.0043	2.38E-96
						RMSSDc	T	C	134055	0.0835	-0.0918	0.0038	1.54E-129
						SDNN	T	C	138394	0.0833	-0.0666	0.004	1.45E-63
						SDNNc	T	C	138370	0.0831	-0.0658	0.0036	6.65E-73
	rs55928223	19	5890670	*NDUFA11*	intronic	HF/pvRSA	C	T	24354	0.0785	-0.2744	0.0185	1.90E-45
						HF/pvRSAc	C	T	25906	0.0785	-0.2744	0.0185	9.22E-56
27d	rs11085154	19	5928894	*RANBP3*	intronic	RMSSDc	C	T	130148	0.5915	0.0155	0.0021	1.02E-13
						RMSSD	C	T	127299	0.5905	0.0159	0.0024	1.64E-11
27e	rs138158774	19	6043745	*RFX2*	intronic	RMSSDc	C	T	118236	0.0145	-0.074	0.0098	3.24E-14
						RMSSD	G	A	111544	0.0143	-0.0693	0.0113	9.67E-10
						SDNN	G	A	122183	0.0147	-0.0567	0.0104	4.34E-08
27f	rs75434750	19	6089572	*RFX2*	intronic	RMSSDc	A	G	118707	0.0274	-0.0411	0.0071	8.19E-09

*Position is based on GRCh37/hg19.

#The multiple SNPs within this sub-cluster for a specific trait are independent (r^2<0.1), but they are in LD with another SNP in this sub-cluster for another trait (r^2>0.1).

SNP: single nucleotide polymorphism; chr: chromosome; EA: effect allele; OA: other allele; EAF: effect allele frequency; SE: standard error; RMSSD: root mean square of successive differences; RMSSDc: heart rate corrected RMSSD; SDNN: standard deviation of normal-to-normal intervals; SDNNc: heart rate corrected SDNN; HF/pvRSA: high frequency power or peak valley respiratory sinus arrhythmia; HF/pvRSAc: heart rate corrected HF/pvRSA.

Discussion

In this large-scale meta-analysis of GWASs for three HRV traits (RMSSD, SDNN, and HF/pvRSA) in up to 143,269 individuals of European ancestry, we identified 59 independent SNPs at 27 genomic loci including 11 loci not previously reported for HRV. The candidate causal genes in the novel loci are mainly involved in cardiomyocyte function, cell communication and cell-cell signaling in cardiac conduction, and neural development.

Our most significantly associated SNP, rs34568271 in the locus on chromosome 19, is in high LD with the non-synonymous SNP (rs12980262; r^2=0.946)[12] in the *NDUFA11* gene, which was already identified for HRV in the first round of the VgHRV consortium and was characterized as deleterious. *NDUFA11* and *FUT5,* which is also located in this locus, have been previously reported for heart rate response to exercise[45], but interestingly not to heart rate at rest[44] .

Calsequestrin 2 (*CASQ2*) on chromosome 1 is one of the newluy identified genes associated with HRV. *CASQ2* is a low-affinity high-capacity Ca^{2+}-binding protein, located in the junctional sarcoplasmic reticulum of mammalian myocardium, where it is involved in the storage and transport of positively charged calcium atoms. A study in mice showed that impairment of the coupled membrane and calcium clock mechanisms by *CASQ2* knock-outs strongly affected SA node rhythm generation[52,53]. In addition, even modest reduction in *CASQ2* increase sarcoplasmic reticulum Ca^{2+} leak and cause ventricular tachycardia susceptibility under stress[54]. Likewise, we hypothesize that the HRV SNP in *CASQ2* increases permeability of calcium ions to speed up depolarization of the pacemaker membrane, thereby increasing heart rate and reducing HRV. This gene has also previously been associated with atrial fibrillation[47] and hypertrophic and dilated cardiomyopathies[55].

Another novel candidate gene reported in our study is gap junction protein alpha 1 (*GJA1*) which encodes Cx43, a connexin family protein and a major component of the cardiac gap junction. Gap junctions are specialized channels that aid in the intercellular exchange of small metabolites, secondary messengers, and ions carrying electric signals between neighboring cells, therefore crucial in electrical coupling of myocytes[56]. Although the role of Cx43 in pacemaker function in the sinus node has not been reported, a SNP in high LD with our lead SNP in this locus has been associated with heart rate and pulse pressure[48,57].

Guanine nucleotide-binding protein subunit gamma-12 (*GNG12*), another newly reported gene in this study, encodes gamma subunits of the G protein heterotrimer composed of three subunits (α, β, and γ). G proteins are involved as a modulator or transducer in various transmembrane signaling systems, including vagal-muscarinergic signaling underlying HRV. The $G_{\beta\gamma}$ subunits interact and activate the G protein-gated inwardly rectifying potassium (GIRK) channel which is an acetylcholine-activated potassium channel (I_{KACh}) composed of GIRK1 and GIRK4 subunits[58]. This causes potassium ions (K^+) to permeate outwardly, which results in a membrane hyperpolarization slowing pacemaker depolarization and subsequently decreasing heart rate. Similarly, a locus on chromosome 1, Ras related GTP binding C (*RRAGC*) encodes a Ras-related GTP-binding protein which act as molecular switches in numerous cell processes and signaling pathways.

As noted above the I_{KACh} channel contributes to slow heart rate triggered by the parasympathetic nervous system. Two of our loci for HRV (*KCNJ3* and *KCNJ5*: potassium inwardly rectifying channel subfamily J member) on chromosomes 2 and 11 encode GIRK1 and GIRK4 respectively. *KCNJ3* was newly identified locus in this study, *KCNJ5* was already found previously as presented in chapter 7 of this thesis. Previous studies showed that *KCNJ5* has been associated with heart rate[44], atrial fibrillation[47], and diastolic blood pressure[48]. Associations with *KCNJ3* have previously been reported with intelligence and cognition related traits, but not with cardiovascular outcomes.

We also identified the genes *CCDC141* (coiled-coil domain containing 141) and *ALG10* (Alpha-1,2-Glucosyltransferase), which were previously reported based on gene-based significance for meta-analyses results of HRV but not significant in SNP-based analyses[12]. These loci have been previously associated with heart rate[44] and heart rate response to exercise[45,46]. The *CCDC141* gene on chromosome 2 plays a crucial role in neuronal development[59]. *ALG10* on chromosome 12 plays a role in glycosylation and has been linked with potassium (K^+) channel regulatory protein (*KCR1*), which may regulate cardiac automaticity[60].

The gene that we found on chromosome 14, *MYH6*, encodes the alpha heavy chain subunit of cardiac myosin. Cardiac muscle myosin is one of the major components of the sarcomere, the building block of the contractile system of cardiac muscle[61]. The role of *MYH6* in HRV has not been previously reported. However, this gene has been previously identified in GWASs of heart rate[44,62] and ECG morphology[50].

The findings of the present study confirmed all previously reported loci for HRV[12] at a genome-wide significant level except *KIAA1755,* also a previously known heart rate gene[44,57] on chromosome 20. Nevertheless, a look-up of its lead SNP (rs6123471) in our summary statistics showed that the SNP had a directionally consistent and nominally significant effect ($p= 6.92x10^{-4}$).

Although several likely causal genes were identified to be associated with HRV, our analysis was restricted to European ancestry, which reduces the generalizability of the results to other ethnicities. The look-up in Hispanic and African-American data revealed that the majority of SNPs identified in European individuals could not be replicated in these ethnicities. This could be because other genetic variants play a role. However, it is more likely to be caused by lack of power, since the sample sizes of the meta-analyzed GWASs of Hispanic and African American individuals were much lower than the European one. Nevertheles, the increasing availability of genomic data from diverse populations, allows for trans-ethnic meta-analyses of GWASs to increase the power to detect novel genetic variants. As an additional benefit, this approach is also helpful to improve the resolution of fine mapping of causal variants by leveraging differences in local linkage disequilibrium structure between ethnic groups[63]. Hence, inclusion of diverse ethnic populations in future studies may give additional insights into the genetic architecture of HRV.

In conclusion, we conducted the largest meta-analysis of HRV GWASs to date and report eleven novel loci associated with HRV. Many of the genes that were newly identified in this study have plausible functions that broaden our mechanistic understanding of biological pathways involved in HRV. Future follow-up experiments, ideally in vivo, should be conducted to elucidate the exact functional role of these genes in HRV. Furthermore, the genetic markers for HRV can be used as instrumental variables to test a potential causal role of the cardiac ANS in cardiometabolic and psychiatric outcomes.

References

1. Malik M, Bigger JT, Camm AJ, et al. Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. *European Heart Journal*. 1996;17(3):354-381. doi: 10.1093/oxfordjournals.eurheartj.a014868
2. Thayer JF, Yamamoto SS, Brosschot JF. The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. *International Journal of Cardiology*. 2010;141(2):122-131. doi: 10.1016/j.ijcard.2009.09.543
3. Rajendra Acharya U, Paul Joseph K, Kannathal N, Lim CM, Suri JS. Heart rate variability: a review. *Medical & biological engineering & computing*. 2006;44(12):1031-1051. doi:10.1007/s11517-006-0119-0
4. Kleiger RE, Miller JP, Bigger JT, Moss AJ. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. *The American Journal of Cardiology*. 1987;59(4):256-262. doi:10.1016/0002-9149(87)90795-8
5. Bishop DG, Wise RD, Lee C, von Rahden RP, Rodseth RN. Heart rate variability predicts 30-day all-cause mortality in intensive care units. *Southern African Journal of Anaesthesia and Analgesia*. 2016;22(4):125-128. doi:10.1080/22201181.2016.1202605
6. Liao D, Cai J, Rosamond WD, et al. Cardiac autonomic function and incident coronary heart disease: A population-based case-cohort study: The ARIC Study. *American Journal of Epidemiology*. 1997;145(8):696-706. doi:10.1093/aje/145.8.696
7. Chalmers JA, Quintana DS, Abbott MJA, Kemp AH. Anxiety disorders are associated with reduced heart rate variability: A meta-analysis. *Frontiers in psychiatry*. 2014; 5:80. doi:10.3389/fpsyt.2014.00080
8. Koch C, Wilhelm M, Salzmann S, Rief W, Euteneuer F. A meta-analysis of heart rate variability in major depression. *Psychological Medicine*. 2019;49(12):1948-1957. doi:10.1017/S0033291719001351
9. de Geus E, Lien R van, Neijts M, Willemsen G. Genetics of autonomic nervous system activity. In: *Oxford Handbook of Molecular Psychology*. 2009:357-390
10. Tegegne BS, Man T, van Roon AM, et al. Heritability and the genetic correlation of heart rate variability and blood pressure in >29 000 Families. *Hypertension*. 2020;76(4):1256-1262. doi:10.1161/HYPERTENSIONAHA.120.15227
11. Newton-Cheh C, Guo C-Y, Wang TJ, O'Donnell CJ, Levy D, Larson MG. Genome-wide association study of electrocardiographic and heart rate variability traits: the Framingham Heart Study. *BMC Medical Genetics*. 2007;8(Suppl 1):S7. doi:10.1186/1471-2350-8-S1-S7
12. Nolte IM, Munoz ML, Tragante V, et al. Genetic loci associated with heart rate variability and their effects on cardiac disease risk. *Nature communications*. 2017;8(1):15805. doi:10.1038/ncomms15805
13. Kerr KF, Avery CL, Lin HJ, et al. Genome-wide association study of heart rate and its variability in Hispanic/Latino cohorts. *Heart Rhythm*. 2017;14(11):1675-1684. doi: 10.1016/j.hrthm.2017.06.018
14. Stolk RP, Rosmalen JGM, Postma DS, et al. Universal risk factors for multifactorial diseases: LifeLines: A three-generation population-based study. *European Journal of Epidemiology*. 2008;23(1):67-74. doi:10.1007/s10654-007-9204-4
15. Sudlow C, Gallacher J, Allen N, et al. UK Biobank: An open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Medicine*. 2015;12(3):e1001779. doi: 10.1371/journal.pmed.1001779
16. Iglesias AI, van der Lee SJ, Bonnemaijer PWM, et al. Haplotype reference consortium panel: Practical implications of imputations with large reference panels. *Human Mutation*. 2017;38(8). doi:10.1002/humu.23247
17. Van Roon AM, Snieder H, Lefrandt JD, De Geus EJC, Riese H. Parsimonious correction of heart rate variability for its dependency on heart rate. *Hypertension*. 2016;68(5). doi:10.1161/HYPERTENSIONAHA.116.08053
18. Draghici AE, Taylor JA. The physiological basis and measurement of heart rate variability in humans. *Journal of Physiological Anthropology*. 2016;35(1):22. doi:10.1186/s40101-016-0113-7

19. Goedhart AD, Van Der Sluis S, Houtveen JH, Willemsen G, De Geus EJC. Comparison of time and frequency domain measures of RSA in ambulatory recordings. *Psychophysiology*. 2007;44(2):203-215. doi:10.1111/j.1469-8986.2006. 00490.x
20. 1000 Genomes Project Consortium, Abecasis GR, Altshuler D, et al. 1000 Genomes Project Consortium: A map of human genome variation from population-scale sequencing. *Nature*. 2010;467(7319):1061-1073. doi:10.1038/nature09534
21. McCarthy S, Das S, Kretzschmar W, et al. A reference panel of 64,976 haplotypes for genotype imputation. *Nature Genetics*. 2016;48(10):1279-1283. doi:10.1038/ng.3643
22. Marchini J, Howie B, Myers S, McVean G, Donnelly P. A new multipoint method for genome-wide association studies by imputation of genotypes. *Nature Genetics*. 2007;39(7):906-913. doi:10.1038/ng2088
23. Aulchenko YS, Struchalin M V., van Duijn CM. ProbABEL package for genome-wide association analysis of imputed data. *BMC Bioinformatics*. 2010;11(1):134. doi:10.1186/1471-2105-11-134
24. Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: A tool for genome-wide complex trait analysis. *American Journal of Human Genetics*. 2011;88(1):76-82. doi: 10.1016/j.ajhg.2010.11.011
25. R Core Team. R: A language and environment for statistical computing. *R Foundation for Statistical Computing*. Published online 2019.
26. Estrada K, Abuseiris A, Grosveld FG, Uitterlinden AG, Knoch TA, Rivadeneira F. GRIMP: A web- and grid-based tool for high-speed analysis of large-scale genome-wide association using imputed data. *Bioinformatics*. 2009;25(20):2750-2752. doi:10.1093/bioinformatics/btp497
27. Zheng X, Davis JW. SAIGEgds - An efficient statistical tool for large-scale PheWAS with mixed models. Schwartz R, ed. *Bioinformatics*. 2021;37(5):728-730. doi:10.1093/bioinformatics/btaa731
28. Loh PR, Tucker G, Bulik-Sullivan BK, et al. Efficient Bayesian mixed-model analysis increases association power in large cohorts. *Nature Genetics*. 2015;47(3):284-290. doi:10.1038/ng.3190
29. Ani A, van der Most PJ, Snieder H, Vaez A, Nolte IM. GWASinspector: comprehensive quality control of genome-wide association study results. Schwartz R, ed. *Bioinformatics*. 2021;37(1):129-130. doi:10.1093/bioinformatics/btaa1084
30. Nolte IM, Munoz ML, Tragante V, et al. Genetic loci associated with heart rate variability and their effects on cardiac disease risk. *Nature Communications*. Published online 2017. doi:10.1038/ncomms15805
31. Willer CJ, Li Y, Abecasis GR. METAL: Fast and efficient meta-analysis of genomewide association scans. *Bioinformatics*. 2010;26(17):2190-2191. doi:10.1093/bioinformatics/btq340
32. Devlin B, Roeder K. Genomic control for association studies. *Biometrics*. 1999;55(4):997-1004. doi:10.1111/j.0006-341X.1999.00997.x
33. Watanabe K, Taskesen E, Van Bochoven A, Posthuma D. Functional mapping and annotation of genetic associations with FUMA. *Nature Communications*. 2017;8(1):1826. doi:10.1038/s41467-017-01261-5
34. Bulik-Sullivan B, Loh PR, Finucane HK, et al. LD score regression distinguishes confounding from polygenicity in genome-wide association studies. *Nature Genetics*. 2015;47(3):291-295. doi:10.1038/ng.3211
35. Vaez A, Jansen R, Prins BP, et al. In silico post genome-wide association studies analysis of C-reactive protein loci suggests an important role for interferons. *Circulation: Cardiovascular Genetics*. 2015;8(3):487-497. doi:10.1161/CIRCGENETICS.114.000714
36. Li H. Tabix: Fast retrieval of sequence features from generic TAB-delimited files. *Bioinformatics*. 2011;27(5):718-719. doi:10.1093/bioinformatics/btq671
37. Danecek P, Auton A, Abecasis G, et al. The variant call format and VCFtools. *Bioinformatics*. 2011;27(15):2156-2158. doi:10.1093/bioinformatics/btr330
38. Purcell S, Neale B, Todd-Brown K, et al. PLINK: A tool set for whole-genome association and population-based linkage analyses. *American Journal of Human Genetics*. 2007;81(3):559-575. doi:10.1086/519795
39. Wang K, Li M, Hakonarson H. ANNOVAR: Functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Research*. 2010;38(16):e164-e164. doi:10.1093/nar/gkq603

40. Sim NL, Kumar P, Hu J, Henikoff S, Schneider G, Ng PC. SIFT web server: Predicting effects of amino acid substitutions on proteins. *Nucleic Acids Research*. 2012;40(W1):W452-W457. doi:10.1093/nar/gks539
41. Buniello A, Macarthur JAL, Cerezo M, et al. The NHGRI-EBI GWAS catalog of published genome-wide association studies, targeted arrays and summary statistics 2019. *Nucleic Acids Research*. 2019;47(D1):D1005-D1012. doi:10.1093/nar/gky1120
42. Warde-Farley D, Donaldson SL, Comes O, et al. The GeneMANIA prediction server: Biological network integration for gene prioritization and predicting gene function. *Nucleic Acids Research*. 2010;38(suppl_2):W214-W220. doi:10.1093/nar/gkq537
43. de Leeuw CA, Mooij JM, Heskes T, Posthuma D. MAGMA: Generalized Gene-Set Analysis of GWAS Data. *PLoS Computational Biology*. 2015;11(4). doi:10.1371/journal.pcbi.1004219
44. Eppinga RN, Hagemeijer Y, Burgess S, et al. Identification of genomic loci associated with resting heart rate and shared genetic predictors with all-cause mortality. *Nature Genetics*. 2016;48(12):1557-1563. doi:10.1038/ng.3708
45. Verweij N, van de Vegte YJ, van der Harst P. Genetic study links components of the autonomous nervous system to heart-rate profile during exercise. *Nature Communications*. 2018;9(1):898. doi:10.1038/s41467-018-03395-6
46. Ramírez J, Duijvenboden S van, Ntalla I, et al. Thirty loci identified for heart rate response to exercise and recovery implicate autonomic nervous system. *Nature Communications*. 2018;9(1):1947. doi:10.1038/s41467-018-04148-1
47. Roselli C, Chaffin MD, Weng LC, et al. Multi-ethnic genome-wide association study for atrial fibrillation. *Nature Genetics*. 2018;50(9):1225-1233. doi:10.1038/s41588-018-0133-9
48. Warren HR, Evangelou E, Cabrera CP, et al. Genome-wide association analysis identifies novel blood pressure loci and offers biological insights into cardiovascular risk. *Nature Genetics*. 2017;49(3):403-415. doi:10.1038/ng.3768
49. Ntalla I, Weng LC, Cartwright JH, et al. Multi-ancestry GWAS of the electrocardiographic PR interval identifies 202 loci underlying cardiac conduction. *Nature Communications*. 2020;11(1):2542. doi:10.1038/s41467-020-15706-x
50. Verweij N, Benjamins JW, Morley MP, et al. The Genetic makeup of the electrocardiogram. *Cell Systems*. 2020;11(3):229-238.e5. doi:10.1016/j.cels.2020.08.005
51. Jones SE, Lane JM, Wood AR, et al. Genome-wide association analyses of chronotype in 697,828 individuals provides insights into circadian rhythms. *Nature Communications*. 2019;10(1):343. doi:10.1038/s41467-018-08259-7
52. Arbel-Ganon L, Behar JA, Gómez AM, Yaniv Y. Distinct mechanisms mediate pacemaker dysfunction associated with catecholaminergic polymorphic ventricular tachycardia mutations: Insights from computational modeling. *Journal of molecular and cellular cardiology*. 2020;143:85-95. doi:10.1016/j.yjmcc.2020.04.017
53. Glukhov A v., Kalyanasundaram A, Lou Q, et al. Calsequestrin 2 deletion causes sinoatrial node dysfunction and atrial arrhythmias associated with altered sarcoplasmic reticulum calcium cycling and degenerative fibrosis within the mouse atrial pacemaker complex1. *European Heart Journal*. 2015;36(11):686-697. doi:10.1093/eurheartj/eht452
54. Chopra N, Kannankeril PJ, Yang T, et al. Modest reductions of cardiac calsequestrin increase sarcoplasmic reticulum Ca2+ leak independent of luminal Ca2+ and trigger ventricular arrhythmias in mice. *Circulation Research*. 2007;101(6):617-626. doi:10.1161/CIRCRESAHA.107.157552
55. Tadros R, Francis C, Xu X, et al. Shared genetic pathways contribute to risk of hypertrophic and dilated cardiomyopathies with opposite directions of effect. *Nature Genetics*. 2021;53(2):128-134. doi:10.1038/s41588-020-00762-2
56. Noorman M, van der Heyden MAG, van Veen TAB, et al. Cardiac cell-cell junctions in health and disease: Electrical versus mechanical coupling. *Journal of Molecular and Cellular Cardiology*. 2009;47(1):23-31. doi:10.1016/j.yjmcc.2009.03.016
57. den Hoed M, Eijgelsheim M, Esko T, et al. Identification of heart rate-associated loci and their effects on cardiac conduction and rhythm disorders. *Nature Genetics*. 2013;45(6):621-631. doi:10.1038/ng.2610

58. Krapivinsky G, Gordon EA, Wickman K, Velimirović B, Krapivinsky L, Clapham DE. The G-protein-gated atrial K+ channel IKAch is a heteromultimer of two inwardly rectifying K+-channel proteins. *Nature*. 1995;374(6518):135-141. doi:10.1038/374135a0
59. Brandon NJ, Sawa A. Linking neurodevelopmental and synaptic theories of mental illness through DISC1. *Nature Reviews Neuroscience*. 2011;12(12):707-722. doi:10.1038/nrn3120
60. Michels G, Er F, Khan IF, et al. K+ channel regulator KCR1 suppresses heart rhythm by modulating the pacemaker current If. Mansvelder H, ed. *PLoS ONE*. 2008;3(1):e1511. doi:10.1371/journal.pone.0001511
61. Holm H, Gudbjartsson DF, Sulem P, et al. A rare variant in MYH6 is associated with high risk of sick sinus syndrome. *Nature Genetics*. 2011;43(4):316-320. doi:10.1038/ng.781
62. Eijgelsheim M, Newton-Cheh C, Sotoodehnia N, et al. Genome-wide association analysis identifies multiple loci related to resting heart rate. *Human Molecular Genetics*. 2010;19(19):3885-3894. doi:10.1093/hmg/ddq303
63. Morris AP. Transethnic meta-analysis of genomewide association studies. *Genetic epidemiology*. 2011;35(8):809-822. doi:10.1002/gepi.20630

Supplements to Chapter 8

Available online at

https://drive.google.com/drive/folders/1VKBxCobA5PO4rfzxWV1Bh1ywIMLxz_mK?usp=sharing