nature COMMUNICATIONS

ARTICLE

Received 9 Mar 2017 | Accepted 8 May 2017 | Published 14 Jun 2017

DOI: 10.1038/ncomms15805 OPEN

Genetic loci associated with heart rate variability and their effects on cardiac disease risk

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Reduced cardiac vagal control reflected in low heart rate variability (HRV) is associated with greater risks for cardiac morbidity and mortality. In two-stage meta-analyses of genome-wide association studies for three HRV traits in up to 53,174 individuals of European ancestry, we detect 17 genome-wide significant SNPs in eight loci. HRV SNPs tag non-synonymous SNPs (in *NDUFA11* and *KIAA1755*), expression quantitative trait loci (eQTLs) (influencing *GNG11*, *RGS6* and *NEO1*), or are located in genes preferentially expressed in the sinoatrial node (*GNG11*, *RGS6* and *HCN4)*. Genetic risk scores account for 0.9 to 2.6% of the HRV variance. Significant genetic correlation is found for HRV with heart rate ($-0.74 < r_g < -0.55$) and blood pressure ($-0.35 < r_g < -0.20$). These findings provide clinically relevant biological insight into heritable variation in vagal heart rhythm regulation, with a key role for genetic variants (*GNG11*, *RGS6)* that influence G-protein heterotrimer action in GIRK-channel induced pacemaker membrane hyperpolarization.

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Heart rate variability (HRV) is a physiological variation in cardiac cycle duration. When measured under supine or sitting conditions, resting HRV is most prominently centred around the frequency of respiration (~0.25 Hz) and the intrinsic blood pressure rhythm (~0.1 Hz). This reflects modulation of tonic activity in the cardiac vagal nerves originating in cortical and subcortical nuclei[1] by oscillatory input at the brainstem level from cardiorespiratory coupling, lung stretch-reflexes, and arterial chemo- and baroreceptors[1,2]. This vagal gating gives rise to oscillatory vagal effects on the pacemaker potentials in the sinoatrial node that scales with the tonic activity in the vagal nerves and provides a source of beat-to-beat variation in heart rate. Due to its good reproducibility[3] and ease of measurement, HRV is a widely used non-invasive research and clinical tool to quantify the degree of vagal control of heart rate[4].

Loss of cardiac vagal control as indexed by low HRV is associated with mortality in patients with cardiovascular disease[5]. Animal research further supports a role for cardiac vagal activity in preventing sudden death and ventricular fibrillation[6]. In addition, hypertension[7], end-stage renal disease[8] and diabetes[9] are all associated with low HRV. Although the above associations may partly reflect impaired cardiac vagal control caused by these diseases, lowered HRV does not simply indicate disease severity as it also predicts all-cause mortality[10] and cardiac morbidity and mortality[11,12] in apparently healthy individuals.

Large inter-individual differences in HRV exist in the basal resting state. Family and twin studies have uniformly confirmed a substantial genetic contribution to resting HRV with heritability estimates between 25 and 71% (ref. 13). Candidate gene studies based on current knowledge of parasympathetic nervous system biology have not yielded results that hold up in replication[14]. To improve our understanding of the genetic basis of HRV, we performed a two-stage meta-analysis of genome-wide association studies (GWAS) in up to 53,174 individuals of European ancestry on three HRV traits (the s.d. of the normal-to-normal inter beat intervals (SDNN), the root mean square of the successive differences of inter beat intervals (RMSSD) and the peak-valley respiratory sinus arrhythmia or high frequency power (pvRSA/HF)). These HRV traits were measured during resting, basal recordings ranging in length from ultrashort 10-s electrocardiograms (ECGs) to up to 90 min of sitting or from 2 to 12 h of daytime recording. Relevance of the identified loci for other ethnicities was examined in data from 11,234 Hispanic/Latino and 6,899 African-American individuals. *In silico* post-GWAS analyses were performed to test for association with cardiac disease risk factors and disease outcomes and to provide insights into the biological mechanisms by which the identified loci influence cardiac vagal control and its effect on HRV.

We detect 17 SNPs in eight loci harbouring several genes preferentially expressed in the sinoatrial node and significant negative genetic correlations of HRV with heart rate and blood pressure. These findings provide clinically relevant biological insight into heritable variation in vagal heart rhythm regulation, with a key role for genetic variants in proteins (RGS6, GNG11) known to influence G-protein heterotrimer action in GIRK-channel induced pacemaker membrane hyperpolarization.

Results

New loci associated with HRV. We meta-analysed results from GWAS on three HRV traits (see Methods section for details) performed by 20 cohorts of European ancestry in up to 28,700 individuals (Fig. 1; Supplementary Figs 1–3; Supplementary Tables 1–4). Using a significance threshold of 1×10^{-6}, 23 single-nucleotide polymorphism (SNPs) in 14 loci that were associated with one or more of these HRV traits were taken forward for wet-lab genotyping or *in silico* replication in 11 cohorts including up to 24,474 additional individuals of European ancestry, followed by a second stage meta-analysis (Supplementary Data 1).

After stage 2, we identified 17 lead SNPs (11 independent) in eight loci (Table 1) that reached genome-wide significance ($P < 5 \times 10^{-8}$). The loci on chromosomes 14 and 15 contained three and two independent signals, respectively, (Supplementary Fig. 3). Conditional analysis confirmed the presence of independently associated variants in these loci (Supplementary Table 5). In total, nine independently associated SNPs in seven loci were detected for SDNN, nine independently associated SNPs in eight loci for RMSSD, and five independently associated SNPs in five loci for pvRSA/HF. Many of the SNPs were associated with at least two of the HRV traits (Supplementary Data 1). In four loci, the lead SNPs differed between traits but were in linkage disequilibrium (LD) with each other ($0.24 < r^2 < 0.90$) (Table 1). Forest plots show little heterogeneity in the genetic associations across the entire set of cohorts for all SNPs (Supplementary Fig. 4). Sex-stratified analyses did not show differences in SNP effects between men and women for the genome-wide associated loci (Supplementary Table 6). Separately meta-analysing across cohorts with short laboratory rest recordings versus longer term ambulatory recordings did not suggest sensitivity of the results to these different recording methods (Supplementary Table 7). Results of VEGAS gene-based analyses corroborated those of the SNP-based analyses (Supplementary Note 1).

Variance explained. Weighted genetic risk scores based on the independent SNPs that reached genome-wide significance after the second stage meta-analysis were computed for the three HRV traits and used to predict RMSSD, SDNN and pvRSA/HF in adults from the Lifelines ($n = 12,101$) and NESDA ($n = 2,218$) cohorts, adolescents from the TRAILS-Pop cohort ($n = 1,191$), and children from the ABCD cohort ($n = 1,094$) (Table 2). The multi-SNP genetic risk scores were all significantly associated with HRV and the percentages of variance explained for the corresponding traits were 1.0–1.4% for SDNN, 1.1–2.4% for RMSSD, and 0.9–2.6% for pvRSA/HF. Cross-trait explained variances of genetic risk scores were close to those for the corresponding trait.

To test the contribution of SNPs that did not reach genome-wide significance, we performed polygenic risk score analyses using increasingly more lenient significance thresholds and determined the percentages of explained HRV in the same four cohorts (Supplementary Fig. 5; Table 3). Maximal variance explained by the polygenic risk score was 0.8–1.4% for SDNN, 0.9–2.3% for RMSSD and 0.9–2.3% for pvRSA/HF. This was reached at relatively small numbers of SNPs (≤ 71) with additional SNPs adding more noise than signal.

The total variance explained by common SNPs (SNP-based heritability) estimated by Genomic Restricted Maximum Likelihood or LD score regression analysis varied between 10.8 and 13.2%, with only small differences in estimates across methods and HRV traits (Supplementary Note 2).

Generalization to other ethnicities. In data from up to 11,234 Hispanic/Latino individuals, five SNPs in five of the eight loci identified for RMSSD, seven SNPs in six of the seven loci for SDNN and three SNPs in three of the five loci for pvRSA/HF showed a statistically significant association that was consistent in direction with the association in individuals of European ancestry (Table 4). In data from 6,899 African–Americans, four SNPs from

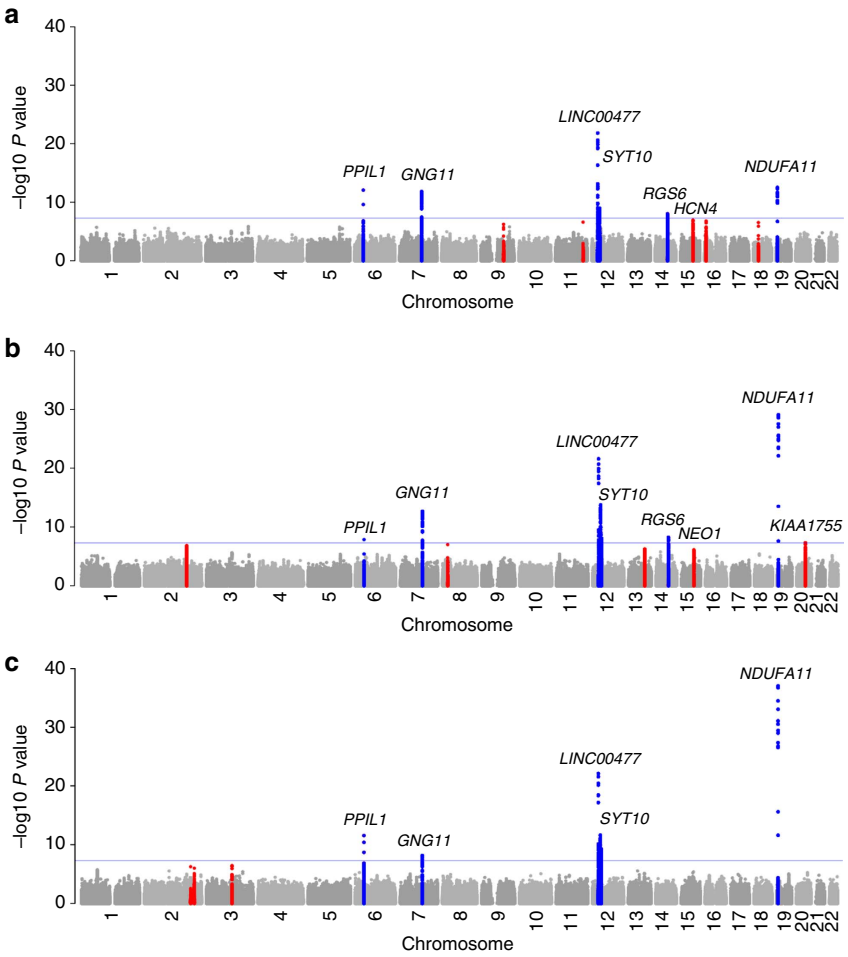

Figure 1 | Manhattan plots of the meta-analyses of stage 1 GWAS results. (**a**) SDNN, (**b**) RMSSD and (**c**) pvRSA/HF in up to 28,700 individuals of European ancestry. Only SNPs with a minor allele frequency >1% and that were present in at least 1/3 of the sample are plotted. Significant loci are shown in blue, suggestive ones in red. The blue horizontal line represents the genome-wide significance threshold. Genes closest to the lead SNPs are indicated for the loci that were genome-wide significantly associated with the trait after the stage 1 + 2 combined meta-analysis.

four of the eight loci were associated with RMSSD, three SNPs in three of the seven loci with SDNN and none with pvRSA/HF. In the combined meta-analysis in a maximum of 71,675 participants from all ethnicities, one SNP (rs6123471 on chromosome 20) was no longer significant (Table 4).

Correcting HRV for heart rate. The strong inverse association between HRV and heart rate reflects the well-established simultaneous biological effect of cardiac vagal activity on heart rate[15] and HRV[16], but it also expresses a mathematical dependency of the variance in inter beat interval (IBI) on the mean IBI that is unrelated to the underlying biology. We conducted three analyses to test whether the association of the HRV SNPs was robust to correction of the HRV traits for heart rate (Supplementary Table 8). First, we used a recently developed analytical technique[17] to obtain the meta-analysis for the coefficient of variation of SDNN and RMMSD from the summary statistics of the HRV and resting heart rate meta-analyses[18]. The coefficient of variation detects the amount of IBI variability relative to the mean IBI of each subject, and deals with the proportionality-based dependence of HRV on heart rate[19]. Second, we established the effect of the 17 HRV SNPs on the coefficients of variation for SDNN and RMSSD in the Lifelines, NESDA and TRAILS-Pop cohorts, and meta-analysed the results. Third, we use a mediation analysis in these same cohorts to see how much of the SNP effects on the three HRV measures was mediated by heart rate. In all three analyses, we find some attenuation of the HRV SNP associations. The average mediation of the association by heart rate was ~28%. However, the correction for heart rate left most of the HRV SNP associations intact, particularly in the first analysis that used the full discovery sample.

Association of the HRV SNPs with resting heart rate. Because the HRV traits reflect cardiac vagal activity, we expected the HRV SNPs to have an effect on resting heart rate. We performed a lookup of the 17 HRV SNPs in a GWAS meta-analysis on resting heart rate in 85,787 individuals[18]. Out of the 17 HRV lead SNPs, 11 were associated with heart rate after correcting for multiple testing (Supplementary Table 9, panel a). All effects were in the expected direction such that the HRV decreasing allele was associated with higher heart rate (Supplementary Fig. 6). Six of the HRV SNPs were not significantly associated with heart rate, including our top hit on chromosome 19 (rs12974991 in *NDUFA11*: p RMSSD $= 4.6 \times 10^{-46}$; p heart rate $= 0.18$). Analysis of summary statistics of the HRV and heart rate meta-analyses as implemented in the *gtx* R package showed that multi-SNP genetic risk scores for HRV were significantly associated with heart rate (Supplementary Table 9, panel b).

Table 1 | Stage 1 + 2 combined meta-analysis results for SDNN, RMSSD and pvRSA/HF of loci that were genome-wide significant ($P < (5 \times 10^{-8})/3$) in the analysis of individuals of European ancestry.

Locus	Chr	SNP	Position (bp) (build 36)	Closest gene	Annotation	Trait	Allele	Stage 1 + 2			
							E/O	*N*	EAF	*β* (s.e.)	*P* value
1	19	rs12974991*	5845584	*NDUFA11*	IN	RMSSD	A/G	43,205	0.078	−0.116(0.008)	**4.57E-46**
		rs12974440*	5845386		IN	pvRSA/HF†	A/G	29,527	0.073	−0.244(0.019)	**1.91E-41**
		rs12980262*	5844058		M	SDNN	A/G	46,046	0.076	−0.060(0.006)	**2.30E-23**
2	12	rs10842383	24663234	*LINC00477 (C12orf67)*	IG, HR[i]	SDNN	C/T	47,808	0.863	−0.049(0.004)	**9.33E-31**
						RMSSD		43,223	0.862	−0.065(0.006)	**2.45E-29**
						pvRSA/HF†		31,085	0.865	−0.124(0.013)	**1.20E-25**
3	6	rs236349	36928543	*PPIL1*	IG	SDNN	G/A	51,379	0.651	−0.033(0.003)	**3.70E-25**
						RMSSD		46,795	0.655	−0.035(0.004)	**9.10E-17**
						pvRSA/HF†		33,654	0.645	−0.069(0.009)	**3.16E-15**
4	12	rs7980799‡	33468257	*SYT10*	IN, HR[ii]	RMSSD	A/C	44,210	0.390	−0.039(0.004)	**3.19E-20**
		rs1351682‡	33490042		IG, HR[iii]	pvRSA/HF†	G/A	30,643	0.437	−0.073(0.009)	**5.70E-15**
		rs1384598‡	33514166		IG, HR[iv]	SDNN	T/A	47,358	0.432	−0.023(0.003)	**7.37E-13**
5	7	rs4262§	93389364	*GNG11*	UTR5, Q, HR[v]	SDNN	C/T	49,005	0.390	−0.028(0.003)	**4.26E-17**
						pvRSA/HF†		31,281	0.388	−0.050(0.010)	**1.84E-11**
		rs180238§	93388383		UP, Q, HR[vi]	RMSSD	C/T	44,420	0.333	−0.034(0.004)	**7.99E-16**
6	14b	rs4899412‖	71534015	*RGS6*	IN, Q	SDNN	T/C	48,252	0.253	−0.026(0.004)	**3.13E-13**
		rs2052015‖	71556806			RMSSD	T/C	45,492	0.165	−0.036(0.006)	**3.56E-10**
	14c	rs2529471	71883022		IN	SDNN	C/A	49,619	0.429	−0.021(0.003)	**1.88E-12**
	14a	rs36423	71422955		IG	SDNN	T/G	48,182	0.129	−0.033(0.005)	**6.25E-13**
						RMSSD		45,419	0.127	−0.040(0.006)	**5.36E-11**
7	15a	rs2680344	71440538	*HCN4*	IN, HR[vii]	SDNN	A/G	51,370	0.777	−0.024(0.004)	**4.88E-11**
	15b	rs1812835	71294557	*NEO1*	IN, Q	RMSSD	A/C	44,421	0.418	−0.025(0.004)	**5.18E-10**
8	20	rs6123471	36273570	*KIAA1755*	UTR3, HR[viii]	RMSSD	T/C	46,789	0.534	−0.024(0.004)	**1.30E-08**

Allele E/O, effect allele/other allele; bp, base pair position based on build 36 (hg18); Chr, chromosome; EAF, effect allele frequency; HR, HRV SNPs that are in pairwise LD (based on SNAP, HapMap release 22 CEU) with identified loci associated with heart rate (HR) from den Hoed *et al.*[18]; IG, intergenic variant; IN, intronic variant; *N*, sample size; M, missense variant; Q, associated with an eQTL; s.e., standard error of *β*; UTR3, variant in the 3′ untranslated region; UTR5, variant in the 5′ untranslated region; UP, upstream variant (within 2kb); *β*, effect size.
NOTE: 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 (dependent) lead SNPs for the different traits. SNPs are sorted according to *P* value of the combined meta-analysis per locus. Genome-wide significant association (two-sided $P < 5 \times 10^{-8}$), corrected for testing three traits (that is, $P < 5 \times 10^{-8}/3$), is shown in bold. Effect alleles were chosen to reflect an increased risk for *low* levels of HRV, hence *β*'s are all negative.
[i] $r^2 = 1$ between rs10842383 and rs17287293[HR]; [ii] same SNP; [iii] $r^2 = 0.782$ between rs1351682 and rs7980799[HR]; [iv] $r^2 = 0.695$ between rs1384598 and rs7980799[HR]; [v] $r^2 = 0.570$ between rs426[2] and rs180242[HR]; [vi] $r^2 = 0.893$ between rs180238 and rs180242[HR]; [vii] $r^2 = 0.505$ between rs2680344 and rs4489968[HR]; [viii] $r^2 = 1$ between rs6123471 and rs6127471[HR].
*these SNPs are all in perfect LD ($r^2 = 1$).
†*P* value, allele, EAF, N from *P* value weighted meta-analysis of all cohorts using METAL and *β*, s.e. from inverse-variance meta-analysis of only HF cohorts using GWAMA.
‡$r^2 = 0.782$ between rs7980799 and rs1351682; $r^2 = 0.695$ between rs7980799 and rs1384598; $r^2 = 0.903$ between rs1351682 and rs1384598.
§$r^2 = 0.600$ between rs4262 and rs180238.
‖$r^2 = 0.237$ between rs4899412 and rs2052015.

In addition, genetic risk scores based on the independent genome-wide significant HRV SNPs from the combined stage 1 and 2 meta-analysis were tested for association with heart rate in the Lifelines, NESDA, TRAILS-Pop and ABCD cohorts (Supplementary Table 9, panel b). The three multi-SNP risk scores of the HRV traits explained a small, but mostly significant percentage of variance in heart rate (0.09–1.13%). Polygenic risk score analysis showed that adding HRV SNPs below the genome-wide significance threshold did not further increase the variance explained in heart rate (Supplementary Fig. 5; Supplementary Table 9, panel c).

The reverse question, whether SNPs with effects on heart rate are associated with HRV, was also investigated. The 21 heart rate SNPs identified by the GWAS meta-analysis on heart rate[18] explained between 0.2 and 0.9% of the variance in the three HRV traits (Supplementary Table 10).

Association with cardiometabolic traits and diseases. In addition to heart rate, we examined the association of the 17 HRV-associated SNPs with other confirmed risk factors for cardiac, metabolic and renal disease traits and endpoints using data from large-scale GWAS meta-analyses (Supplementary Table 11). Multi-SNP risk scores were computed based on our 17 top SNPs and we tested their association with the outcomes.

No effects of risk scores using the 17 HRV SNPs were observed for systolic or diastolic blood pressure, body mass index, renal function, heart failure, sudden cardiac death, coronary artery disease, atrial fibrillation or type 2 diabetes. Only for atrial fibrillation we observed individually significant SNPs. These two highly significant SNPs (rs10842383 near *LINC00477*, $P = 3.45 \times 10^{-7}$ and rs2680344 in *HCN4*, $P = 4.34 \times 10^{-7}$ (Supplementary Table 12) had large opposite effects on atrial fibrillation, while both decreased HRV. In addition to these lookups that were restricted to genome-wide significant SNPs, we employed bivariate LD score regression[20] that uses the full GWAS summary statistics of the HRV and cardiometabolic traits and diseases to compute genetic correlations. The genetic correlations systematically pointed to an overlap in the genetic variants causing low HRV and increased risk for disease (that is, negative

Table 2 | Explained variance in HRV traits in the Lifelines ($n=12,101$), NESDA ($n=2,118$), TRAILS-Pop ($n=1,191$) and ABCD ($n=1,094$) cohorts by the weighted multi-SNP genetic risk score based on the independent genome-wide significant SNPs in the stage 1 + 2 meta-analysis.

Trait	Risk score	Lifelines			NESDA			TRAILS-Pop			ABCD		
		No. SNPs	*P* value	ΔR^2	No. SNPs	*P* value	ΔR^2	No. SNPs	*P* value	ΔR^2	No. SNPs	*P* value	ΔR^2
SDNN	SDNN	9	6.3E-33	1.00%	9	4.9E-10	1.39%	10	8.0E-05	1.28%	10	7.8E-04	1.03%
SDNN	RMSSD	7	1.1E-30	0.93%	11	7.5E-10	1.35%	11	6.3E-06	1.69%	11	3.5E-05	1.56%
SDNN	pvRSA/HF	5	6.4E-25	0.75%	5	5.2E-07	0.89%	5	8.7E-07	1.99%	4	4.3E-03	0.75%
RMSSD	SDNN	9	8.3E-37	1.13%	9	2.0E-11	1.54%	10	4.4E-06	1.73%	10	4.7E-04	1.11%
RMSSD	RMSSD	7	8.8E-37	1.13%	11	6.1E-12	1.62%	11	5.3E-08	2.42%	11	2.5E-06	2.01%
RMSSD	pvRSA/HF	5	1.5E-30	0.93%	5	2.0E-11	1.54%	5	2.1E-09	2.92%	4	8.1E-04	1.02%
pvRSA/HF	SDNN	7	NA	NA	9	1.5E-13	1.58%	10	4.3E-05	1.38%	10	2.0E-03	0.87%
pvRSA/HF	RMSSD	6	NA	NA	11	7.1E-14	1.62%	11	1.3E-06	1.93%	11	1.5E-05	1.70%
pvRSA/HF	pvRSA/HF	5	NA	NA	5	7.7E-17	2.01%	5	1.4E-08	2.64%	4	1.8E-03	0.89%

NA, not available.
NOTE: ΔR^2 is the difference in percentage of explained variance by the multi-SNP genetic or polygenetic risk score between the models with and without the risk score while adjusting both for age, sex and principal components.
For Lifelines, NESDA and TRAILS-Pop the weights (that is, effects sizes) and number of genome-wide significant SNPs included in the risk score were adjusted by analytically extracting the cohort's effect size and s.e. from the meta effect size and s.e., respectively, and recalculating the *P* value based on these adjusted effect sizes and s.e.'s, since these cohorts were included in stage 1 and/or 2.

correlations with systolic and diastolic blood pressure, coronary artery disease, heart failure, sudden cardiac death, BMI and type 2 diabetes) compatible with clinical relevance of the HRV SNPs identified, although significance was reached only for systolic and diastolic blood pressure after correction for number of outcomes tested (Supplementary Table 11).

Potential functional impact of the HRV variants. To identify functional variants tagged by the 17 HRV SNPs, we performed various post-GWAS annotation (Supplementary Fig. 7). *In silico* annotation (Supplementary Data 2) showed that the lead SNP for SDNN on chromosome 19 was a non-synonymous SNP (rs12980262 in *NDUFA11*) and that the lead SNPs for RMSSD (rs12974991) and pvRSA/HF (rs12974440) were in perfect LD with this SNP (Table 1; Supplementary Data 2). SNP rs129080262 was characterized as deleterious, with a sorting intolerant from tolerant (SIFT) score of 0.01 and a polymorphism phenotyping (PolyPhen) score of 0.753 indicating a possibly damaging effect. Functional variant analyses using RegulomeDB confirmed that rs12980262 and rs12974440 in *NDUFA11* on chromosome 19 likely have functional consequences (Supplementary Table 13) by binding to transcription factors or influencing the chromatin state. SNP rs6123471 in the locus on chromosome 20 was in high LD with two non-synonymous SNPs in the *KIAA1755* gene (rs3746471 [$r^2=0.94$] and rs760998 [$r^2=0.55$]) that are predicted to yield tolerated, benign amino acid changes (Supplementary Data 2).

We examined if the 17 HRV SNPs were eQTLs in a large whole blood database. Four of the HRV SNPs were significantly (false discovery rate $<5\%$) associated with gene expression in blood (Supplementary Table 14): rs1812835 with expression of *NEO1*, rs4899412 with expression of *RGS6*, and rs180238 and rs4262 with expression of *GNG11*. These four SNPs were all in strong LD with the top eQTL SNPs for these genes ($r^2>0.70$) and lost significance after conditioning on the corresponding top eQTL. The eQTLs for *NEO1* and *RGS6* were replicated in at least one other whole blood eQTL study (Supplementary Table 14). The eQTL for *GNG11* was replicated in the medulla ($P=2.8\times10^{-4}$) and the anterior tibialis artery ($P=8.1\times10^{-9}$). None of the 17 SNPs reached significance in a smaller heart eQTL database.

Nine of the 17 HRV SNPs were in high LD ($r^2>0.70$) with SNPs associated with methylation level of one or multiple CpG sites (methylation quantitative trait loci [mQTLs]) in whole blood (Supplementary Table 15). Two of the HRV SNPs that were eQTLs also influenced methylation of the same gene in whole blood, strongly suggestive of a regulatory function for those SNPs. eQTL rs1812835 in *NEO1* was associated with methylation level of cg11357013, cg19281068, cg11552023 and cg17150474. eQTL rs4262 was associated with methylation level of cg08038054 and cg06439941 in *GNG11*. The other two eQTL SNPs did not achieve genome-wide significance level for an association with methylation, but eQTL rs4899412 in *RGS6* was in high LD with a proxy SNP (rs2238280) that was associated with methylation level of cg19493789, which is located in a CpG island shelf near *RGS6*.

Five other HRV SNPs were (in high LD with) mQTLs but were not themselves eQTLs. For example, HRV SNPs rs12974991, rs12974440 and rs12980262 (chromosome 19) were associated with methylation level of multiple CpG sites (cg22854549, cg03715305 and cg19211619) located in or nearby *NDUFA11*, but were not associated with expression level of *NDUFA11* in whole blood. Such mQTLs may well exert a regulatory effect on *NDUFA11* in other tissues. DEPICT tissue enrichment analysis (Supplementary Data 3; Supplementary Table 16, Supplementary Fig. 8) showed *NDUFA11* expression was weak in blood, but enriched in heart, sensory and endocrine tissues.

Discussion

This meta-analysis of GWAS for HRV yielded 17 lead SNPs (11 independent) in eight loci that were genome-wide significantly associated, six of which generalized to individuals of African-American and Hispanic/Latino ethnicity. Various ways that correct HRV for its mathematical dependency on resting heart rate attenuated the SNP effects, but largely left the associations intact. Together, the hits in the eight loci explained 0.9–2.6% of the variance in resting HRV in four independent cohorts of European ancestry. Details of known biological functions of the genes closest to these loci are given in the Supplementary Note 6.

We noted a strong enrichment of our HRV loci in a previously conducted meta-analysis of GWAS for resting heart rate[18], a known risk factor for cardiac morbidity and mortality[21,22]. SNPs in five of the 21 resting heart rate loci (that is, *LINC00477 (C12orf67)*, *SYT10*, *GNG11*, *HCN4* and *KIAA1755*) were associated with HRV at genome-wide significance level and six more attained nominal significance, with associations always in the expected direction. Genetic risk

Table 3 | Explained variance in HRV traits in the Lifelines ($n=12,101$), NESDA ($n=2,118$), TRAILS-Pop ($n=1,191$) and ABCD ($n=1,094$) cohorts by the optimal polygenic risk scores computed at the *P* value threshold that explained the largest percentage of phenotypic variance.

Trait	Risk score	Cohort	*P* cutoff	No. SNPs	*P* value	ΔR^2
SDNN	SDNN	Lifelines	<5E-7	13	6.8E-27	0.82%
		NESDA	<5E-8	6	2.6E-08	1.16%
		TRAILS-Pop	<5E-5	64	1.1E-04	1.23%
		ABCD	<5E-5	71	9.4E-05	1.39%
SDNN	RMSSD	Lifelines	<5E-8	8	2.4E-23	0.71%
		NESDA	<5E-6	23	1.2E-07	1.05%
		TRAILS-Pop	<5E-8	8	1.2E-04	1.23%
		ABCD	<5E-7	13	2.8E-06	2.00%
SDNN	pvRSA/HF	Lifelines	<5E-8	7	3.1E-19	0.58%
		NESDA	<5E-8	4	3.5E-05	0.64%
		TRAILS-Pop	<5E-7	6	9.7E-06	1.61%
		ABCD	<5E-5	67	9.2E-04	1.01%
RMSSD	SDNN	Lifelines	<5E-7	13	8.9E-31	0.95%
		NESDA	<5E-8	6	1.6E-10	1.46%
		TRAILS-Pop	<5E-8	7	8.3E-06	1.63%
		ABCD	<5E-5	71	1.6E-04	1.30%
RMSSD	RMSSD	Lifelines	<5E-7	12	2.8E-30	0.94%
		NESDA	<5E-7	10	2.7E-10	1.43%
		TRAILS-Pop	<5E-7	11	3.4E-07	2.13%
		ABCD	<5E-7	13	3.8E-07	2.34%
RMSSD	pvRSA/HF	Lifelines	<5E-8	7	1.4E-25	0.78%
		NESDA	<5E-8	4	3.6E-09	1.25%
		TRAILS-Pop	<5E-7	6	3.7E-08	2.47%
		ABCD	<5E-8	67	8.4E-04	1.02%
pvRSA/HF	SDNN	NESDA	<5E-8	6	1.1E-12	1.52%
		TRAILS-Pop	<5E-8	7	5.0E-05	1.36%
		ABCD	<5E-5	71	5.4E-04	1.09%
pvRSA/HF	RMSSD	NESDA	<5E-7	10	5.6E-14	1.69%
		TRAILS-Pop	<5E-7	11	3.3E-06	1.78%
		ABCD	<5E-7	13	1.9E-06	2.06%
pvRSA/HF	pvRSA/HF	NESDA	<5E-8	4	4.4E-13	1.58%
		TRAILS-Pop	<5E-7	6	1.6E-07	2.25%
		ABCD	<5E-5	67	1.6E-03	0.90%

NA, not available.
NOTE: Weighted polygenic risk score was determined based on independent SNPs in the stage 1 meta-analysis. For NESDA and TRAILS-Pop the weights (that is, effects sizes) and *P* values were adjusted by analytically extracting the cohort's effect size and s.e. from the meta effect size and s.e., respectively, and recalculating the *P* value based on these adjusted effect size and s.e., since these cohorts were included in stage 1.

scores for HRV traits were also significantly associated with heart rate and LD score regression confirmed that the allelic variants that decrease HRV in parallel increase heart rate. This suggests to us that part of the HRV SNPs exert their effect on heart rate through oscillatory modulation of pacemaker activity by the vagal nerves.

Supplementary Fig. 9 depicts the two routes by which acetylcholine released by the vagal nerves in the sinoatrial node is known to influence heart rate, both of which are supported by our results in *GNG11*, *RGS6* and *HCN4*. By binding the muscarinic type 2 receptor (M_2R) and dissociating the G-protein heterotrimer ($G\alpha\beta\gamma$) into a $G\alpha_{i/o}$ subunit and a $G\beta\gamma$ component, acetylcholine inhibits the ongoing depolarization of the pacemaker cells by β1/β2-adenylatecyclase activation of funny (I_f) channels and calcium channels[23]. In parallel, it acts to actively hyperpolarize the pacemaker cells by activation of the GIRK1/4 channel. Each route accounts for about half of the tonic decrease in heart rate upon vagal stimulation[23], but the response time for M_2R-GIRK effects on the sinus rate is much shorter than for the M_2R-HCN2/4 or the β1/β2-adenylatecyclase signalling pathways. Only signalling through the $G\beta\gamma$ component is fast enough ($\sim$0.3 s) to rapidly track changes in vagal outflow to the sinoatrial node, for example, as they occur within the duration of a single respiration ($\sim$4.5 s), whereas signalling through the α subunit is too slow (>3 s) to track such phasic changes in acetylcholine release[1,24]. GIRK signalling, therefore, accounts for most of HRV due to the phasic oscillation in vagal activity[24], but it accounts for only half of the tonic vagal effects on heart rate.

The above leads to HRV only partially capturing the vagal effects on heart rate. Additional reasons for the imperfect relation between HRV and vagal effects on heart rate[1,2] are individual differences in: (i) resting respiration rate and depth; (ii) the amplitude of the intrinsic 0.1 Hz oscillations related to both vagal

Table 4 | Meta-analysis results for the identified loci in other ethnicities and combined meta-analysis results with European ancestry.

Locus	Chr	SNP	Trait	Allele	Hispanic/Latino				African American				EUR + HIS + AfAm
				E/O	*N*	EAF	*β* (s.e.)	*P* value	*N*	EAF	*β* (s.e.)	*P* value	*P* value
1	19	rs12974991	RMSSD	A/G	11,233	0.065	−0.162 (0.018)	**7.05E-20**	6,673	0.455	−0.077 (0.033)	**1.10E-02**	**1.86E-63**
		rs12974440	pvRSA/HF*	A/G	404	0.048	−0.518 (0.174)	**3.06E-03**	1900	0.019	−0.189 (0.158)	3.41E-01	**4.53E-41**
		rs12980262	SDNN	A/G	11,233	0.048	−0.161 (0.070)	**1.04E-02**	6675	0.093	−0.046 (0.030)	6.48E-02	**1.57E-24**
2	12	rs10842383	SDNN	C/T	11,233	0.854	−0.053 (0.012)	**2.45E-06**	6676	0.955	0.056 (0.026)	9.83E-01	**7.61E-33**
			RMSSD		11,233	0.854	−0.064 (0.012)	**1.38E-07**	6673	0.955	0.065 (0.030)	9.86E-01	**4.23E-32**
			pvRSA/HF*		404	0.830	−0.140 (0.095)	1.40E-01	1901	0.959	0.068 (0.104)	6.79E-01	**4.98E-25**
3	6	rs236349	SDNN	G/A	11,234	0.684	−0.034 (0.009)	**6.15E-05**	6676	0.724	−0.017 (0.011)	6.57E-02	**1.76E-28**
			RMSSD		11,234	0.684	−0.034 (0.009)	**1.67E-04**	6673	0.724	−0.021 (0.013)	**4.79E-02**	**5.88E-20**
			pvRSA/HF*		404	0.704	−0.164 (0.080)	**4.13E-02**	1901	0.729	0.004 (0.043)	4.87E-01	**4.64E-15**
4	12	rs7980799	RMSSD	A/C	11,234	0.269	−0.031 (0.010)	**1.23E-03**	6488	0.097	−0.029 (0.021)	7.70E-02	**1.57E-22**
		rs1351682	pvRSA/HF*	G/A	404	0.348	−0.166 (0.077)	**3.19E-02**	1901	0.142	−0.082 (0.058)	6.91E-02	**2.00E-14**
		rs1384598	SDNN	T/A	11,234	0.307	−0.026 (0.009)	**1.80E-03**	6676	0.146	−0.024 (0.015)	5.41E-02	**2.88E-15**
5	7	rs4262	SDNN	C/T	11,234	0.427	−0.016 (0.008)	**2.39E-02**	6676	0.608	−0.028 (0.011)	**5.87E-03**	**5.36E-19**
			pvRSA/HF*		404	0.410	−0.014 (0.074)	8.46E-01	1901	0.618	−0.055 (0.043)	1.15E-01	**1.50E-11**
		rs180238	RMSSD	C/T	11,234	0.367	−0.024 (0.009)	**4.05E-03**	6673	0.474	−0.032 (0.011)	**2.77E-03**	**8.07E-19**
6	14b	rs4899412	SDNN	T/C	11,234	0.329	−0.012 (0.009)	8.27E-02	6676	0.419	−0.009 (0.010)	1.86E-01	**5.96E-13**
		rs2052015	RMSSD	T/C	11,234	0.173	−0.015 (0.012)	9.94E-02	6673	0.098	−0.001 (0.020)	4.83E-01	**1.94E-09**
	14c	rs2529471	SDNN	C/A	11,233	0.485	−0.018 (0.008)	**1.38E-02**	6676	0.543	0.003 (0.010)	3.83E-01	**2.08E-12**
	14a	rs36423	SDNN	T/G	11,234	0.193	−0.021 (0.011)	**2.41E-02**	6676	0.160	−0.030 (0.015)	**1.79E-02**	**1.60E-14**
			RMSSD		11,234	0.193	−0.017 (0.011)	7.05E-02	6673	0.160	−0.034 (0.016)	**1.72E-02**	**1.02E-11**
7	15a	rs2680344	SDNN	A/G	11,234	0.681	−0.005 (0.009)	2.97E-01	6676	0.450	−0.024 (0.011)	**1.32E-02**	**2.90E-11**
	15b	rs1812835	RMSSD	A/C	11,234	0.426	−0.012 (0.009)	8.83E-02	1388	0.140	−0.009 (0.033)	3.98E-01	**5.30E-10**
8	20	rs6123471	RMSSD†	T/C	11,234	0.560	−0.001 (0.009)	4.40E-01	6673	0.739	0.020 (0.013)	5.67E-02	5.14E-06

AfAm, African American; Allele E/O, effect allele/other allele; Chr, chromosome; bp, base pair position based on build 36 (hg18); EAF, effect allele frequency; EUR, European; HIS, Hispanic/Latino; *N*, sample size; s.e., standard error of *β*; *β*, beta/effect size.
NOTE: SNPs sorted as in Table 1 according to the European ancestry combined meta-analysis *P* value per locus. Significant Ps are shown in bold (see text for criteria). Effect alleles were chosen to reflect an increased risk for low levels of HRV, hence *β*'s are all negative.
**P* value, allele, EAF, *N* from *z*-score weighted meta-analysis of all cohorts using METAL and *β*, s.e. from inverse-variance meta-analysis of only HF cohorts using GWAMA.
†*β* of participants of European ancestry differs significantly from that of participants from African-American (diff $\beta = 0.044$, $P = 0.0012$) or Hispanic/Latino ancestry (diff $\beta = -0.023$, $P = 0.0195$).

and sympathetic blood pressure regulation through the baroreflex loops; (iii) mechanotransduction or intracellular pathways stimulated by sinoatrial stretch or (iv) the efficiency of the actual vagal gating process. These processes can have a strong impact on HRV, but less so on mean heart rate. We found six SNPs in four loci, including our top hit (rs12974991 in *NDUFA11*), that may act on the individual differences in these processes as they had no discernible effect on heart rate, in spite of their significant impact on HRV.

The genome-wide significant SNPs in *GNG11*, *RGS6* and *NEO1* were eQTLs and in strong LD with the top mQTLs and eQTLs for the corresponding genes. Two of these (*GNG11*, *RGS6*) readily provide a biological hypothesis to account for the associations detected in the meta-analysis. The C alleles of rs4262 and rs180238 of *GNG11* coding for the γ11 subunit of the heterotrimeric G-protein complex Gαβγ cause decreased expression of this subunit and were associated with lower HRV. The effects of the *GNG11* eQTLs associated with lower HRV are likely to lower the availability of the γ11 subunit, thereby reducing Gβγ component-induced GIRK activation. This potentially blunts the heart rate change in response to the oscillatory changes in cardiac vagal activity.

The regulator of heterotrimeric G-protein complex signalling, type 6 (*RGS6*) gene on chromosome 14 was found to be linked to three independent signals for SDNN and RMSSD. RGS6 acts as a critical negative regulator of M_2R signalling in the sinoatrial node of the heart rapidly terminating Gβγ signalling and thus curtailing vagal lowering of the heart rate[25,26]. The results of our meta-analysis are consistent with a role for *RGS6* in decreasing HRV previously hinted at by animal experimentation[23,27] and a human case report[27,28]. The T allele of our eQTL *RGS6* SNP (rs4899412) causes increased expression of *RGS6*. By increasing *RGS6* expression, the T allele acts as a gain-of-function mutation that gives rise to a decrease in GIRK-channel signalling and the observed decrease in HRV. Of note, $Rgs6^{-/-}$ mice, that show the expected increase in HRV, are characterized by a strong bradycardia and an increased susceptibility to AV block and atrial fibrillation which is attributed to an enhancement of GIRK-induced sinoatrial and atrioventricular node hyperpolarization by removing the negative regulation of Gβγ by RGS6 (refs 23,26,28).

The association of the rs2680344 SNP in *HCN4* is puzzling because HCN signalling does not involve the fast M_2R-GIRK channels and cannot translate rapid vagal fluctuation into beat-to-beat variation in IBI length, that is, HRV. The effect of the *HCN4* SNP on HRV may be secondary to its effects on the average slope of the diastolic depolarization[29]. The HCN4 protein is a key component of the I_f channel[30–32] that generates the pacemaker potential by a gradual depolarization of the sinoatrial myocyte cell membrane during diastole. This 'pacemaker depolarization' phase is known to be slowed by loss-of-function mutations in the *HCN4* that lead to lower heart rate[31] and the I_f is the known site of action for ivabradine and other therapeutic agents used to slow heart rate in angina patients[32]. Of note, both ivabradine treatment[33] and loss-of-function mutations increase the risk for atrial fibrillation[34]. In contrast, gain-of-function mutations in the sensitivity of *HCN4* for cAMP lead to higher heart rate[30]. This leads us to hypothesize that the A allele of rs2680344 in *HCN4* either is itself a gain-of-function mutation or tags such a mutation because it increases heart rate[18].

High HRV is associated with lower morbidity and mortality in patients with cardiovascular disease[5], hypertension[7], end-stage

renal disease[8] and diabetes[9], but also in apparently healthy individuals[11,12]. Using LD score regression on meta-GWAS summary statistics from various risk factors and endpoints we find some evidence for overlap in the genetic variants causing low HRV and increased risk for disease, but significance was reached only for systolic and diastolic blood pressure after correction for multiple outcomes tested. These genetic correlations are compatible with causal effects of cardiac vagal control in the aetiology of disease, but they could also be ascribed to reversed causality, where the disease process leads to lower cardiac vagal control. A strength of this study in this regard is that analyses were confined to individuals in good cardiac health, that is, cohorts excluded patients with existing cardiovascular diseases or medication potentially impacting HRV. Because we selected individuals in good cardiac health reverse effects of disease on HRV seem less likely, although some latent pathology could have been present. However, an alternative explanation that is harder to rule out is that the genetic correlation derives from pleiotropic effects of genetic variants common to both outcomes.

Further strengths of this study were the consistency of results across the different HRV traits used to capture cardiac vagal control and the generalization of the HRV SNP effects to different ancestries, in spite of known ethnic differences in absolute resting HRV[35]. Results also held in men and women separately and across a very large range of mean cohort ages spanning from early childhood to the late middle ages; in spite of a strong reduction in HRV values with aging[36]. Although effects of age and sex on HRV were taken into account in the analyses, many other factors were not. The ideal design would have corrected for the known effects of respiration depth and rate on HRV, which are independent of vagal activity[37]. These could not be added as covariates because they were not available in most cohorts. We were liberal in excluding other covariates like BMI, smoking and exercise in the GWAS analyses. These traits are substantially heritable themselves and adjusting for heritable covariates can bias the genome-wide association effects[38] or even induce non-existing associations through collider bias[39]. Finally, instructions on pre-ECG recording behaviours like physical activity, and caffeine, alcohol or nicotine use were not rigorously standardized across cohorts.

Direct clinical relevance of most current GWAS findings is still low and our study is no exception. Potential future clinical use of our findings hinges on the ability of our genetic variants to capture (sub)cortical, brainstem and medullary transmission of tonic vagal activity to the sinoatrial node, not just the impact of that activity on heart rate. Subcortical generation of tonic vagal activity is an important biomarker for cardiovascular health and potentially modifiable by interventions on psychosocial stress[40] and lifestyle habits[41]. It can even be a transdiagnostic biomarker for psychopathology and executive cognitive functioning possibly by reflecting the integrity of prefrontal cortex functioning[42]. Genetic markers for HRV may prove useful as instrumental variables in Mendelian randomization[43] to test causal hypotheses on the effects of centrally generated vagal activity on behavioural and health outcomes.

In conclusion, this meta-analysis detects a critical role for genetic variation in Gβγ and HCN signalling in explaining individual differences in HRV. The HRV variants detected can help guide further investigations of the functional consequences and potential therapeutic implications of individual differences in sinoatrial Gβγ signalling.

Methods

Study cohorts. Appropriate IRB approval and informed consent from participants in all participating cohorts was obtained. Full information on consent procedures and details of the IRB boards are provided in the Supplementary Note 8.

HRV measurement. In this study, we investigated three HRV traits: SDNN, the RMSSD and pvRSA or HF. SDNN and RMSSD were derived from the IBI time series obtained from the R waves in the ECG[4]. HF was calculated from Wavelet or Fourier decomposition with power obtained from a high frequency band of either 0.15–0.40 Hz or 0.15–0.50 Hz. A time domain measure of RSA was derived by pvRSA using a respiratory signal co-registered with the ECG. Estimates of pvRSA are 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.

HRV traits were extracted from the IBI time series preferably based on 2–10 min periods of ECG in a standardized setting, at rest and in a sitting/supine position. If ambulatory data were available, we advised cohorts to extract a period of sitting still in the evening, when this proved feasible. Supplementary Table 2 lists the actual way HRV was assessed by the participating cohorts. For the cohorts analysed in stage 2, we extended our HRV measurements to include cohorts with 10 s and/or 20 s ECG recordings, as RMSSD and SDNN based on these ultra-short recordings have shown a good agreement with 4–5 min recordings[3]. Furthermore, since IBI time series require reliable detection of the R-wave only, a three-lead ECG was considered sufficient while the use of more leads was encouraged. For pvRSA, an additional respiration signal of sufficient quality to detect beginning and end of inspiration and expiration was needed.

SDNN and RMSSD have prevailed in epidemiological studies because they are more easily assessed in large cohorts and, as noted above, can be obtained even from short ECG recordings. HF and pvRSA were available in fewer cohorts, but they better reflect the cardiorespiratory coupling that drives the oscillatory modulation of vagal effects in the sinoatrial node. In the typical resting respiratory frequency range, these time- and frequency-domain measures of RSA are much less contaminated by oscillations in cardiac sympathetic control than SDNN (and other measures of HRV that span a broader frequency range). This is due to the temporal dynamics of the sinoatrial node signalling pathway that acts as a low pass filter allowing only oscillations in vagal effects to translate into HRV, whereas for sympathetic effects or vagal effects at progressively higher respiratory frequencies the node acts as a leaky integrator causing more tonic changes in heart rate[1]. Phasic modulation of vagal effects is therefore captured most purely by pvRSA or HF. Because pvRSA and HF are conceptually similar and highly correlated with each other ($r > 0.80$) across a wide range of values for respiration and heart rates[44], we grouped the analyses on pvRSA and HF under the label pvRSA/HF.

Study population. Cohorts that had data on at least one of the three HRV traits and genome-wide data were invited to participate in the first (discovery) stage of the Genetic Variance in Heart Rate Variability (V_gHRV) consortium. The stage 1 discovery analysis was performed in up to 28,700 individuals of European ancestry from a maximum of 20 cohorts. Independent cohorts with either genome-wide or gene-centred array data or with the ability to perform wet-lab genotyping on the single-nucleotide polymorphism (SNPs) taken forward from the first stage were included in the second (replication) stage. This stage included additional data from up to 24,474 individuals from 11 cohorts of European ancestry (see Supplementary Tables 1–4 for cohort descriptions and details).

Association analysis: stage 1 (discovery). The following exclusion criteria were applied a priori: (1) individuals with heart disease (for example, angina, past myocardial infarction, left ventricular failure) and (2) individuals known to use antidepressants (particularly tricyclic antidepressants) and all anticholinergic agents (for example, digoxin, atropine and acetylcholinesterase inhibitors) because of the strong effects that these drugs have on HRV. Individuals reporting over the counter use of anticholinergic agents were not excluded.

Imputation of SNPs was done to extend and create similar SNP databases between cohorts using different genotyping platforms. Most of the cohorts used the HapMap Phase II release 22 CEU panel as reference, but later releases (for example, release 24) or other reference data sets (for example, 1000 Genomes) were also used (Supplementary Table 4).

Each cohort performed linear regression analyses on all available HRV traits using an additive SNP model adjusting for age at the time of ECG recording, sex, principal components—to adjust for population stratification—and other study-specific parameters; all HRV traits were log-transformed because of the skewness of their distributions. Only autosomal associations were examined. Analyses were performed for all individuals as well as for men and women separately.

Stage 1 meta-analysis. Before meta-analysis, quality control of all uploaded cohort files was performed using the QCGWAS package[45]. In case of issues the cohorts were notified and problems were solved. Using the QCGWAS results, specific imputation quality and allele frequency thresholds were set for each cohort.

An inverse-variance, fixed-effects meta-analysis was performed for RMSSD and SDNN for which SNPs of the different cohorts were merged based on rs-id. For pvRSA/HF, we performed a sample size weighted meta-analysis using z-scores with METAL[46], since we combined results of two HRV phenotypes (pvRSA and HF) that have different units and ranges, and therefore incomparable SNP effect sizes. To get an idea of the size of the SNP effect on pvRSA/HF, we obtained effect sizes

and s.e.'s from an additional fixed-effect meta-analysis on the GWAS results of the (majority of) cohorts that measured HF. Results of the meta-analyses were double genomic control corrected[47] to control for potential inflation as a result of population stratification within and between cohorts. The results included all SNPs that met the following selection criteria: (a) a minor allele frequency in the meta-analysis of >1%, and (b) present in at least one third of the cohorts. This resulted in 2,555,913 SNPs being analysed for SDNN, 2,534,714 SNPs for RMSSD and 2,628,894 SNPs for pvRSA/HF. For each trait separately, SNPs with a $P<1\times10^{-6}$ were clumped for LD using pairwise LD checking in SNAP[48] to ascertain independent primary and secondary signals ($r^2<0.1$). A total of 23 lead SNPs in 14 loci were selected for follow-up in the second (replication) stage.

Stage 2 meta-analysis. Stage 2 cohorts applied the same exclusion criteria and performed the same association analysis as in the discovery stage, but analyses were restricted to the 23 lead SNPs. If a SNP was not available in a cohort, the best available proxy was used instead based on strongest LD according to the 1000 Genomes database. To verify homogeneity of the results in the stage 2 cohorts with those in the stage 1 cohorts, the stage 1 meta-analysis effect sizes of the 23 SNPs were correlated to the effect sizes obtained in each cohort for each of the HRV traits. If a negative correlation ($r<0$) was found, the cohort/trait pair was excluded from stage 2 analysis. For this reason results from one cohort for SDNN were excluded. The replication results were then meta-analysed per trait using an inverse-variance fixed-effects meta-analysis for RMSSD and SDNN and a sample size P weighted meta-analysis using z-scores in METAL[46] for pvRSA/HF. SNPs were matched based on rs-id. Next the association results from both stages were combined in the same way. A SNP was only considered to be significantly associated to HRV if it satisfied the following criteria: (1) it had $P<1\times10^{-6}$ in stage 1, (2) it had a one-sided $P<0.05$ in the stage 2 meta-analysis congruent with the direction of effect in the stage 1 meta-analysis and (3) it had a genome-wide significant $P<5\times10^{-8}/3$ (two-sided) in the combined meta-analysis of stage 1 and 2 results, correcting for the testing of three separate traits.

Conditional analysis. In the discovery stage independent SNPs were selected for follow-up based on LD clumping ($r^2<0.1$). To confirm independence between these SNPs within the loci on chromosome 14 and 15, we applied the conditional-and-joint analysis as implemented in the Genome-wide Complex Trait Analysis software package[49] to the stage 1 summary statistics of RMSSD and SDNN with the genotype data of the NESDA cohort[50] of 1,925 individuals as the LD reference data set. In addition, cohort-level individual data on log-transformed RMSSD and SDNN of 12,101 individuals from the Dutch Lifelines cohort[51] were analysed using linear regression analysis with age and sex as covariates conditioned on the other associated SNP(s) within the locus.

Gene-based association analysis (VEGAS). We performed gene-based testing with the full set of ~2.5 M HapMap SNPs from GWAS results of all three phenotypes, using VEGAS (Supplementary Table 17). This software has the advantage of accounting for LD structure and the possibility to define a range beyond the gene bounds to include promoter, 5′UTR, intronic and 3′UTR regions into the analysis. We defined a 50 kb extra window beyond the genes, considered every SNP in this window for the gene-based analysis, and ran the analyses per chromosome with up to 10^6 permutations. A $P<2.5\times10^{-6}$ ($=0.05/\sim20{,}000$ genes) was considered as the threshold for significance.

Variance explained. The Lifelines and NESDA cohorts were used for genetic risk score and polygenic risk score analyses to determine the percentage of variance explained by independent HRV SNPs that were genome-wide significant, and by SNPs meeting increasingly lenient significance thresholds, respectively. Lifelines and NESDA represent examples of a population-based cohort and a cohort ascertained on case-control status (for major depressive disorder). Both recruited adult participants. To test the stability of explained variance across the life span, we repeated this analysis in two other Dutch cohorts, the adolescent TRAILS-Pop cohort[52] (age 10–18) and the ABCD cohort consisting of young children (age 5–7) (ref. 53).

For the genetic risk score, stage 1 + 2 summary statistics were used for the selection of HRV SNPs. No correction was needed for ABCD as genotyping in this cohort had finished only after completion of the meta-analyses. However, the NESDA cohort had been included in both stage 1 and 2, TRAILS-Pop in stage 1, and Lifelines in stage 2, so the effect sizes and s.e.'s of the HRV SNPs were corrected to subtract the effects of those cohorts to obtain independent validation cohorts[54]. Also, only SNPs were used in the genetic risk score if they remained genome-wide significant after analytically subtracting these cohort's effects from the meta-analysis. Genetic risk scores of the remaining SNPs (Lifelines: SDNN(9), RMSSD(7), pvRSA/HF(5); NESDA: SDNN(9), RMSSD(11), pvRSA/HF(5); TRAILS-Pop: SDNN(10), RMSSD(11), pvRSA/HF(5)) weighted by the adjusted effect size were calculated for the participants of all four cohorts and regressed on the three HRV traits (pvRSA/HF was not available in Lifelines). Explained variance was computed as the change in R^2 from a model with and without the genetic risk score, while adjusting both for age, sex and principal components.

To compute the polygenic risk scores, the imputed genotypes were first converted to best-guess genotypes. This was done regardless of the imputation quality, since it was previously shown that even low-quality SNPs might contribute to the variance explained by SNPs (ref. 54). The SNP set was further pruned for LD using PriorityPruner (http://prioritypruner.sourceforge.net/) to select independent SNPs, taking the significance of the SNP in the discovery meta-analysis of each of the HRV traits into account. This provided three LD-pruned SNP sets. Polygenic risk scores were then calculated in PLINK[55] using significance thresholds of 5×10^{-8}, 5×10^{-7}, 5×10^{-6}, 5×10^{-5}, 5×10^{-4}, 0.005, 0.05, 0.5 and 1 and associated with the three HRV traits and resting heart rate in the Lifelines, NESDA, TRAILS-Pop and ABCD cohorts. For NESDA and TRAILS-Pop pruning and polygenic risk score analysis was based on analytically corrected results, since these cohorts were part of stage 1 of our study[54].

Heritabilities and genetic correlations. We applied genomic restricted maximum likelihood analysis implemented in the Genomic Complex Trait Analysis software package[56] in the Lifelines cohort (Supplementary Table 18) to estimate the percentages of additive phenotypic variance that can be explained by common SNPs (that is, common SNP heritability denoted as h^2_{SNP}). For this analysis, SNPs from the HapMap Phase 3 project were selected to obtain a set of independent SNPs. We further used LD score regression to estimate the heritabilities of the three HRV traits and the genetic correlation among HRV traits and with heart rate[20]. The GWAS meta-analysis summary statistics for RMSSD, SDNN and pvRSA/HF were obtained from stage 1 of the current study, and the GWAS meta-analysis summary statistics for heart rate from the discovery stage of a recent GWAS meta-analysis for heart rate[18]. The LD scores required by the method were computed using 1000 Genomes data of Europeans. The heritabilities of the three HRV measurements were estimated using the univariate model of this method. Cross-phenotype LD score regression analysis was performed using the LDSC tool (LD SCore) to estimate genetic correlations between pairs of phenotypes[20].

In addition, we used the Oman Family Study[57] to perform univariate and bivariate analyses in five multigenerational highly inbred pedigrees to estimate the heritabilities for and the genetic correlations between log-transformed RMSSD, SDNN, HF and heart rate using SOLAR (v7.2.5) (ref. 58).

Generalization to other ethnicities. We further examined the generalization of loci identified after meta-analysis of stage 1 and 2 results to other ethnicities using data from 11,234 individuals of two Hispanic/Latino cohorts, and 6,899 individuals from five African-American cohorts (Supplementary Tables 1–4). Stage 3 meta-analyses were performed in the same way as in stage 2 of this study to assess the effect of the HRV-associated SNPs in individuals of Hispanic/Latino and African-American ancestry, in the combined set of European and Hispanic/Latino ancestry, in the combined set of European and African-American ancestry, and in all three ethnicities combined. Here, we applied the same criteria for significance as in stage 2 described above, that is, a SNP was only considered to be significantly associated to HRV if: (1) it had $P<1\times10^{-6}$ in stage 1 meta-analysis in European individuals, (2) it had a one-sided $P<0.05$ in the new ethnicity specific meta-analysis congruent with the direction of effect in the stage 1 meta-analysis in European individuals and (3) it had a genome-wide significant $P<5\times10^{-8}/3$ (two-sided) in the combined meta-analysis.

Correcting HRV for heart rate. The well-known inverse association between HRV and heart rate in part reflects a dependency of the variance in IBI on the mean IBI that is unrelated to cardiac vagal activity[59]. That is, the slower the heart rate, the longer the IBI, and therefore, any proportionally minor beat-to-beat differences in IBI are more pronounced at slower heart rates. This occurs on top of the well-established dual effect of cardiac vagal activity that lowers heart rate and increases HRV[15,16]. Although these two mechanisms (biological, mean-variance dependency) are impossible to completely separate, we conducted three analyses to test whether the HRV SNPs were robust to correction for the mean IBI.

First, we corrected SDNN and RMSSD for their dependency on mean IBI by using the coefficient of variation, which is a more parsimonious solution[19] than the logarithmic approach suggested by Monfredi *et al.*[29]. We obtained the summary statistics for the resting heart rate GWAS meta-analysis[18] from: https://walker05.u.hpc.mssm.edu/ and used the GWIS procedure[17] to infer a GWA analysis of the coefficient of variation of the SDNN and the RMSSD. We approximated the coefficients of variation by (SDNN/X) × 100% and (RMSSD/X) × 100%, respectively, where X equals 60,000 per heart rate. Transformation from heart rate to IBI is required as both terms in the coefficient of variation (HRV and IBI) are in milliseconds, whereas the heart rate GWAS meta-analysis used heart rate in beats per minute. As the coefficients of variation were skewed, we used a log-transformation. As an example of the linear approximation by GWIS we assume that the increaser effect of one allele for an SDNN SNP is +0.2 with the same SNP reducing heart rate by −0.1. Given a mean SDNN of 100 and mean heart rate of 60, we can then approximate (omitting some nuances adequately explained in Nieuwboer *et al.*[17]) the effect of the SNP on

the coefficient of variation of the SDNN as:

$$\ln\left(\frac{100+0.2}{60{,}000/(60-0.1)}\right)-\ln\left(\frac{100}{60{,}000/(60)}\right)=0.00033$$

We used the delta method to approximate a s.e. for the effect of the SNP given that we know the s.d.'s for the SNP effects on SDNN and HR, and their dependence. We obtain the dependence from analysis with LD score regression[20].

Second, we performed association analyses for our 17 top SNPs on the actual log-transformed coefficients of variation of SDNN and RMSSD computed in the Lifelines, NESDA and TRAILS-Pop cohorts and then meta-analysed these results. Because pvRSA and HF are expressed on different scales, such a meta-analysis was not feasible for pvRSA/HF.

Third, we repeated the association analysis for our 17 top SNPs on SDNN, RMSSD and pvRSA/HF in the Lifelines, NESDA and TRAILS-Pop cohorts with and without adjusting for heart rate as a covariate and performed mediation tests with the Sobel test to assess the mediation effect of heart rate on the HRV SNP association. Significance of the Sobel *t*-value was determined using a bootstrap procedure ($n=10{,}000$ permutations). The mediation *P* values of the three cohorts for SDNN and RMSSD and two for pvRSA/HF (as this was not available in Lifelines) were next meta-analysed to determine the significance of mediation and to compute the percentage of the SNP effect on HRV that was mediated through its effects on heart rate. We note that this is likely an overcorrection because the HRV SNPs are expected to influence heart rate through a common biological mechanism, that is, changes in cardiac vagal activity.

Association of the HRV SNPs with heart rate. We conducted a lookup of the 17 (11 independent) HRV lead SNPs identified in this study using the results of a recent GWAS meta-analysis for heart rate[18]. A HRV-associated SNP was considered to be significantly associated with resting heart rate if the GWAS meta-analysis result for heart rate was $<0.05/11=0.0045$. Three separate HRV weighted multi-SNP genetic risk scores were calculated from 10 (SDNN), 11 (RMSSD) and five (pvRSA/HF) HRV SNPs, respectively, (based on all genome-wide significant SNPs for the respective HRV trait in the stage $1+2$ meta-analysis). These were tested for their effect α on resting heart rate using the gtx package in R (https://cran.r-project.org/web/packages/gtx), which approximated α by $(\Sigma\omega\times\beta\times se_{\beta}^{-2})/(\Sigma\omega^2\times se_{\beta}^{-2})$ with $se_{\alpha}\cong\sqrt{(1/\Sigma\omega^2\times se_{\beta}^{-2})}$, where ω is the effect of the SNP on HRV, β is the effect of the SNP on heart rate and se_{β} is the s.e. of β. This approximation requires only single SNP association summary statistics extracted from GWAS results[60]. The effects of the multi-SNP genetic risk scores were considered as statistically significant when the *P* was less than 0.0045 (correcting for 11 traits; heart rate and the 10 cardiometabolic traits described below).

In addition to these lookups that were restricted to genome-wide significant SNPs, we employed LD score regression[20] that uses the full summary statistics of the HRV and heart rate GWAS meta-analyses to compute genetic correlations.

We further examined the variance in resting heart rate explained by multi-SNP genetic risk scores (based on the lead SNPs only) and of the full polygenic risk scores for HRV in the four Dutch cohorts Lifelines, NESDA, TRAILS-Pop and ABCD. The identical approach was used as done previously for the computation of variance explained in the HRV traits themselves.

Association of heart rate SNPs with HRV. We also performed reverse analyses to detect the effects of heart rate SNPs on the HRV traits. In our GWAS meta-analysis results for SDNN, RMSSD and pvRSA/HF, we performed a lookup for the 21 previously identified heart rate SNPs by den Hoed *et al.*[18]. A heart rate associated SNP was considered to be significantly associated with HRV if the *P* was $<0.05/21=0.0024$. The 21 heart rate SNPs were tested in a multi-SNP risk score for their effect on the HRV traits using the *gtx* approach as described above.

To examine the variance explained in the HRV traits by the 21 heart rate SNPs, multi-SNP genetic risk scores and polygenetic risk scores based on the heart rate SNPs were computed in the Lifelines, NESDA, TRAILS-Pop and ABCD cohorts and these were tested for association with the available HRV traits. For the multi-SNP genetic risk scores weights were either the original SNP effect sizes on heart rate (for NESDA, TRAILS-Pop and ABCD) or corrected because of participation of the cohort in the GWAS meta-analysis (Lifelines). Only 15 of the 21 SNPs were used in the Lifelines cohort because five SNPs lost genome-wide significance after subtracting the SNP effects of the Lifelines cohort. One other SNP (rs826838) was removed because it was in LD ($r^2=0.15$) in Lifelines with a more significant heart rate SNP (rs7980799).

Association with cardiometabolic traits and diseases. We estimated the joint effect of the HRV SNPs on cardiometabolic and renal disease traits and endpoints. The traits included were systolic and diastolic blood pressure, body mass index and urinary albumin excretion as well as estimated glomerular filtration rate based on creatinine. The clinical outcomes used were heart failure, coronary artery disease, atrial fibrillation, sudden cardiac death and type 2 diabetes. The relevant consortia (Supplementary Table 11) and/or corresponding authors of the studies were contacted with the request to perform a lookup and provide summary GWAS meta-analysis results for our list of 17 SNPs.

The association analyses consisted of the same three steps as used for heart rate. First, we checked the *P* of our HRV SNPs (or their proxies) in the cardiometabolic trait or disease GWAS meta-analysis results. Second, three separate HRV weighted genetic risk scores were calculated from 11 (RMSSD), 10 (SDNN) and five (pvRSA/HF) HRV SNPs, respectively (based on all genome-wide significant SNPs for the respective HRV trait in the stage $1+2$ meta-analysis). These were tested for their effect on the clinical outcomes using a regression model in the *gtx* package in R as described above for the association of the HRV SNPs with heart rate. The effects of the genetic risk scores were considered as statistically significant when the *P* was less than 0.0045 (0.05/11, correcting for heart rate and the 10 traits and diseases).

In addition to these lookups that were restricted to genome-wide significant SNPs, we employed LD score regression[20] that uses the full GWAS summary statistics of the HRV and cardiometabolic traits and diseases to compute genetic correlations.

Search for known functional SNPs (*in silico* annotation). We followed an *in silico* bioinformatics-based approach[61] to search and annotate SNPs in the regions surrounding the 17 identified HRV SNPs. For this purpose SNP positions were converted from National Center for Biotechnology Information (NCBI) build 36, Human Genome 18, to NCBI build 37, Human Genome 19, (GRCh37/hg19) using the NCBI Genome Remapping service tool (http://www.ncbi.nlm.nih.gov/genome/tools/remap). For ± 1 Mb regions surrounding the SNPs, we downloaded the according variance call format file from the 1000 Genomes Project. We used data of 503 European ancestry individuals from 1000 Genomes Project Phase 3 (version 5.a.) to calculate LD between the HRV SNP and all other SNPs within the area. SNPs in moderate to high LD ($r^2\geq 0.5$) were subsequently selected and annotated by ANNOVAR software[62] for functionality. For all non-synonymous SNPs loss-of-function and gain-of-function was determined by using the SIFT and PolyPhen prediction scores. A SNP was categorized as deleterious if the SIFT score was ≤ 0.05 or the PolyPhen score was between 0.957 and 1 (probably damaging).

We used RegulomeDB to integrate results from the RoadMap Epigenomics and ENCODE projects to identify variants that are likely to have functional consequences using the lead SNPs identified for the three HRV traits. We distilled information on transcription factor binding and chromatin states for SNPs that showed most evidence of being functional, that is, for SNPs with a RegulomeDB score <4.

Finally, all the HRV SNPs and those that were in high LD ($r^2\geq 0.8$) with them were looked up in the National Human Genome Research Institute GWAS catalogue to check for association with other complex traits or diseases identified in previous GWAS studies[63].

eQTL analyses. We performed expression quantitative trait locus (eQTL) analysis in whole blood to identify regulatory variants that were associated with the HRV SNPs using the gene-expression database from NESDA[50] and NTR[64] cohorts. The sample used for this analysis consisted of 4,896 individuals of European ancestry. For complete details on the sample and the procedures, see[65].

eQTL effects were tested with a linear model approach using MatrixeQTL[66] with expression level as dependent variable and SNP genotype values as independent variable. In this study we only tested *cis* effects for our HRV SNPs, meaning that the probe was at a distance <1 Mb from the SNP on the genome according to GRCh37/hg19. For each probe set that displayed a statistically significant association with at least one SNP in the *cis* region, we identified the most significantly associated SNP (top eQTL). Conditional eQTL analysis was carried out by first residualizing probe set expression using the corresponding top eQTL and then repeating the eQTL analysis using the residualized data.

All HRV SNPs with significant results in the NESDA/NTR eQTL data were looked up in two other independent whole blood eQTL databases, eQTLs in lymphoblastoid cell lines, eQTLs in 10 different brain regions, and a heart eQTL database.

mQTL analyses. We obtained mQTL results from a previously published study[67]. In short, genome-wide DNA methylation data were generated using Illumina 450 k arrays for 3,841 whole blood samples. Corresponding genotype data were imputed using the Genome of The Netherlands[68] reference panel. To determine the effect of nearby genetic variation on methylation levels (*cis*-mQTLs) a Spearman rank correlation and corresponding *P* value was computed for each CpG-SNP pair, in which the CpG and SNP location were no further than 250 kb apart. To control for multiple testing, we used a permutation procedure to empirically control the false discovery rate at 5%. The distribution of observed *P* values was compared to the *P* value distribution obtained from the analyses on permuted data. For a permutation the sample identifiers of the genotype data set were shuffled, breaking the link between the genotype data set and the methylation data set. This was repeated 10 times to obtain a stable distribution of *P* values under the null hypothesis. To determine the false discovery rate only the strongest effect per CpG in both the real analysis and in the permutations were selected.

Gene prioritization using four bioinformatics approaches. Potentially causal genes for the associations identified by GWAS were identified using four previously

described bioinformatics tools: ToppGene, Endeavour, MetaRanker and DEPICT (Supplementary Table 19). To this end, we first retrieved positional coordinates for all lead SNPs according to GRCh37/hg19. These coordinates were used to extract all genes located within ±40 kb of lead SNPs using the UCSC genome browser. The identified genes subsequently served as input for ToppGene and Endeavour, together with two genes with established roles in sinus node function (*HCN4*) and synaptic signal transmission (*ACHE*) that served as training genes. For MetaRanker, we first combined results of the stage 1 + 2 meta-analyses of GWAS for the three HRV traits, retained the association with the lowest *P* for lead SNPs that were identified for multiple traits, and subsequently provided SNPs, *P* values, and the same two test genes (*HCN4* and *ACHE*) as input. For DEPICT—arguably the most powerful and informative of the four methods—we used results from the stage 1 meta-analysis for all SNPs that reached a *P* for association $<10^{-5}$ as input, for each of the three HRV outcomes separately. In order for genes to be prioritized by the combined four approaches, they needed to be either: (1) selected by DEPICT for at least one of the three HRV outcomes; or (2) identified by at least two of the three remaining tools (ToppGene, Endeavour and/or MetaRanker).

Network and functional enrichment analyses. We performed gene network and enrichment analysis using the GeneMANIA algorithm, which uses data resources on genetic interactions, protein–protein, co-expression, shared protein domains and co-localization networks. To build a functional interaction network, we selected genes as input for this analysis using the following criteria: (a) genes implicated by gene prioritization using the four bioinformatics approaches described above, (b) the genes closest to our 17 HRV SNPs, (c) genes to which linked ($r^2>0.50$) non-synonymous SNPs mapped, (d) genes to which other linked ($r^2>0.80$) SNPs mapped, (e) genes identified by VEGAS, and (f) expression probe gene names significantly associated with HRV eQTLs (false discovery rate <0.01). The input gene list was extended to 100 by their most strongly interacting genes and a weighted composite functional association network was constructed[61]. Subsequently, functional enrichment analysis of all genes of the constructed interaction network against Gene Ontology (GO) terms was performed to find the most enriched GO terms (Supplementary Table 20). Significantly enriched GO terms (false discovery rate <0.10) were visualized as highlighted boxes within their corresponding GO tree depicted by the RamiGO R package[69] (Supplementary Fig. 10).

Tissue and gene-set enrichment analyses. We used DEPICT for a tissue enrichment analysis to tabulate tissues that are enriched for expression of genes located within ±40 kb of SNPs with a $P<10^{-5}$ association with the HRV traits. DEPICT calculates the likelihood of every known gene to be a member of, amongst others, KEGG, GEO or REACTOME-based gene sets ($N=14{,}461$) to create reconstituted gene sets. It then determines which of these reconstituted gene sets are enriched for the HRV genes. A graphical representation of DEPICT's reconstituted gene-set enrichment analysis ($P<0.05$ after Bonferroni correction for examining three HRV traits) was generated using a script that is based on an affinity propagation clustering algorithm by Frey *et al.*[70]. Interactions between gene sets are considered significant if the Pearson coefficient, which is based on the number of genes that are shared between gene sets, is >0.3.

Data availability. Summary statistics of the meta-analyses are available on request from the corresponding authors after a formal data access request procedure and approval by the VgHRV consortium.

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Acknowledgements

A full list of acknowledgements appears in the Supplementary Information. Funding sources had no involvement in the collection, analysis and interpretation of the data.

Author contributions

H.S. and E.d.G. designed and overviewed the project; I.M.N., M.L.M. and V.T. did the quality control of the individual GWA results and performed the meta-analyses; C.Al., D.G.B., P.I.W.d.B., R.B., D.B., P.T.E., O.H.F., M.A.G., C.A., A.H., H.H., M.J., M.Ku., C.C.L., C.M.L., A.P.M., G.N., D.T.O., J.O., A.P., B.M.P., O.T.R., V.B.R., J.A.S., K.S., J.-C.T., A.T., A.B.Z., D.C., M.K.E., P.v.d.H., M.H., E.I., M.-R.J., S.K., M.Kä., C.K., D.K., T.L., L.L., C.M.N., C.J.O., A.J.O., B.P., A.P.R., H.R., J.D.R., J.I.R., T.S., B.H.S., H.T., T.G.M.V., F.W.A., B.J.J.M.B., E.A.W., M.d.H., H.S. and E.d.G. were Principal Investigators of the participating cohorts; M.L.M., R.J., D.J., A.K., A.Mi., J.F.T., S.A., C.Al., A.Al., J.A., R.J.B., D.B., A.R.B., A.D., M.E., J.S.F., P.F., M.G.J.G., M.A.G., V.G., H.H., N.H.-K., X.J., J.J., M.J., A.M.K., J.A.K., T.K., J.D.L., D.L., M.C.L., M.Ne., K.N., D.T.O., S.P., A.P., B.M.P., O.T.R., V.B.R., M.S., D.S., J.H.S., N.L.S., E.Z.S., N.S., J.A.S., P.K.S., K.S.-S., J.S., C.A.S., A.Vo., G.W., Z.-M.Z., H.K.G., M.H., E.I., S.K., M.Kä., M.Ki., D.K., T.L., L.L., C.M.N., H.R., A.M.v.R., T.G.M.V., B.J.J.M.B., S.R.H., E.A.W. and E.d.G. performed HRV phenotyping; R.J., P.G., L.-P.L, A.X.M., M.M.-N., E.S., A.W., M.H.Z., A.Ab., T.A., M.B., M.G.J.G., J.-J.H., X.J., J.J., M.Ku., Y.Li., C.M.L., H.M.z.S., Y.M., N.M., A.P.M., M.A.N., D.T.O., D.S., J.H.S., A.S., K.D.T., L.E.T., A.G.U., M.W., K.C.W., A.B.Z., M.K.E., M.-R.J, M.Kä., D.K., T.L., C.M.N., C.J.O., H.R., J.D.R., T.G.M.V. and H.S. performed genotyping and imputation; I.M.N., M.L.M., V.T., A.T.A., R.J., A.Va, B.v.d.H., C.L.A., J.C.B., B.D., J.v.D., S.M.G., P.G., J.H, V.H., S.-J.H., D.J., K.F.K., A.K., B.P.K., J.K., S.W.v.d.L., L.-P.L., A.X.M., A.Mi., P.J.v.d.M., M.M.-N., M.Ni., E.S., J.D.S., J.F.T., N.V., A.W., D.Z., M.H.Z., A.Ab., F.A., J.A., P.I.W.d.B., M.B., G.B., A.R.B., I.C., G.B.E., J.F.F., J.S.F., D.H., J.-J.H, A.M.K., T.K., J.D.L., Y.Li, H.J.L., C.M.L., S.A.L., A.Ma., B.M., Y.M., A.P.M., M.A.N., K.E.N., V.B.R., J.A.S., P.K.S., A.M.S., K.S.-S, T.A.T., J.v.S., A.Vo., Q.W., C.M.N. and A.M.v.R. performed data analysis; I.M.N., M.L.M., V.T., S.R.H., E.A.W., M.d.H., H.S. and E.d.G. drafted and edited the manuscript. All authors contributed to and critically reviewed the manuscript.

Additional information

Supplementary Information accompanies this paper at http://www.nature.com/naturecommunications

Competing interests: M.A.G. has an equity interest in San Diego Instruments. B.M.P. serves on the DSMB of a clinical trial funded by the manufacturer (Zoll LifeCor) and on the Steering Committee of the Yale Open Data Access Project funded by Johnson & Johnson. The remaining authors declare no competing financial interests.

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How to cite this article: Nolte, I. M. *et al.* Genetic loci associated with heart rate variability and their effects on cardiac disease risk. *Nat. Commun.* **8**, 15805 doi: 10.1038/ncomms15805 (2017).

Publisher's note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

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SI GUIDE

Type of file: pdf
Size of file: 4,288 KB
Title of file for HTML: Supplementary Information
Description: Supplementary Figures, Supplementary Tables, Supplementary Notes and Supplementary references.

Supplementary Data

Type of file: XLSX
Size of file: 37 KB
Title of file for HTML: Supplementary Data 1
Description: Stage 1, stage 2, and combined stage 1+2 meta-analysis results for SDNN, RMSSD, and pvRSA/HF for the 23 SNPs that were genome-wide suggestive ($p<1x10-6$) after stage 1 in the meta-analysis for any of the traits.

Type of file: XLSX
Size of file: 63 KB
Title of file for HTML: Supplementary Data 2
Description: In silico annotation and GWAS catalog lookup results of the 17 HRV SNPs identified in this study.

Type of file: XLSX
Size of file: 58 KB
Title of file for HTML: Supplementary Data 3
Description: DEPICT tissue enrichment analysis.
Type of file: pdf
Size of file: 1,017 KB
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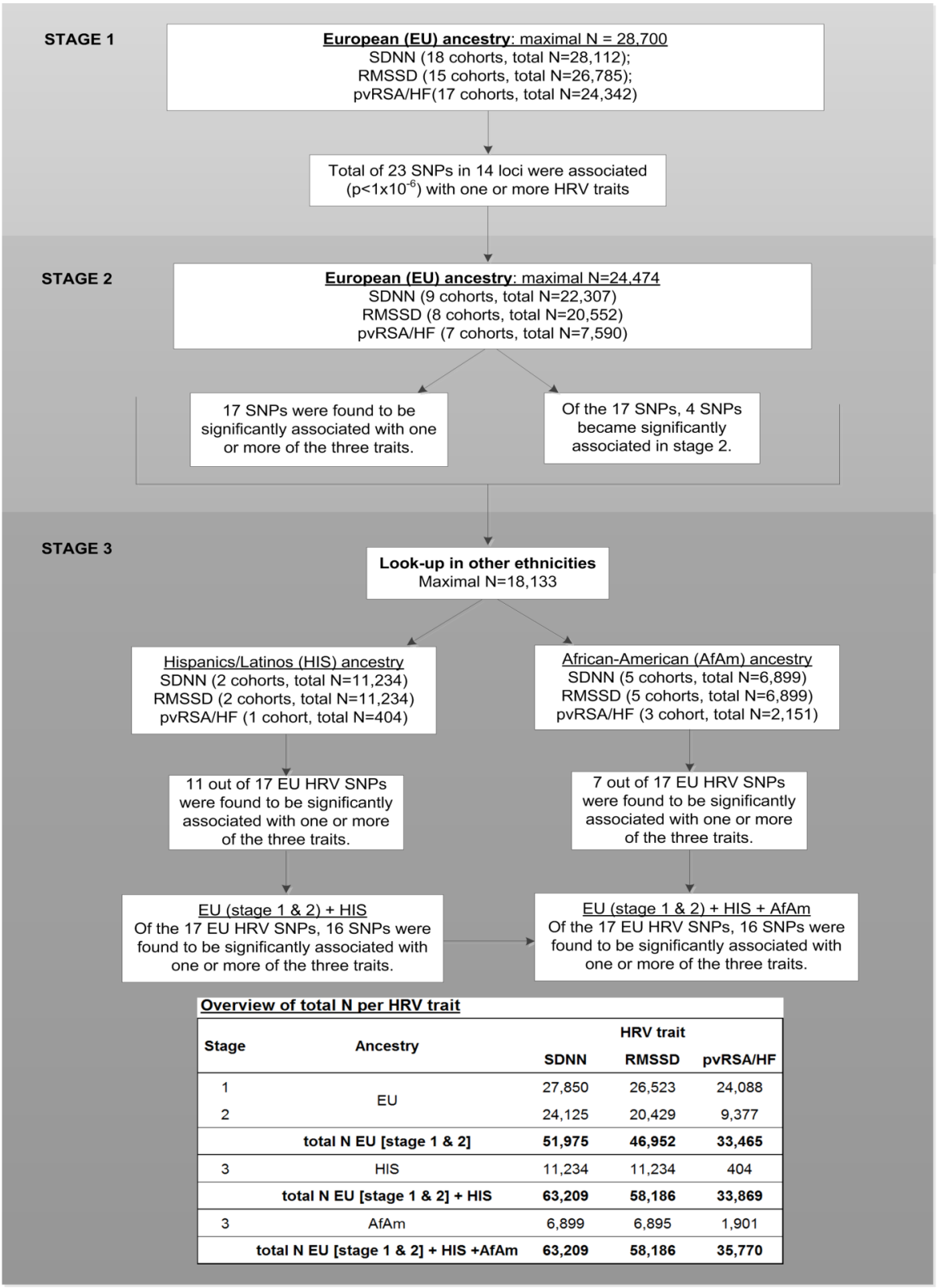
Description:

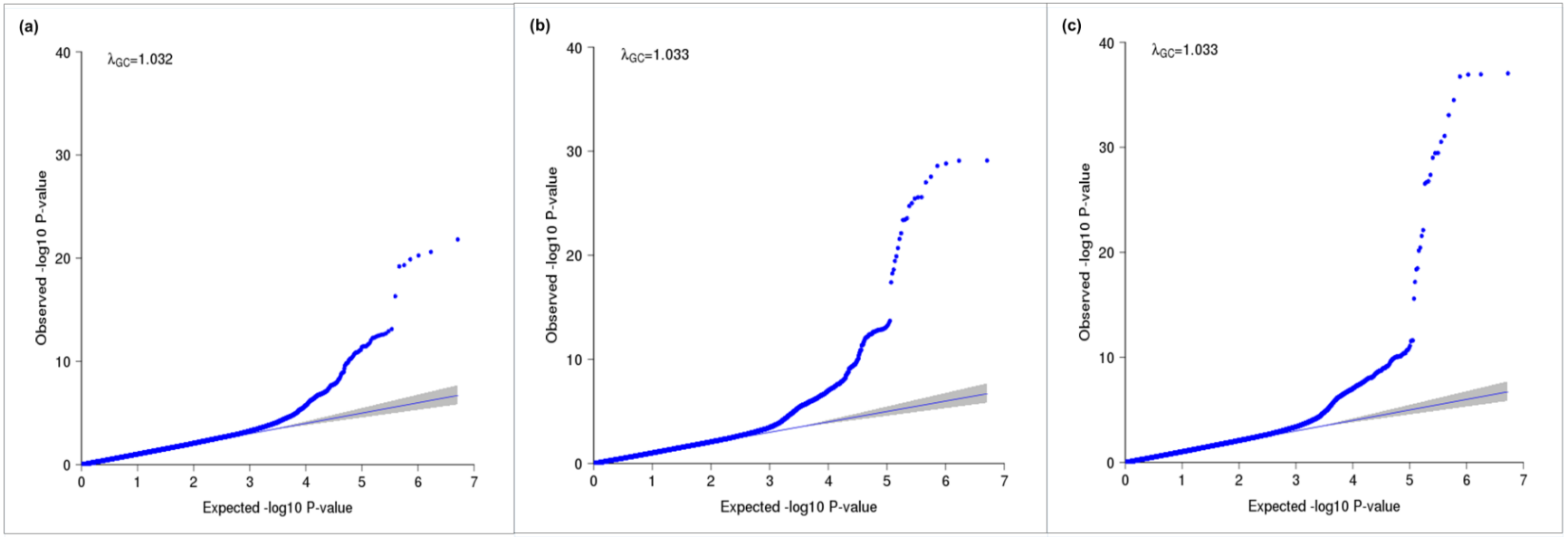
SUPPLEMENTARY FIGURES



Overview of total N per HRV trait

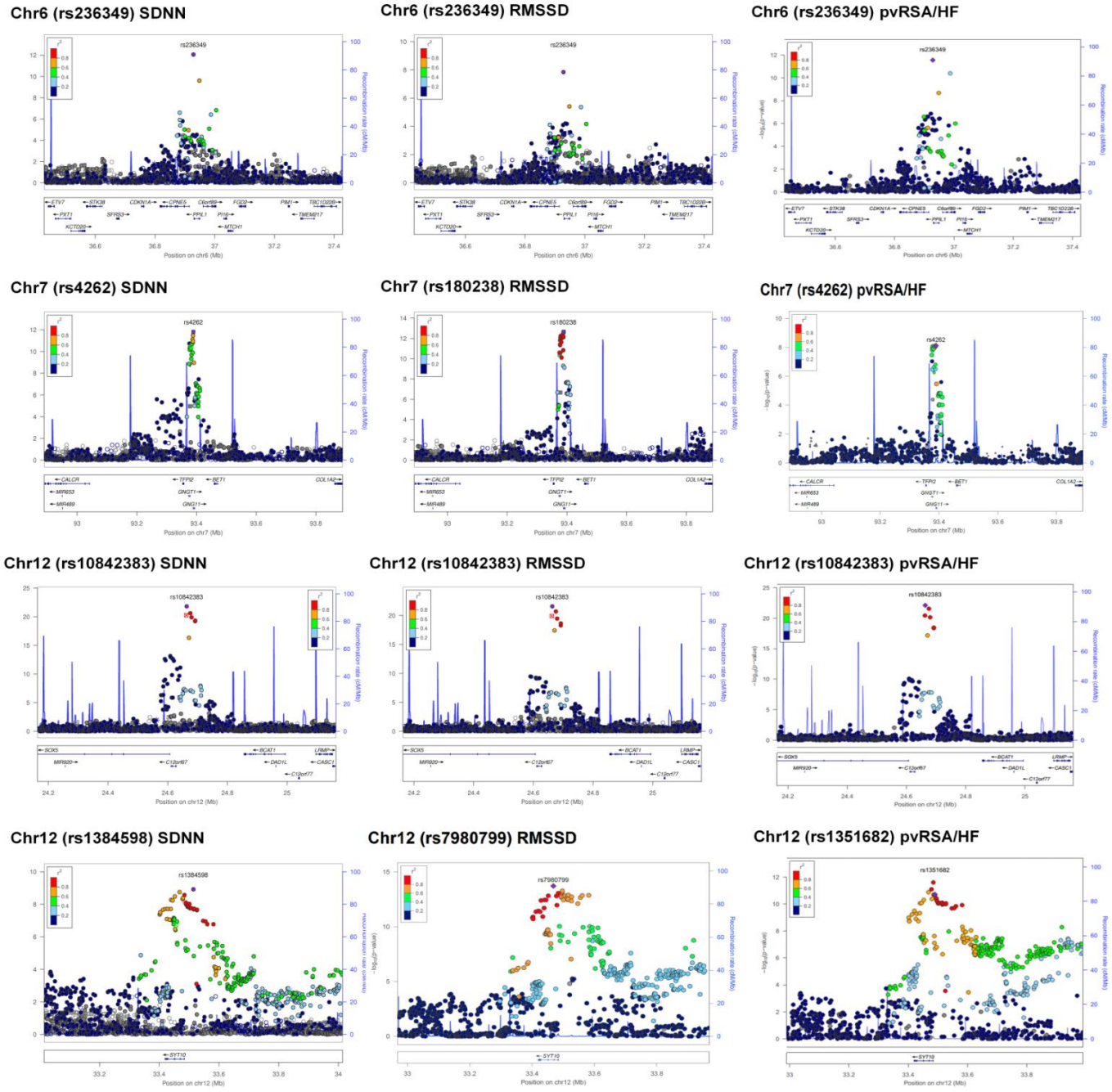
Stage	Ancestry	HRV trait		
		SDNN	RMSSD	pvRSA/HF
1	EU	27,850	26,523	24,088
2		24,125	20,429	9,377
	total N EU [stage 1 & 2]	**51,975**	**46,952**	**33,465**
3	HIS	11,234	11,234	404
	total N EU [stage 1 & 2] + HIS	**63,209**	**58,186**	**33,869**
3	AfAm	6,899	6,895	1,901
	total N EU [stage 1 & 2] + HIS +AfAm	**63,209**	**58,186**	**35,770**

Supplementary Figure 1: V_gHRV GWA meta-analysis study design.



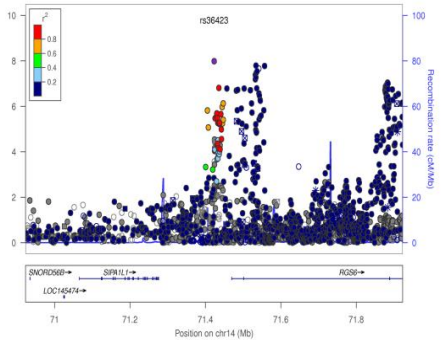
Supplementary Figure 2: Quantile-quantile-plots of the three meta-analyses of GWAS results of (a) SDNN, (b) RMSSD, and (c) pvRSA/HF for the stage 1 meta-analysis on individuals of European ancestry.

NOTE: Expected $-\log_{10}(p\text{-values})$ assuming a normal distribution are shown on the x-axis. The y-axis depicts the observed $-\log_{10}(p\text{-values})$. Only SNPs with a minor allele frequency >1% and that were present in at least 1/3 of the sample are shown.

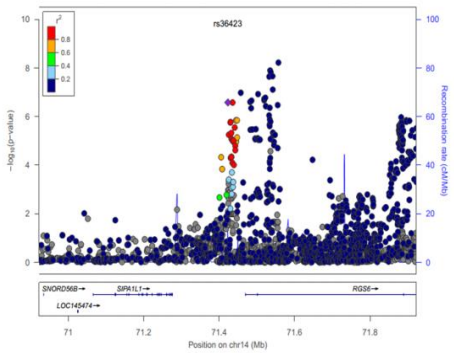


Supplementary Figure 3: Regional association plots of the 17 HRV SNPs from the stage 1 meta-analysis on individuals of European ancestry.
NOTE: Lead SNP and HRV trait are indicated in the top line. In the plots rs-numbers of the lead SNPs are given. Color coding reflects the LD (r^2) of the SNP with this lead SNP according to the legend. The left y-axis represent -$\log_{10}$(*p*-value) of association; the right y-axis indicates recombination rate. The x-axis depicts the position on the chromosome (Mb) and lists known genes near the locus. Symbols annotation: framestop (triangle), splice (triangle), non-synonymous (inverted triangle), synonymous (square), UTR (square), TFBScon [in a conserved region predicted to be a transcription factor binding site] (eight pointed star), MCS44 [in a region highly conserved within placental mammals] (square with two diagonal lines) and none-of-the-above (filled circle).

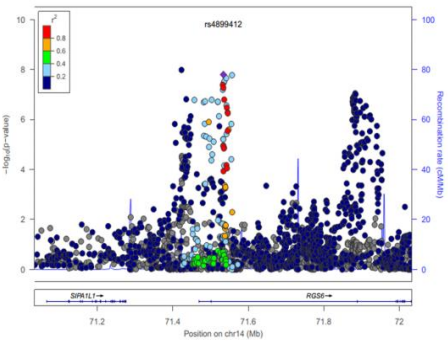
Chr14 (rs36423) SDNN



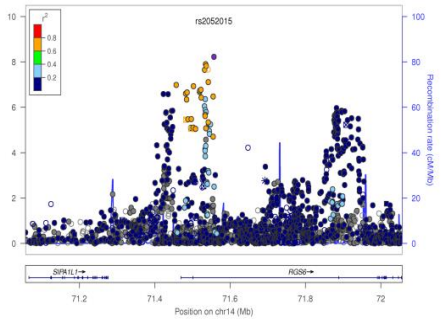
Chr14 (rs36423) RMSSD



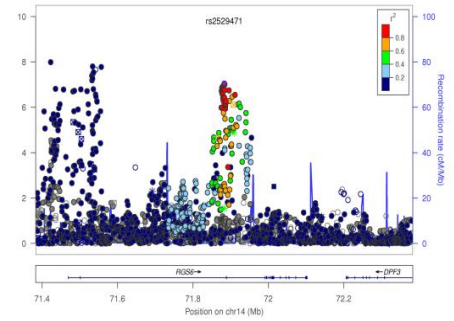
Chr14 (rs4899412) SDNN



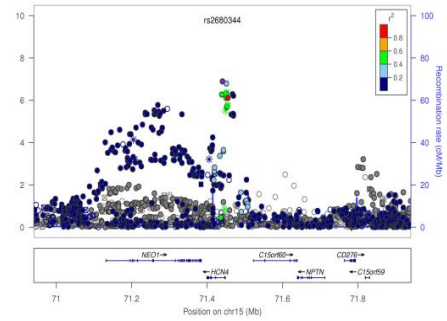
Chr14 (rs2052015) RMSSD



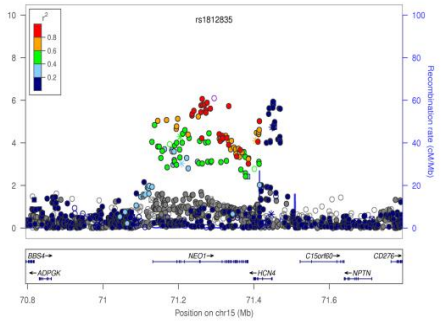
Chr14 (rs2529471) SDNN



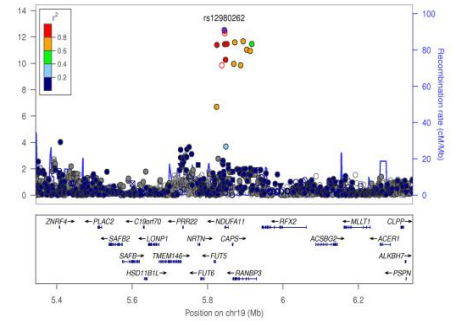
Chr15 (rs2680344) SDNN



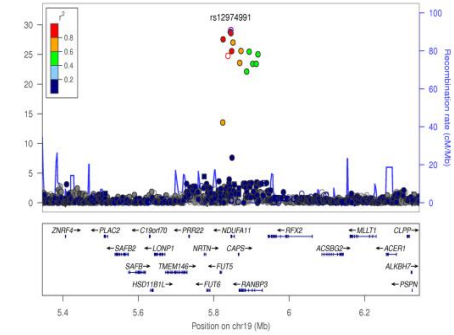
Chr15 (rs1812835) RMSSD



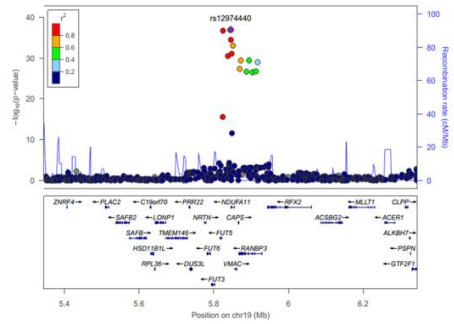
Chr19 (rs12980262) SDNN



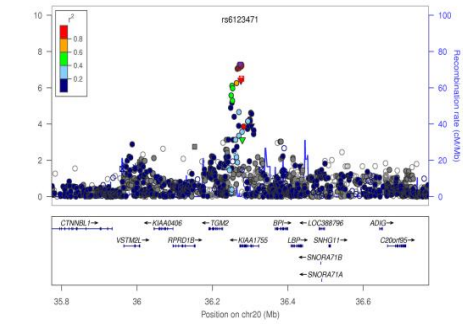
Chr19 (rs12974991) RMSSD



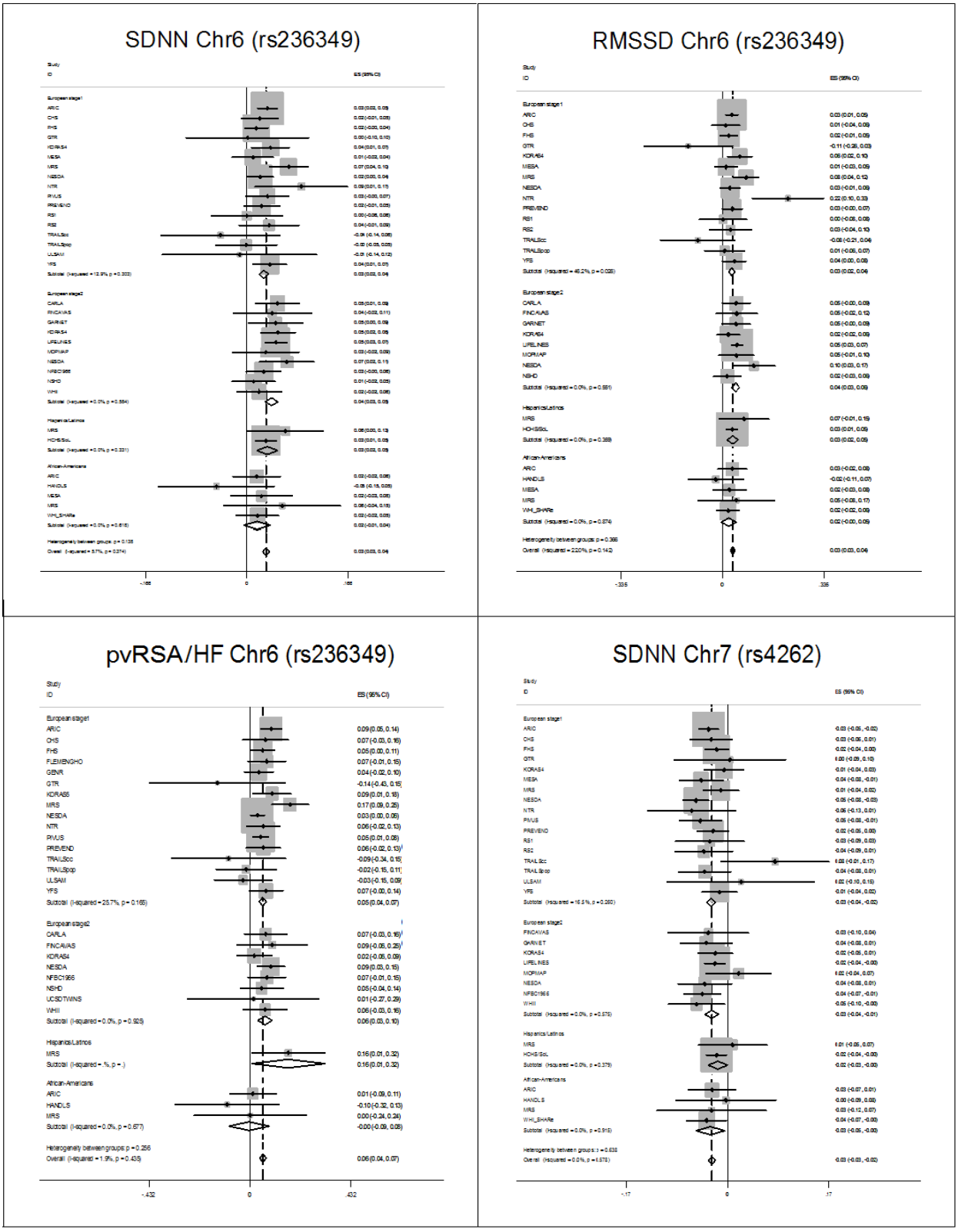
Chr19 (rs12974440) pvRSA/HF



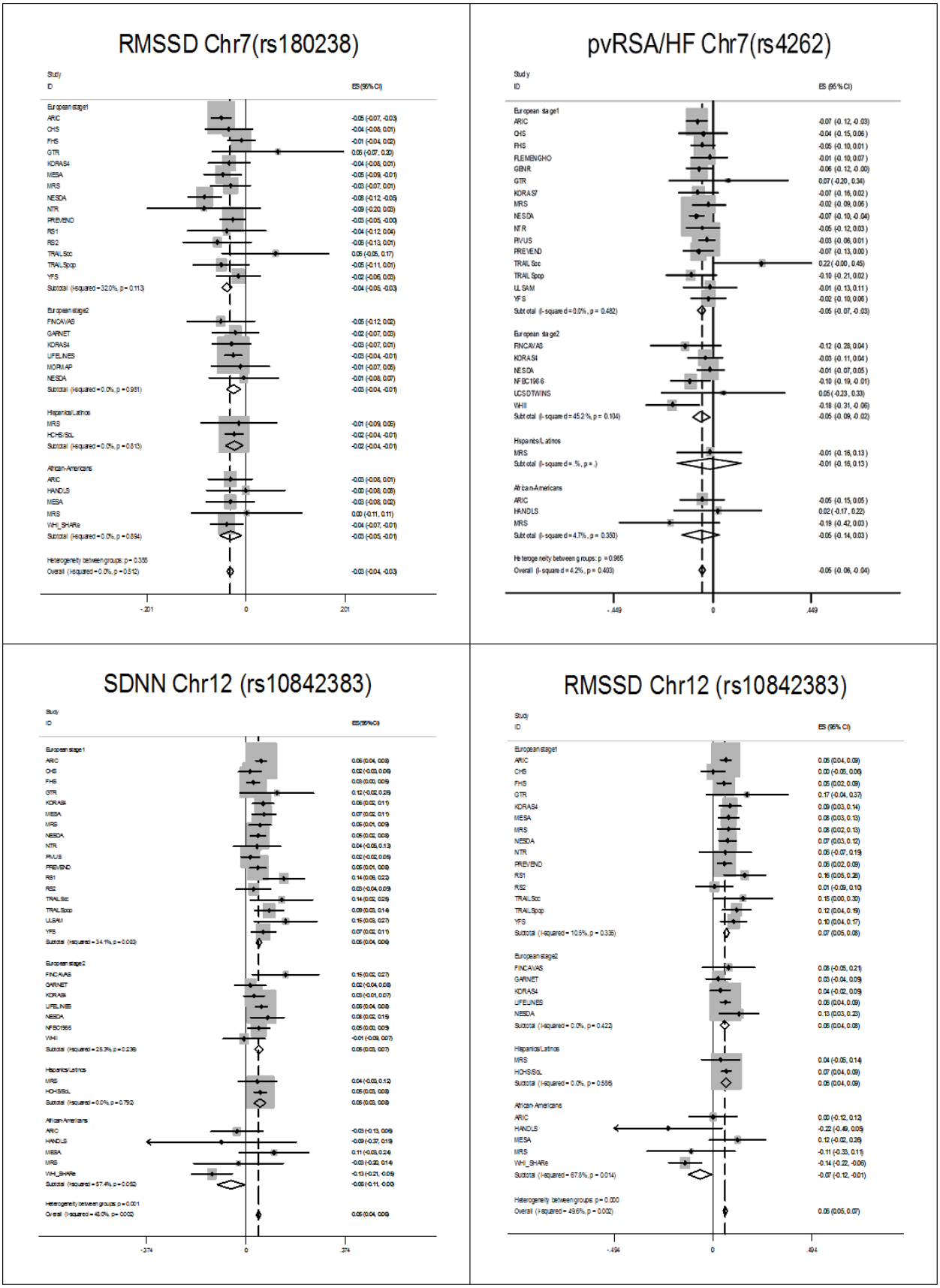
Chr20 (rs6123471) RMSSD



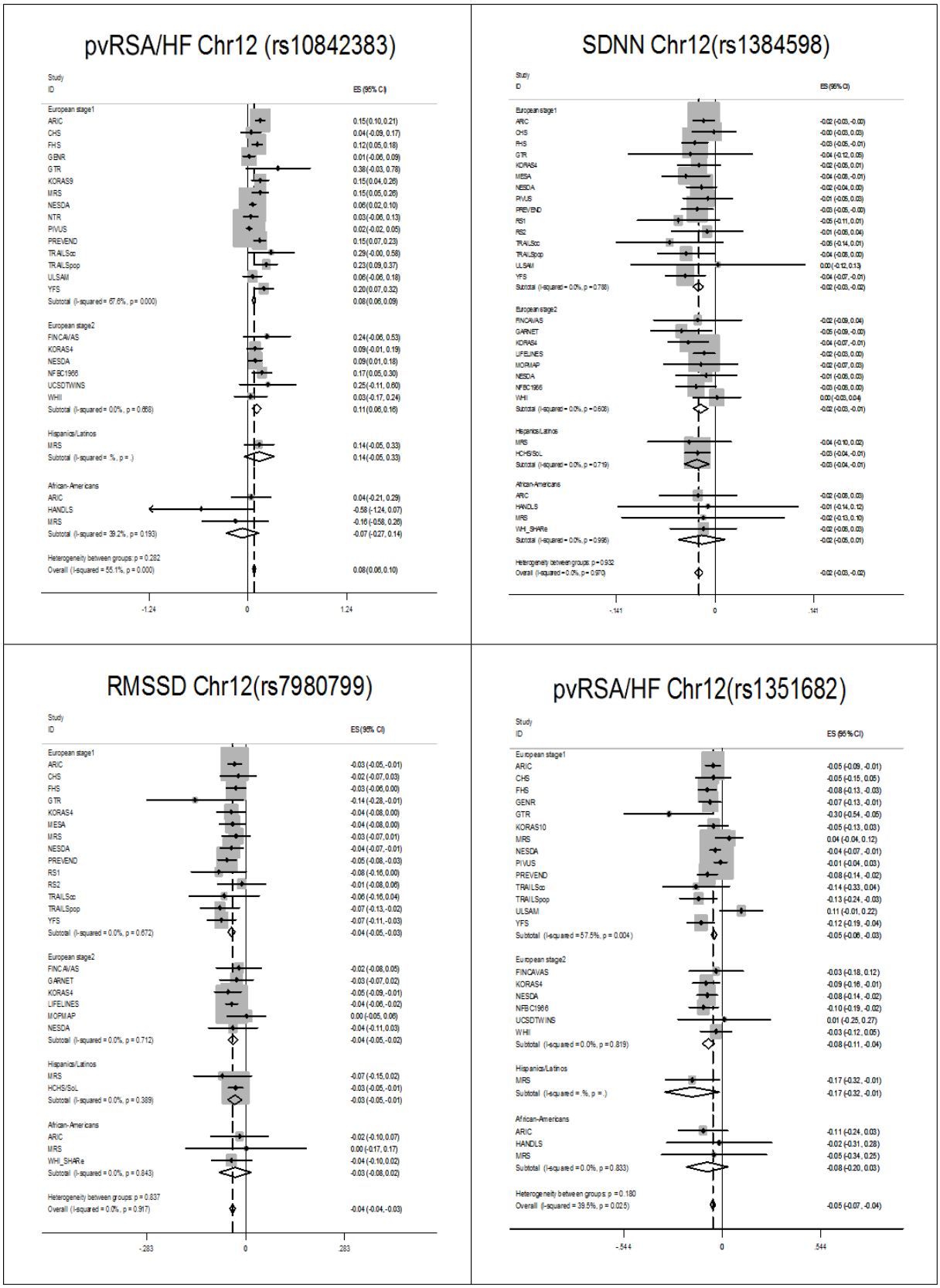
Supplementary Figure 3 (continued)



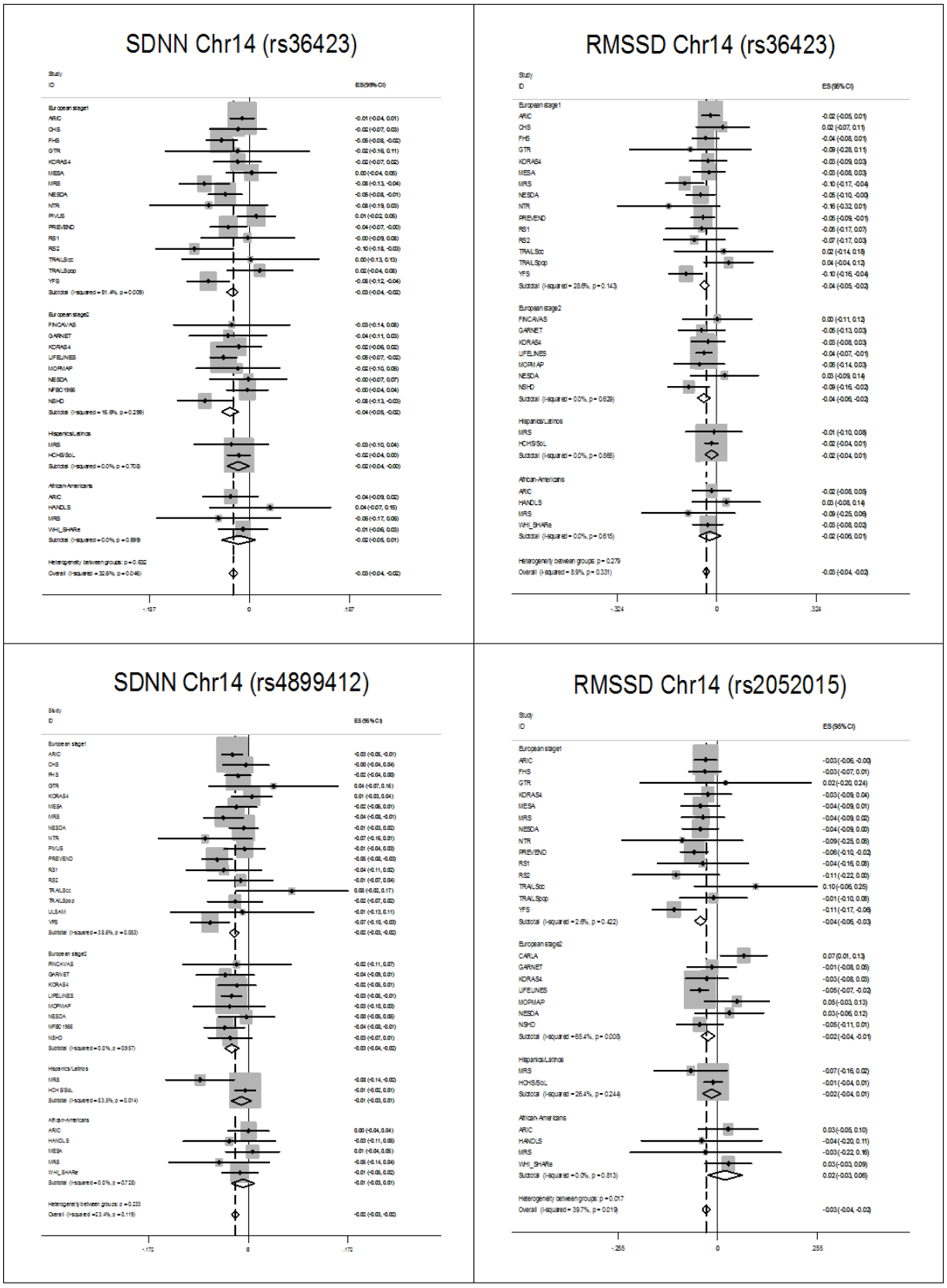
Supplementary Figure 4: Forest plots of the 17 HRV SNPs showing the effect sizes from the individual cohorts and from the stage 1, 2, and 3 meta-analyses on individuals of European ancestry, Hispanic/Latino ancestry, and African American ancestry.
NOTE: Meta-analysis derived effect sizes and 95% CI are given per stage and ancestry. The lower lines represent the heterogeneity test and the overall effect size based on all individuals from all ethnicities.



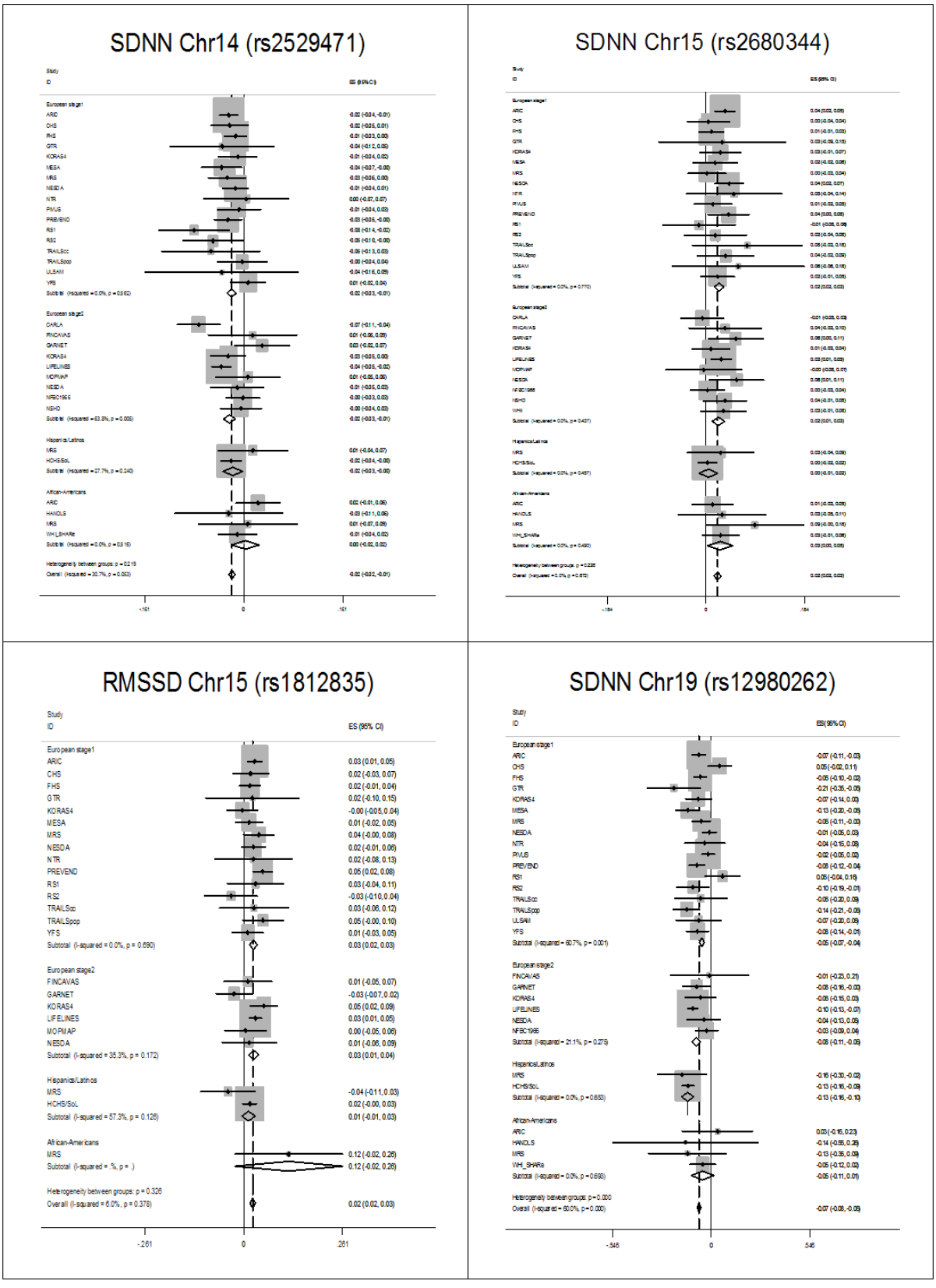
Supplementary Figure 4 (continued)



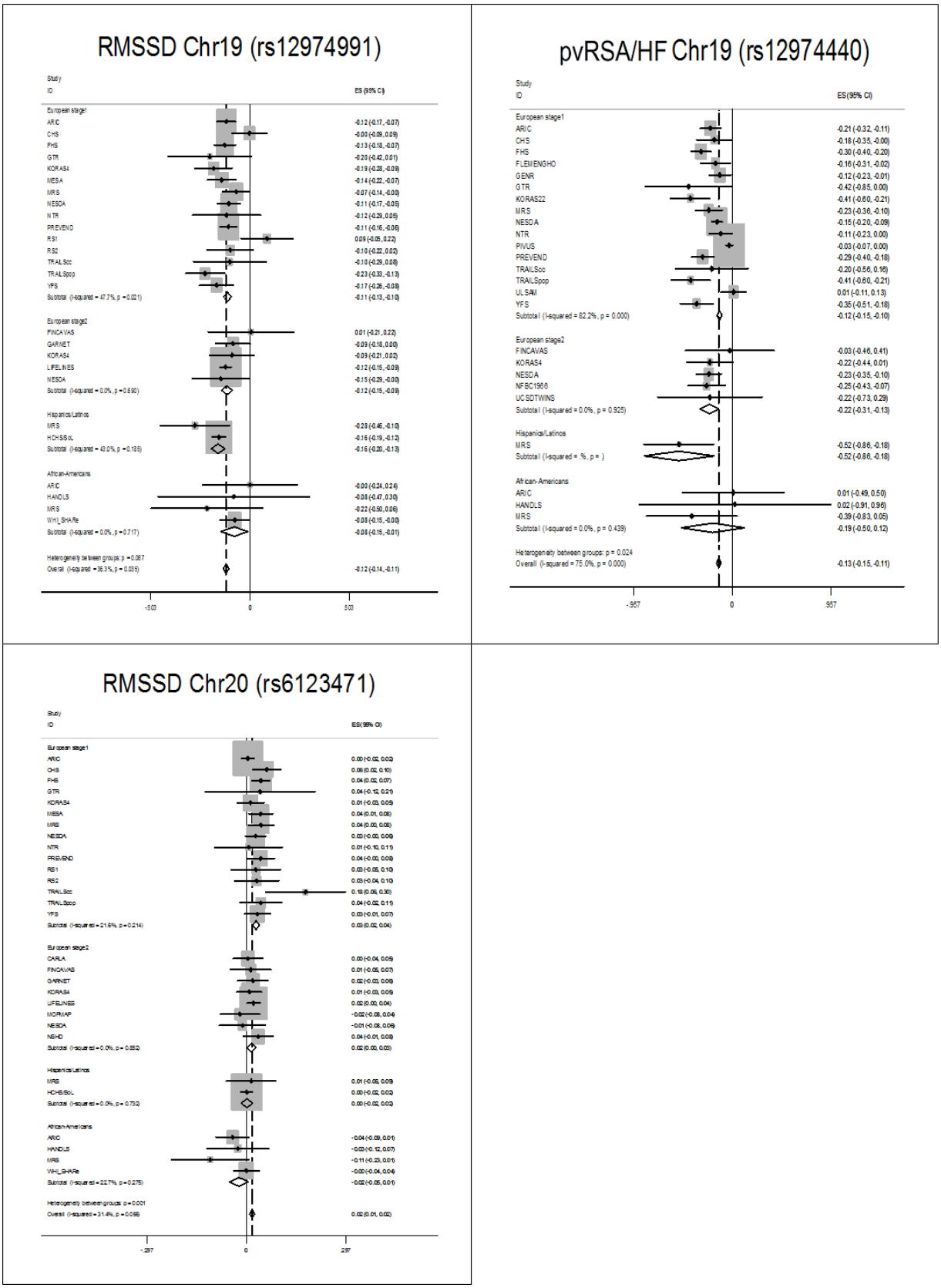
Supplementary Figure 4 (continued)



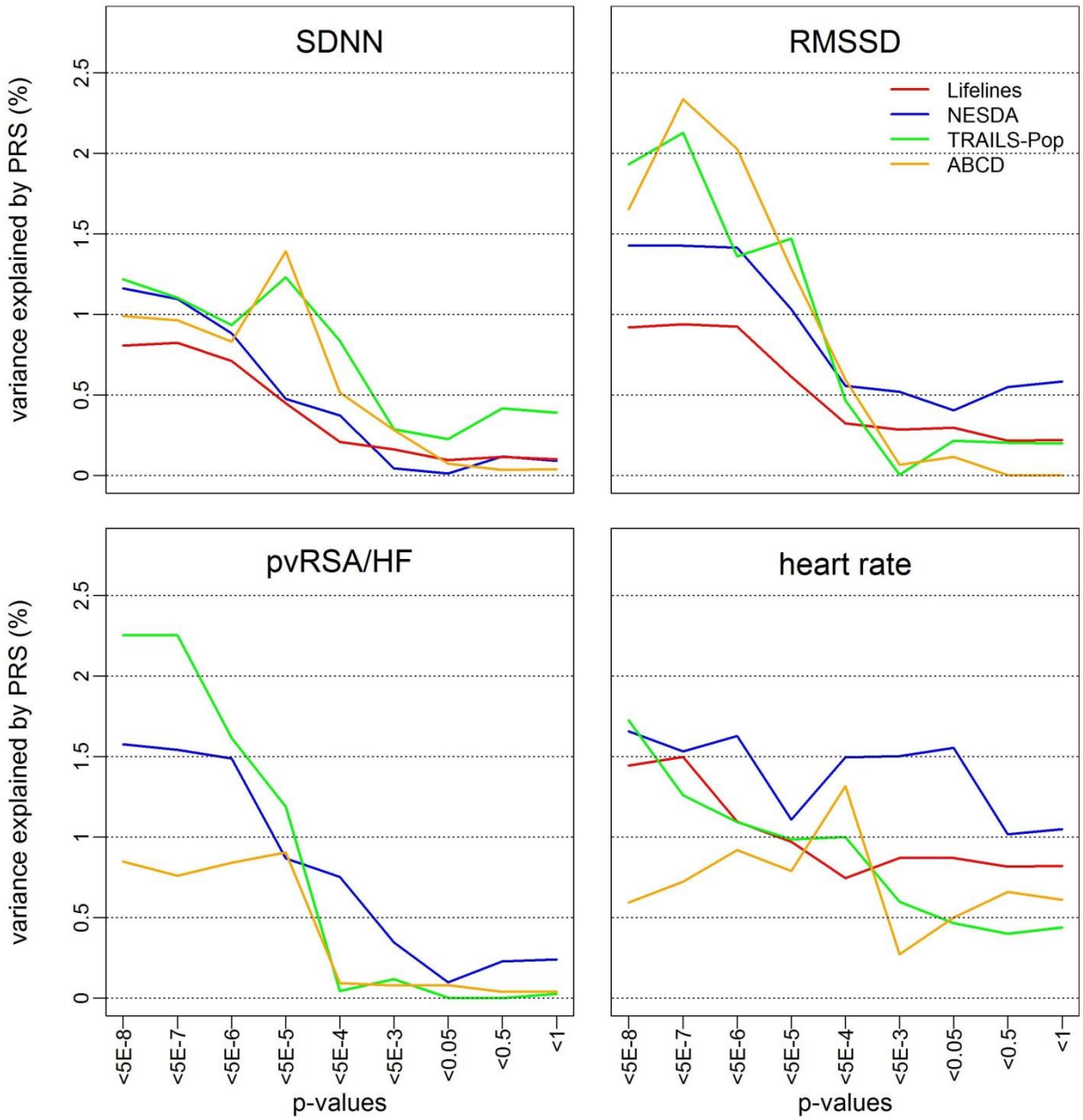
Supplementary Figure 4 (continued)



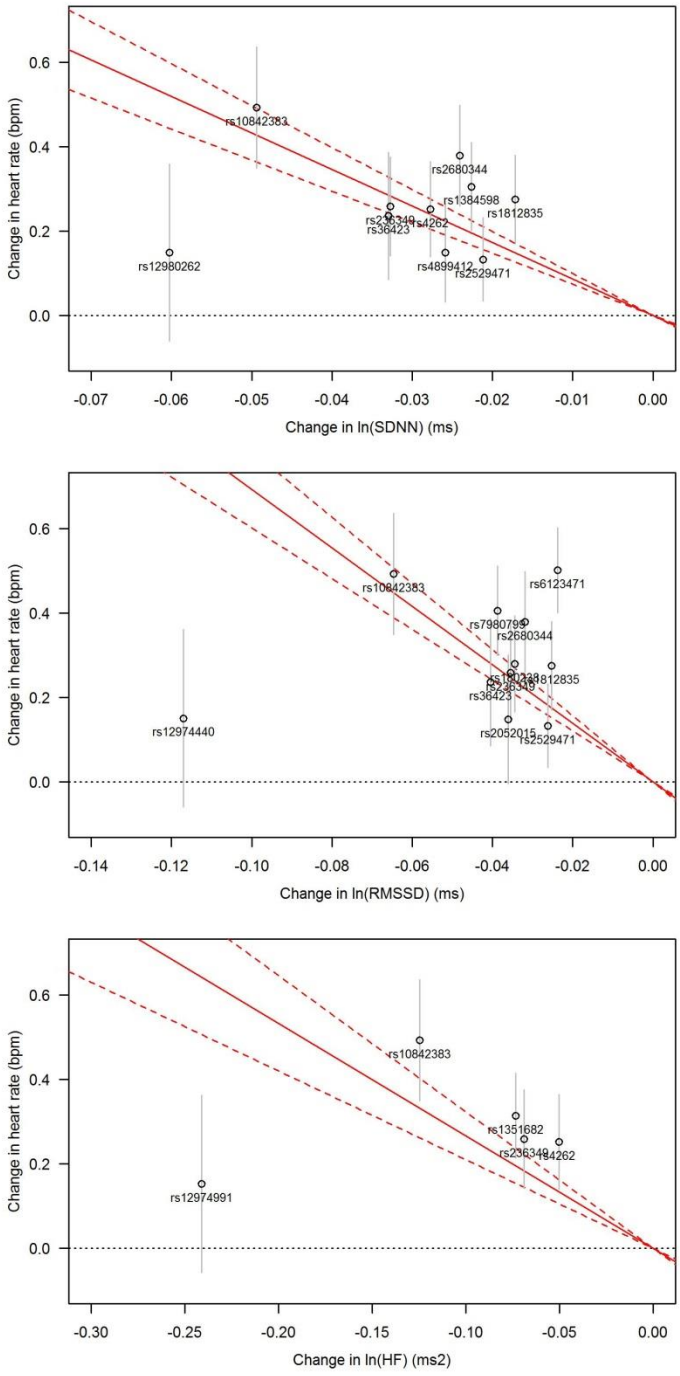
Supplementary Figure 4 (continued)



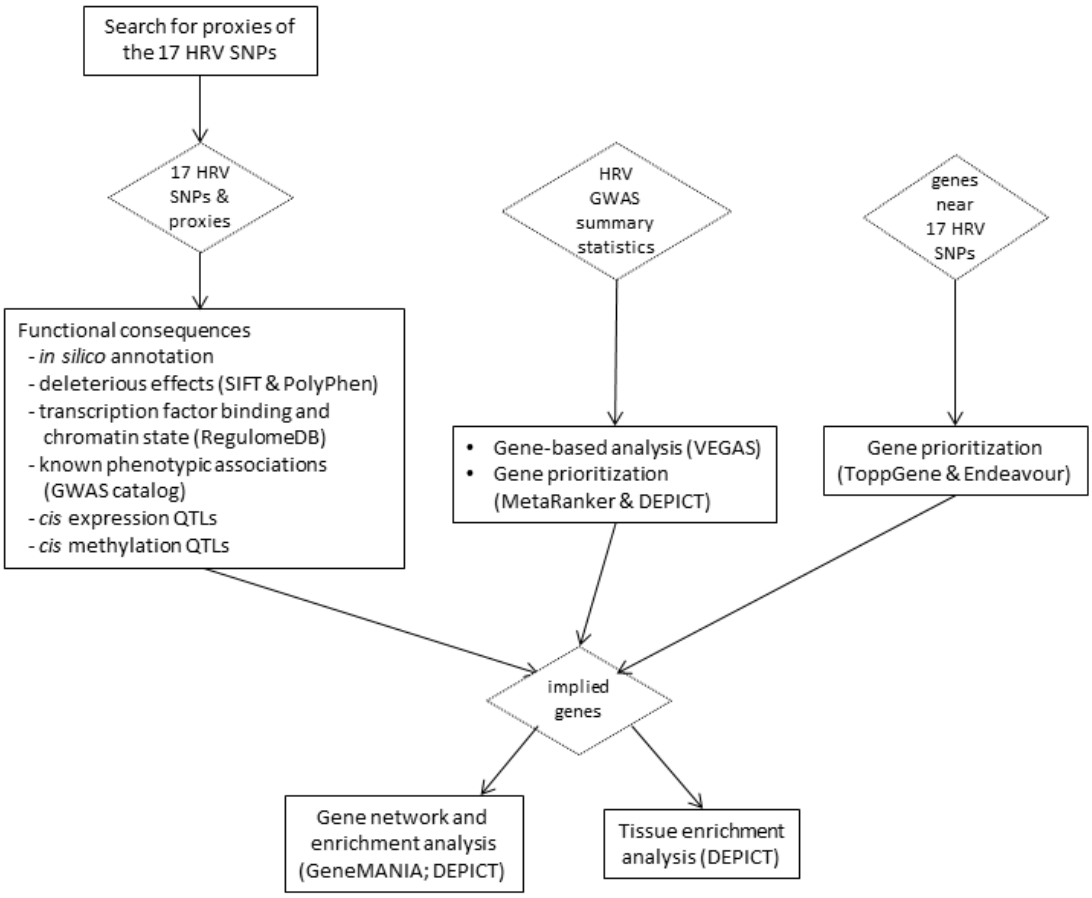
Supplementary Figure 4 (continued)



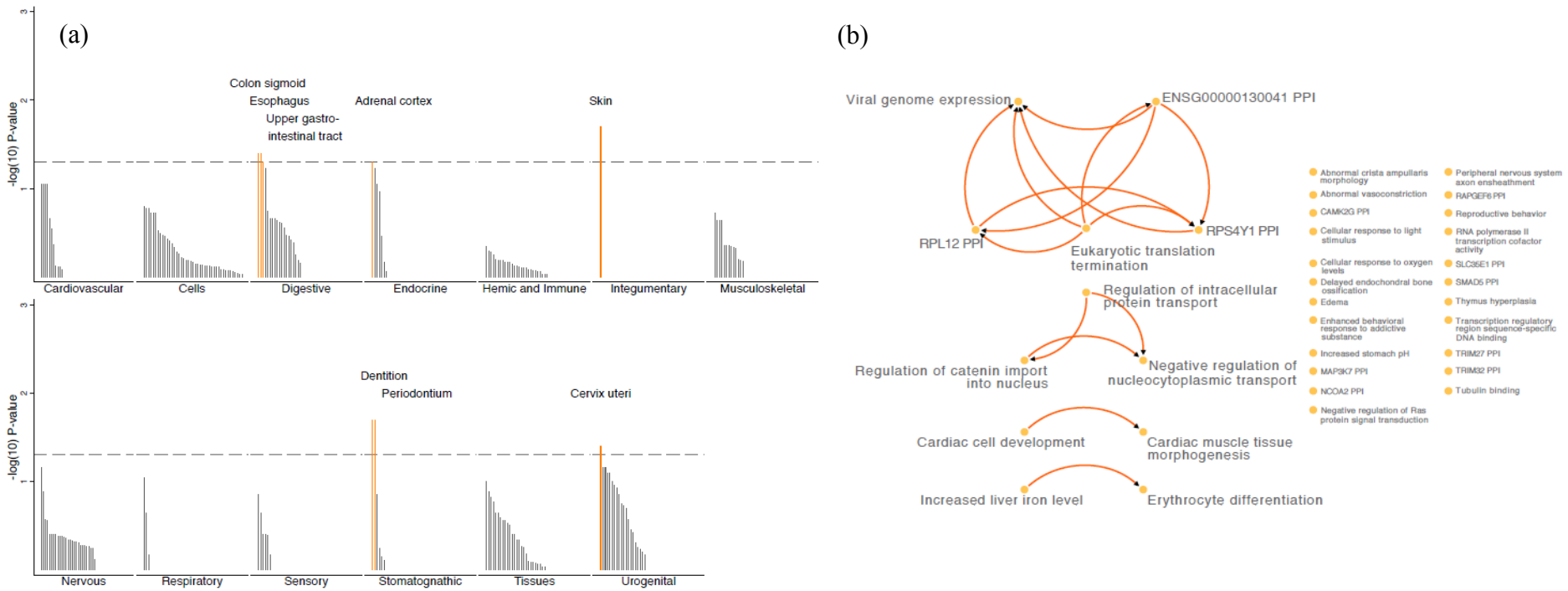
Supplementary Figure 5: Explained variance in HRV traits and heart rate by polygenic risk scores (PRS) for HRV in the Lifelines (n=12,101), NESDA (n=2,118), TRAILS-Pop (n=1,191), and ABCD (n=1,094) cohorts.
NOTE: Significance thresholds to include HRV SNPs in the polygenic risk scores are shown on the x-axis and were $p < 5.0\text{x}10^{-8}$; $p < 5.0\text{x}10^{-7}$; $p < 5.0\text{x}10^{-6}$; $p < 5.0\text{x}10^{-5}$; $p < 5.0\text{x}10^{-4}$; $p < 5.0\text{x}10^{-3}$; $p < 0.05$; $p < 0.5$; and $p < 1$. The y-axis depicts the percentage of variance explained by the polygenic risk score.



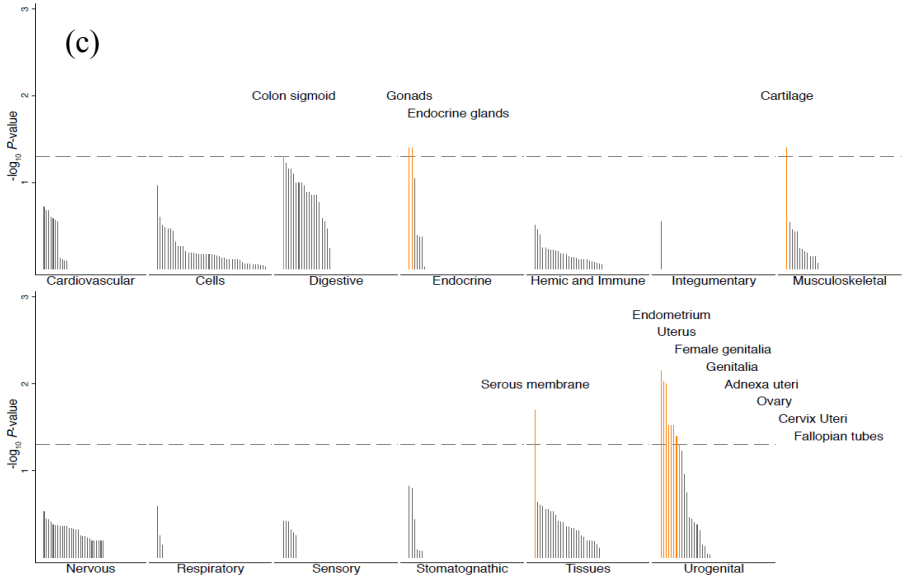
Supplementary Figure 6: Diagnostic plot for the association between genetic risk alleles for the HRV traits and heart rate.
NOTE: Each SNP is plotted by its decreasing effect on HRV per risk effect allele (x-axis) versus the estimated effect of that allele on the risk for high heart rate (y-axis). A solid red line shows the effect size estimate, called α, for the risk score on HRV. The 95% CI of α is represented by red dashed lines. The grey vertical lines indicate the 95% confidence interval (CI) of the effect on heart rate for each individual SNP. The estimated effects on heart rate are in beats per minute (bpm). Graphs from top to bottom are for SDNN, RMSSD, and pvRSA/HF, respectively.

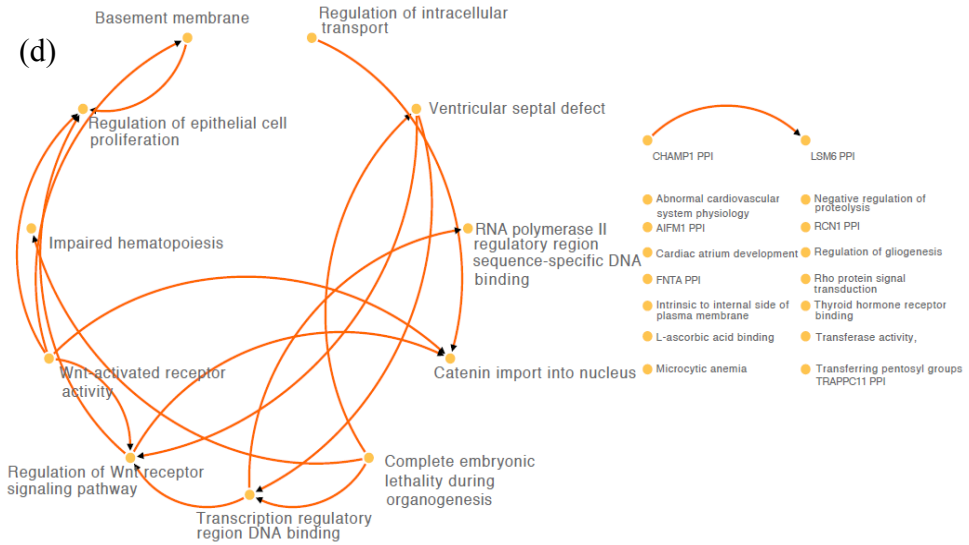


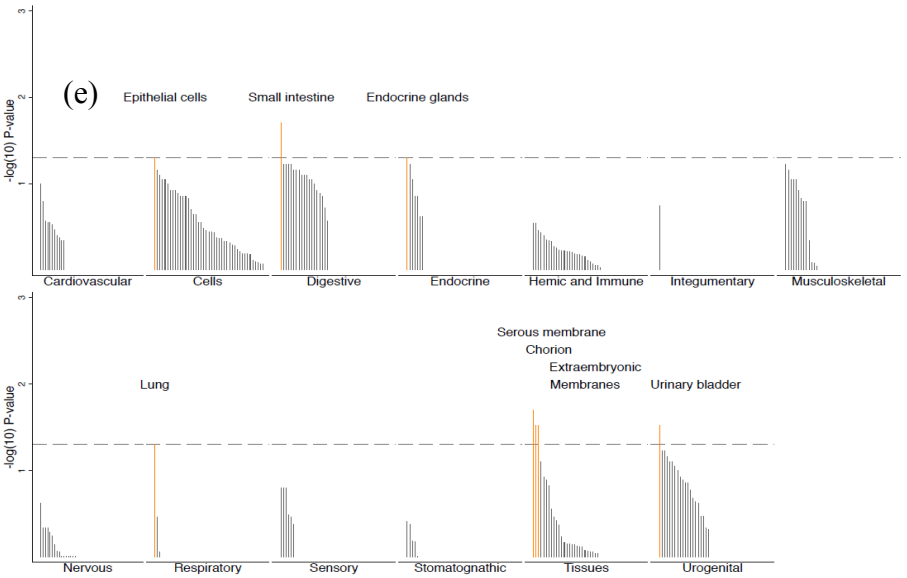
Supplementary Figure 7: Flowchart depicting the post-GWAS steps to annotate the HRV SNPs using public online resources**:** SIFT [1], PolyPhen [2], RegulomeDB [3] , VEGAS [4], MetaRanker [5], DEPICT, [6], ToppGene [7], Endeavour [8], and GeneMANIA [9].

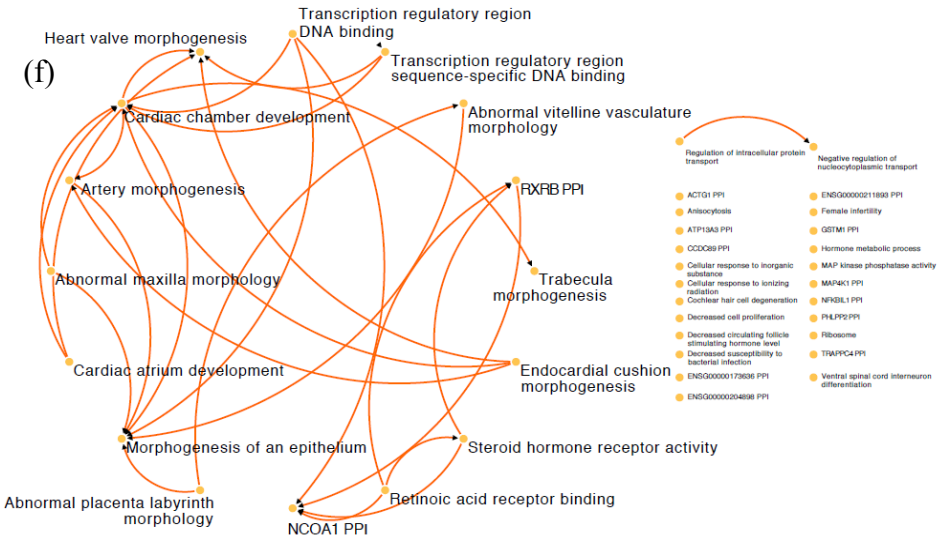


Supplementary Figure 8: Tissue enrichment and reconstituted gene set enrichment analysis using DEPICT for (a-b) SDNN; (c-d) RMSSD; and (e-f) pvRSA/HF.
NOTE: In panels (a), (c), and (e) graphical representations of DEPICT's tissue enrichment analysis are provided, highlighting annotated tissues that are enriched for expression of genes located within ±40kb of SNPs associated with $p < 10^{-5}$ to SDNN, RMSSD, and pvRSA/HF, respectively (enrichment in orange $p < 0.05$). In panels (b), (d), and (f) graphical representations of DEPICT's reconstituted gene set enrichment analysis are shown ($p < 0.05$ after Bonferroni correction) for examining SDNN, RMSSD, and pvRSA/HF, respectively. DEPICT calculates every gene's likelihood to be a member of KEGG, GEO, or REACTOME-based gene sets amongst others (N=14,461) resulting in reconstituted gene sets. Shown are the reconstituted gene sets enriched for genes in SDNN-, RMSSD-, and pvRSA/HF-associated loci (nodes), respectively, and the significant interactions between these gene sets (edges).

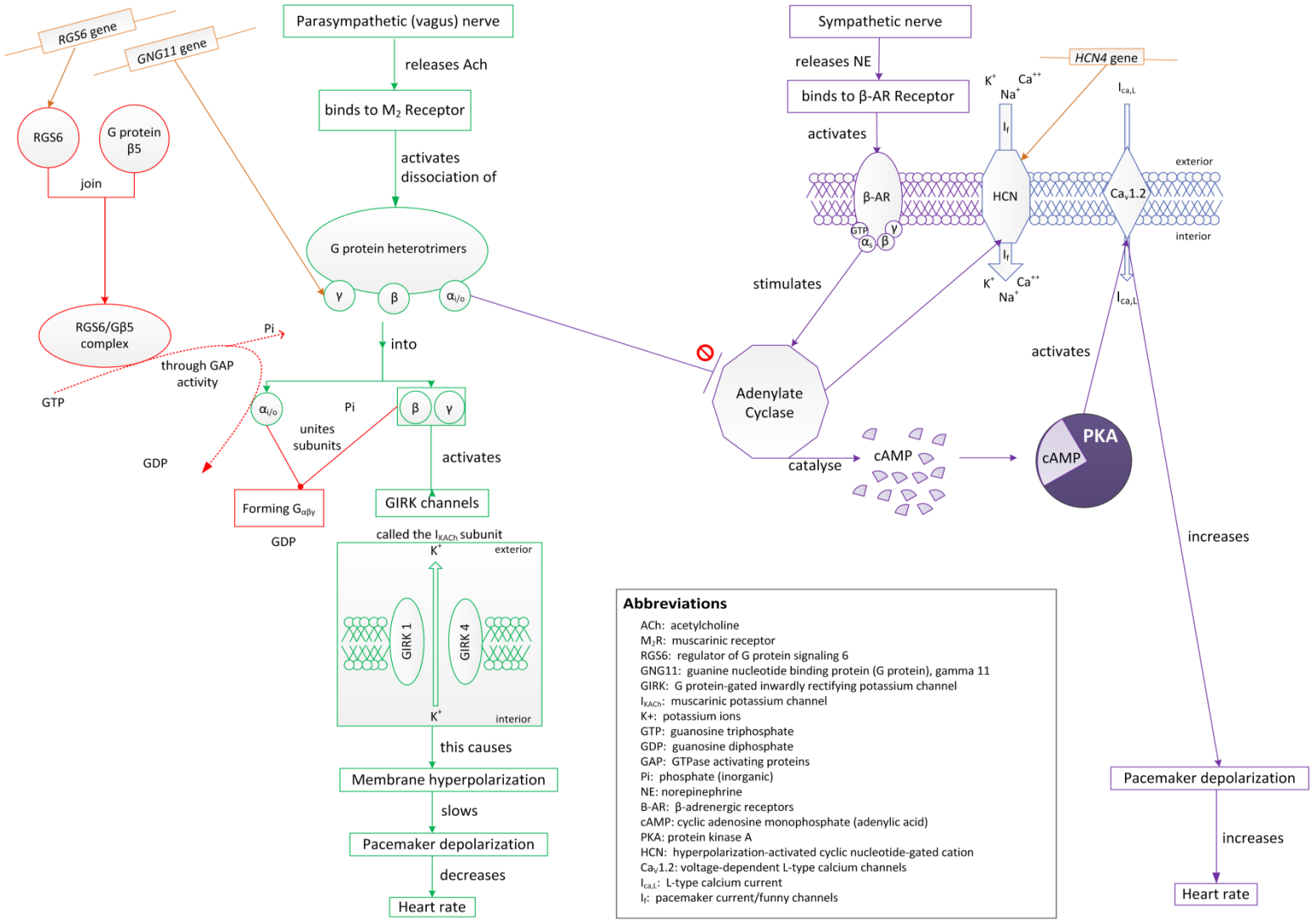








Supplementary Figure 8 (continued)



Supplementary Figure 9: A schematic representation of known cardiac vagal effects on the sinoatrial node and the potential roles of HRV SNPs in *GNG11*, *RGS6*, and *HCN4*. See for more details on next page.

Various pathways convey the effects of cardiac autonomic activity on heart rate regulation[10, 11]. To these known pathways we added the hypothesized effects of some of the HRV SNPs.

Green colored pathway: The vagal nerve releases acetylcholine (ACh), which binds to the muscarinic (M2R) receptor. This dissociates the inactive G protein heterotrimer (Gαβγ) composed of three subunits (α, β, and γ) into two components, namely the α subunit and a Gβγ component. The Gβγ component then interacts and activates the G protein-gated inwardly rectifying potassium (GIRK) channel, which is a potassium channel (I_{KACh}) composed of GIRK1 and GIRK4 subunits. This causes potassium ions (K^+) to permeate outwardly, which results in a membrane hyperpolarization slowing pacemaker depolarization and subsequently decreasing heart rate. We hypothesize that HRV SNPs in *GNG11* may lower the availability of the γ11 subunit and could reduce Gβγ component induced GIRK activation, which is expected to blunt the effects of phasic changes in cardiac vagal activity, thereby decreasing HRV.

Red colored pathway: RGS6 binds with G-protein β5 to create the RGS6/Gβ5 dimer complex, which activates GTPase activating proteins (GAPs), regulatory proteins that hydrolyze guanosine triphosphate (GTP) breaking a phosphate bond (Pi) to make guanosine diphosphate (GDP) on the α subunit. This causes the Gαi/o subunit and a Gβγ component to rejoin into the inactive Gαβγ heterotrimer. This ends GIRK channel activation and leads to an increase in heart rate.

We hypothesize the HRV SNP in *RGS6* to increase the availability of RGS6, which gives rise to a decrease in GIRK channel signaling, blunting the effects of phasic changes in cardiac vagal activity, thereby decreasing HRV.

Purple colored pathway: Catecholamine-mediated activation of β-adrenergic receptors also act as a guanine nucleotide exchange factor to dissociate the Gαβγ, with the GTP-Gαs then causing adenylate-cyclase to catalyze cAMP production. cAMP-dependent protein kinases then activate the L-type Ca,1,2 channels that increase depolarization of the pacemaker membrane, which leads to an increase in tonic heart rate.

Blue colored pathway: Through this same adenylate/cAMP signaling pathway, vagal activation of the M2R also impacts on the funny channels (I_f) of which the hyperpolarization-activated cyclic nucleotide-gated channel isoform 4 (HCN4) is the predominant molecular constituent. The I_f is permeable to K^+, Na^+ and Ca^{++} yielding a net inward current that plays a key role in the generation of the pacemaker potential[12, 13]. The unique property of reverse voltage dependence of the funny channel causes a spontaneous gradual depolarization of the pacemaker membrane until the action potential threshold, at which potential the systolic phase of the next heartbeat commences, whereas the sympathetic Gαs subunit speeds up I_f diastolic depolarization, the vagal Gαi/o subunit counters this by slowing the I_f diastolic depolarization.

We hypothesize the HRV SNP in *HCN4* to increase permeability of the HCN channel to speed up depolarization of the pacemaker membrane, thereby increasing tonic heart rate and reducing HRV.

(a) See VgHRV_Supplementary_Figure10a.png

(b) See VgHRV_Supplementary_Figure10b.png

Supplementary Figure 10: Functional enrichment analysis based on 24 query genes resulting from the GWAS meta-analyses and post-GWAS analyses on the three HRV traits.
NOTE: The following genes were used as input for GeneMANIA: *NDUFA11*, *FUT5*, *CAPS*, *PPIL1*, *C6orf89*, *SYT10*, *GNG11*, *GNGT1*, *RGS6*, *HCN4*, *NEO1*, *KIAA1755*, *CCDC141*, *TFPI2*, *ALG10*, *ALG10B*, *CPNE8*, *NRTN*, *FUT6*, *FUT3*, *VMAC*, *RFX2*, *RANBP3*, and *CPNE5*. The resulting significantly enriched gene ontology terms (false discovery rate<0.10) are visualized as highlighted boxes within their corresponding gene ontology tree depicted by RamiGO R package, as (a) yellow category: terms related to cell membrane signal transduction, and (b) green category: terms related to cellular anabolic, catabolic, and respiratory processes. The relations between the boxes have standard color scheme: green, red, black, blue, and light blue represent 'positively regulates', 'negatively regulates', 'regulates', 'is a', and 'part of', respectively (http://www.geneontology.org/GO.ontology-ext.relations.shtml).

SUPPLEMENTARY TABLES

Supplementary Table 1: Description of stage 1 discovery, stage 2 replication, stage 3 other ethnicity lookup, and post-GWAS cohorts and lookup trait/disease consortia used in the V_gHRV analyses.

Study acronym	Study full name	Study design	Country	Ethnicity	Study reference
STAGE 1 DISCOVERY COHORTS					
ARIC	Atherosclerosis Risk in Communities study	prospective population-based cohort study	USA	EUR	PMID: 2646917
CHS	Cardiovascular Health Study	prospective population-based cohort study	USA	EUR	PMID: 1669507
FHS	Framingham Heart Study	prospective population-based cohort study	USA	EUR	PMID: 14819398
FINGESTURE	FINnish GEnetic STUdy of aRrhythmic Events	prospective case-control study (cases: post-AMI patients)	Finland	EUR	ClinicalTrials.gov Identifier: NCT02075866
FLEMENGHO-EPOGH	FLEMish study on Environment, Genes and Health Outcomes – European Project on Genes in Hypertension	prospective population-based cohort study	Belgium, Romania, Poland, Italy, Russian Federation and Czech Republic	EUR	PMID: 12640246 & 22184326
GenR	Generation R Study	prospective population-based cohort study of infants	Netherlands	EUR	PMID: 16826450
GTR	Groningen Twin Registry	prospective twin study	Netherlands	EUR	PMID: 23186546
KORA S4	KOoperative gesundheitsforschung in der Region Augsburg – Survey 4	prospective population-based cohort study	Germany	EUR	PMID: 16032513 & 16032514
MESA	Multi-Ethnic Study of Atherosclerosis	prospective population-based cohort study	USA	EUR	PMID: 12397006
MRS	Marine Resiliency Study	prospective population-based cohort study	USA	EUR	DOI: http://dx.doi.org/10.5888/pcd9.110134; PMID: 25456346

NESDA	Netherlands Study of Depression and Anxiety	prospective case-control study (cases: MDD patients)	Netherlands	EUR	PMID: 18763692
NTR	Netherlands Twin Register	prospective twin-family study	Netherlands	EUR	PMID: 12537867
PIVUS	Prospective Investigation of the Vasculature in Uppsala Seniors	prospective population-based cohort study	Sweden	EUR	PMID: 16141402
PREVEND	Prevention of Renal and Vascular ENd-stage Disease	prospective population-based cohort study enriched for individuals with microalbuminuria	Netherlands	EUR	PMID: 11004219
RS(1+2)	Rotterdam Study	prospective population-based cohort study	Netherlands	EUR	PMID: 24258680
TRAILS-CC	TRacking Adolescents Individual Lives Survey – CliniCal cohort	prospective high-risk cohort study of adolescents	Netherlands	EUR	PMIDs: 25431468, 25066533
TRAILS-Pop	TRacking Adolescents Individual Lives Survey – POPulation cohort	prospective population-based cohort study of adolescents	Netherlands	EUR	see TRAILS-CC
ULSAM	Uppsala Longitudinal Study of Adult Men	prospective population-based cohort study	Sweden	EUR	PMID: 1216390; http://www2.pubcare.uu.se/ULSAM/
YFS	Cardiovascular Risk in Young Finns Study	prospective population-based cohort study	Finland	EUR	PMID: 18263651; http://youngfinnsstudy.utu.fi/
STAGE 2 REPLICATION COHORTS					
CARLA	CARdiovascular disease, Living and Ageing in Halle	prospective population-based cohort study	Germany	EUR	PMID: 19199053
FINCAVAS	FINnish CArdioVAscular Study	prospective population-based cohort study	Finland	EUR	PMID: 16515696
KORA S4	KOoperative gesundheitsforschung	prospective population-based cohort study	Germany	EUR	PMID: 16032513

	in der Region Augsburg – Survey 4				
Lifelines	Lifelines Cohort Study	prospective population-based cohort study	Netherlands	EUR	PMID: 18075776
MRC NSHD	Medical Research Council National Survey of Health and Development	prospective population-based birth cohort study	UK	EUR	PMID: 21345808; http://www.nshd.mrc.ac.uk
NESDA	Netherlands Study of Depression and Anxiety	prospective case-control study (cases: MDD patients)	Netherlands	EUR	PMID: 18763692
NFBC 1966	Northern Finland Birth Cohort 1966	prospective population-based birth cohort study	Finland	EUR	PMID 19060910
UCSD TWINS	University of California San Diego TWINS	prospective twin study	USA	EUR	PMID: 22676942
WHI CT – GARNET	Women's Health Initiative Clinical Trials (Genomics And Randomized Trials Network) controls	prospective cohort study	USA	EUR	PMID: 9492970 and http://www.genome.gov/27541119
WHI CT – MOPMAP	Women's Health Initiative Clinical Trials (Modification Of PM-Mediated Arrhythmogenesis in Populations) controls	prospective cohort study	USA	EUR	PMID: 9492970 and http://projectreporter.nih.gov/project_info_description.cfm?aid=7984809&icde=19283008
WHII	Whitehall-II study	prospective population-based cohort study	UK	EUR	PMID: 15576467
STAGE 3 LOOKUP COHORTS OF OTHER ETHNICITY					
HCHS/SOL	Hispanic Community Health Study/Study of Latinos	multicenter community-based cohort study	USA	HIS	PMID: 20609343, 20609344
MRS	Marine Resiliency Study	prospective population-based cohort study	USA	HIS	DOI: http://dx.doi.org/10.5888/pcd9.110134; PMID: 25456346
ARIC	Atherosclerosis Risk in Communities study	prospective population-based cohort study	USA	AfAm	PMID: 2646917

HANDLS	Healthy Aging in Neighborhoods of Diversity across Life Span	prospective community-based study	USA	AfAm	PMID: 20828101
MESA	Multi-Ethnic Study of Atherosclerosis	prospective population-based cohort study	USA	AfAm	PMID: 12397006
MRS	Marine Resiliency Study	prospective population-based cohort study	USA	AfAm	DOI: http://dx.doi.org/10.5888/pcd9.110134; PMID: 25456346
WHI CT – SHARe	Women's Health Initiative Clinical Trials (Single nucleotide polymorphism Health Association Resource)	prospective cohort study	USA	AfAm	PMID: 9492970 and https://www.nhlbi.nih.gov/resources/geneticsgenomics/programs/share.htm
POST-GWAS COHORTS & CONSORTIA providing lookups					
ABCD	Amsterdam Born Children and their Development	population-based	Netherlands	EUR	PMID: 20813863
OFS	Oman Family Study	five large inbred pedigrees	Oman	Arabic	PMID: 15767758
CHARGE-HF	CHARGE-Heart Failure Working Group	GWAS Consortium	multiple	EUR	PMID 20445134
CKDGen	CKDGen Consortium	GWAS Consortium	multiple	EUR	PMID: 21355061, 22479191
ICBP	International Consortium for Blood Pressure	GWAS Consortium	multiple	EUR	PMID: 21909115
AFGen Consortium	AFGen Consortium	GWAS Consortium	multiple	EUR	PMID: 22544366
CHARGE-SCD	CHARGE-SCD Consortium	GWAS Consortium	multiple	EUR	PMID:21738491

EUR: European; HIS: Hispanic/Latino; AfAm: African American; PMID: PubMed ID; NCT: ClinicalTrails.gov identifier; DOI: digital object identifier.

Supplementary Table 2: Phenotyping information of stage 1 discovery, stage 2 replication, stage 3 other ethnicity lookup, and post-GWAS cohorts used in the V_gHRV analyses.

Study acronym	Analysis sample size (N)	HRV assessment method	HRV measurement	Female/male participation
STAGE 1 DISCOVERY COHORTS				
ARIC	8262	3-lead ECG; 2 minutes; supine	RMSSD; SDNN; HF	Men and women
CHS	759	24hr Holter monitor	RMSSD; SDNN; HF	Men and women
FHS	1944	2hr ambulatory ECG	RMSSD; SDNN; HF	Men and women
FINGESTURE	494	24hr Holter monitor	SDNN; HF	Men and women
FLEMENGHO-EPOGH	196	12-lead ECG & nasal thermistor for RSA: PSA to estimate HF ranges ; ECG recording for 15 min ; supine	pvRSA	Men and women
GenR	392	3-pole ECG & breathing pattern using a piëzo-electric transducer ; 100-180 seconds ; sitting	HF	Men and women
GTR	134	type II 3-lead ECGs & respiration with a flexible band around upper thorax; 5 minutes; sitting	RMSSD; SDNN; HF	Women
KORA S4	1617	2-lead ECG ; 5 minutes ; supine	RMSSD; SDNN; HF	Men and women
MESA	2401	12-lead ECG; average from 3 sequential 10-second ECGs; supine; resting	RMSSD; SDNN	Men and women
MRS	1383	finger photoplethysmograph ; 5 minutes ; sitting	RMSSD; SDNN; HF	Men
NESDA	1740	type II, 3-lead ECG & breathing recorded from thorax impedance ; ~90 minutes ; sitting	RMSSD; SDNN; pvRSA	Men and women
NTR	439	type II, 3-lead ECG & breathing recorded from respitrace ; 8 minutes ; sitting	RMSSD; SDNN; pvRSA	Men and women
PIVUS	766	6-precordial-lead ECG & breathing recorded using custom-made impedance device ; 5-minutes ; supine ; controlled breathing (12 breaths/min)	SDNN; pvRSA	Men and women
PREVEND	2793	beat-to-beat blood pressure pulse wave recording on middle finger (Portapres); 15 minutes; supine	RMSSD; SDNN; HF	Men and women
RS1	972	12-lead ECG ; 10 seconds; resting	RMSSD; SDNN	Men and women
RS2	985	12-lead ECG ; 10 seconds; resting	RMSSD; SDNN	Men and women
TRAILS-CC	307	type II 3-lead ECG; 4 minutes (T1); supine	RMSSD; SDNN; HF	Men and women
TRAILS-Pop	1222	type II 3-lead ECG; 4 minutes (T1), 5 minutes (T3); supine	RMSSD; SDNN; HF	Men and women
ULSAM	67	6-precordial-lead ECG & breathing recorded using custom-made impedance device from a 24hr recording during normal activity	SDNN; pvRSA	Men
YFS	1827	2-lead ECG ; 3 minutes ; supine	RMSSD; SDNN; HF	Men and women

STAGE 2 REPLICATION COHORTS				
CARLA	1367	12-lead ECG ; 5 minutes for HRV analysis ; supine	RMSSD; SDNN; HF	Men and women
FINCAVAS	542	12-lead ECG ; 1 minute ; supine	RMSSD; SDNN; HF	Men and women
KORA S4	1959	2-lead ECG ; 5 minutes ; supine	RMSSD; SDNN; HF	Men and women
Lifelines	12101	12-lead ECG; 10 seconds; supine	RMSSD; SDNN	Men and women
MRC NSHD	1127	3 lead ECG ; 6 minutes ; supine	RMSSD; SDNN; HF	Men and women
NESDA	606	type II, 3-lead ECG & breathing recorded from thorax impedance ; ~90 minutes ; sitting	RMSSD; SDNN; pvRSA	Men and women
NFBC 1966	1941	lead-II ECG recording ; 3 minutes ; sitting	SDNN; HF	Men and women
UCSD TWINS	230	3 lead ECG ; 5 minutes; sitting	HF	Men and women
WHI CT – GARNET	1648	12-lead ECG; 10 seconds; resting; supine	RMSSD; SDNN	Women
WHI CT – MOPMAP	1198	12-lead ECG; 10 seconds; resting; supine	RMSSD; SDNN	Women
WHII	1755	12-lead ECG; 5 minutes; resting; supine	SDNN; HF	Men and women
STAGE 3 LOOKUP COHORTS OF OTHER ETHNICITY				
HCHS/SOL	10830	12-lead ECG; one 10-second ECG; supine; resting	RMSSD; SDNN	Men and women
MRS	404	finger photoplethysmograph & (simultaneous)2-lead ECG recording ; 5 minutes ; sitting	RMSSD; SDNN; HF	Men
ARIC	1582	3-lead ECG; 2 minutes; supine	RMSSD; SDNN; HF	Men and women
HANDLS	188	Portapres ambulatory HR and BP monitor; 5 minutes; sitting	RMSSD; SDNN; HF	Men and women
MESA	1480	12-lead ECG; average from 3 sequential 10-second ECGs; supine; resting	RMSSD; SDNN	Men and women
MRS	131	finger photoplethysmograph ; 5 minutes ; sitting	RMSSD; SDNN; HF	Men
WHI CT – SHARe	3518	12-lead ECG; 10 seconds; supine	RMSSD; SDNN	Women
POST-GWAS COHORTS				
ABCD	1094	type II, 3-lead ECG & breathing recorded from thorax impedance ; ~4 minutes ; sitting	RMSSD; SDNN; pvRSA	Men and women
OFS	1326	type II, 6-lead ECG ; 10 minutes; supine	RMSSD; SDNN; HF	Men and women

ECG: electrocardiogram; RSA: respiratory sinus arrhythmia; PSA: proportion of specific agreement; HF: high frequency; HR: heart rate; BP: blood pressure.

Supplementary Table 3: Clinical characteristics of stage 1 discovery, stage 2 replication, and stage 3 other ethnicity, and post-GWAS cohorts used in the V_gHRV analyses.

Study acronym	Analysis sample size (N)	Women (%)		Age	SDNN	ln(SDNN)	RMSSD	ln(RMSSD)	pvRSA/HF	ln(pvRSA/HF)
				Mean (SD)						
STAGE 1 DISCOVERY COHORTS										
ARIC	8.262	54.9	overall	54.04 (5.65)	37.58 (19.14)	3.52 (0.46)	27.87 (21.73)	3.15 (0.58)	16.89 (44.33)	2.03 (1.27)
			women	53.74 (5.63)	36.27 (18.16)	3.49 (0.45)	27.96 (20.37)	3.16 (0.58)	17.96 (37.96)	2.16 (1.24)
			men	54.41 (5.66)	39.18 (20.16)	3.56 (0.46)	27.75 (23.28)	3.14 (0.58)	15.59 (51.00)	1.87 (1.29)
CHS	759	58.1	overall	71.2 (4.5)	96.7 (28.4)	4.5 (0.3)	21.5 (11.8)	2.9 (0.4)	115.8 (172.1)	4.3 (0.8)
			women	70.6 (4.1)	93.1 (25.4)	4.5 (0.3)	20.3 (9.1)	2.9 (0.4)	104.9 (116.6)	4.3 (0.8)
			men	72.0 (4.9)	101.6 (32.0)	4.6 (0.3)	23.2 (14.8)	3.0 (0.5)	130.8 (227.7)	4.3 (0.9)
FHS	1.944	53.86	overall	51.70 (12.85)	91.76 (27.97)	4.47 (0.31)	33.54 (16.61)	3.41 (0.44)	34.07 (38.51)	5.44 (0.88)
			women	52.23 (13.06)	93.60 (27.79)	4.45 (0.32)	33.94 (16.76)	3.40 (0.44)	33.91 (37.67)	5.44 (0.89)
			men	51.09 (12.60)	89.61 (28.18)	4.49 (0.30)	33.08 (16.43)	3.42 (0.44)	34.26 (39.47)	5.43 (0.86)
FINGESTURE	494	23.68	overall	61 (9.96)	97.84 (31.86)	4.53 (0.34)	n.a.	n.a.	525.4 (885.4)	5.83 (0.84)
			women	64 (9.56)	87.88 (32.36)	4.41 (0.36)	n.a.	n.a.	630.8 (1400.1)	5.85 (0.89)
			men	60 (9.89)	100.72 (31.17)	4.58 (0.32)	n.a.	n.a.	493.7 (661.1)	5.82 (0.83)
FLEMENGHO-EPOGH	196	48.98	overall	52.74 (11.44)	n.a.	n.a.	n.a.	n.a.	n.a.	3.07 (0.40)
			women	53.00 (10.54)	n.a.	n.a.	n.a.	n.a.	n.a.	3.02 (0.37)
			men	52.50 (12.30)	n.a.	n.a.	n.a.	n.a.	n.a.	3.11 (0.41)
GenR	392	49.49	overall	1.22 (0.08)	n.a.	n.a.	n.a.	n.a.	n.a.	2.85 (0.42)
			women	1.22 (0.08)	n.a.	n.a.	n.a.	n.a.	n.a.	2.86 (0.44)
			men	1.22 (0.07)	n.a.	n.a.	n.a.	n.a.	n.a.	2.84 (0.38)
GTR	134	100	overall	23.43 (3.39)	51.05 (20.96)	3.86 (0.39)	37.17 (25.04)	3.45 (0.55)	1034.68 (1684.47)	6.29 (1.1)
			women	23.43 (3.39)	51.05 (20.96)	3.86 (0.39)	37.17 (25.04)	3.45 (0.55)	1034.68 (1684.47)	6.29 (1.1)
			men	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
KORA S4	1.617	51.64	overall	53.46 (8.82)	35.93 (19.15)	3.47 (0.46)	25.26 (19.11)	3.04 (0.61)	61.20 (215.59)	3.25 (1.25)
			women	53.26 (8.77)	35.36 (19.34)	3.46 (0.46)	25.79 (19.32)	3.06 (0.60)	61.43 (106.10)	3.37 (1.24)
			men	53.68 (8.87)	36.54 (18.94)	3.49 (0.46)	24.69 (18.89)	3.01 (0.61)	60.96 (290.09)	3.12 (1.26)
MESA	2.401	52.35	overall	62.3 (10.1)	33.6 (16.4)	2.9 (0.6)	25.0 (22.5)	3.0 (0.66)	n.a.	n.a.
			women	62.3 (10.2)	22.5 (14.5)	2.9 (0.6)	25.3 (19.5)	3.0 (0.64)	n.a.	n.a.
			men	62.4 (9.9)	22.6 (18.1)	2.9 (0.6)	24.7 (25.4)	2.9 (0.67)	n.a.	n.a.
MRS	1.383	0	overall	22.75 (3.48)	65.43 (27.43)	4.10 (0.41)	59.51 (34.98)	3.94 (0.54)	4304.72 (5277.67)	7.86 (1.04)
			women	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
			men	22.75 (3.48)	65.43 (27.43)	4.10 (0.41)	59.51 (34.98)	3.94 (0.54)	4304.72 (5277.67)	7.86 (1.04)
NESDA	1.740	68.05	overall	41.88 (12.59)	61.19 (25.20)	4.11 (3.23)	33.14 (20.66)	3.50 (3.03)	42.95 (24.23)	1.57 (0.24)
			women	40.95 (12.69)	60.31 (24.43)	4.10 (3.20)	33.96 (20.83)	3.53 (3.04)	45.65 (24.60)	1.60 (0.23)
			men	43.86 (12.15)	63.07 (26.71)	4.14 (3.29)	31.38 (20.20)	3.45 (3.01)	37.19 (22.35)	1.50 (0.25)

NTR	439	65.15	overall	30.83 (14.42)	77.09 (32.75)	4.21 (0.46)	61.02 (34.41)	3.97 (0.55)	76.73 (46.56)	4.18 (0.58)
			women	29.84 (14.71)	60.65 (34.16)	3.98 (0.54)	75.22 (31.28)	4.24 (0.44)	79.52 (45.94)	4.23 (0.57)
			men	32.67 (7.84)	81.28 (35.47)	4.30 (0.50)	61.80 (41.20)	3.97 (0.59)	71.51 (47.41)	4.09 (0.62)
PIVUS	766	53.66	overall	70.20 (0.18)	34.56 (18.19)	3.43 (0.46)	n.a.	n.a.	243.20 (385.89)	4.79 (1.20)
			women	70.27 (0.15)	34.60 (18.61)	3.44 (0.45)	n.a.	n.a.	240.6 (335.90)	4.84 (1.17)
			men	70.13 (0.18)	34.52 (17.73)	3.43 (0.47)	n.a.	n.a.	246.2 (437.10)	4.73 (1.24)
PREVEND	2.793	49.48	overall	53.22 (11.68)	35.64 (17.46)	3.47 (0.45)	29.35 (19.70)	3.24 (0.51)	438.32 (1089.07)	5.39 (1.13)
			women	53.90 (12.06)	36.15 (18.19)	3.48 (0.47)	28.11 (19.76)	3.19 (0.51)	398.76 (1109.34)	5.27 (1.13)
			men	52.55 (11.25)	35.14 (16.71)	3.46 (0.44)	30.57 (19.58)	3.28 (0.51)	477.12 (1067.78)	5.50 (1.12)
RS1	972	37.55	overall	79.13 (4.79)	36.70 (31.39)	3.37 (0.64)	35.66 (45.39)	3.13 (0.86)	n.a.	n.a.
			women	78.80 (4.47)	43.07 (39.77)	3.48 (0.71)	43.30 (57.93)	3.23 (3.23)	n.a.	n.a.
			men	79.23 (4.97)	79.23 (4.97)	3.31 (0.59)	31.06 (35.05)	5.66 (3.08)	n.a.	n.a.
RS2	985	58.78	overall	72.13 (5.00)	35.10 (27.72)	3.38 (0.56)	31.91 (45.54)	3.07 (0.77)	n.a.	n.a.
			women	72.25 (5.18)	32.87 (25.68)	3.33 (0.52)	29.49 (44.97)	3.03 (0.72)	n.a.	n.a.
			men	71.96 (4.71)	38.27 (30.15)	3.44 (0.60)	35.34 (46.18)	3.12 (0.85)	n.a.	n.a.
TRAILS-CC	307	30.62	overall	11.11 (0.48)	75.79 (38.05)	6.51 (0.50)	89.21 (54.25)	4.31 (0.62)	4277.19 (5828.07)	7.64 (1.27)
			women	11.07 (0.55)	73.52 (30.44)	6.51 (0.44)	82.15 (42.75)	4.26 (0.59)	3509.59 (3736.52)	7.60 (1.16)
			men	11.13 (0.45)	76.79 (40.98)	6.51 (0.52)	92.31 (58.41)	4.33 (0.64)	4619.12 (6526.94)	7.65 (1.31)
TRAILS-Pop	1.222	52.7	overall	11.46 (1.57)	70.74 (35.84)	4.13 (0.51)	77.61 (51.06)	4.14 (0.68)	3715.66 (5230.48)	7.47 (1.32)
			women	11.42 (1.54)	67.49 (33.42)	4.09 (0.49)	73.04 (48.02)	4.09 (0.66)	3327.63 (4981.83)	7.36 (1.30)
			men	11.50 (1.60)	74.34 (38.05)	4.18 (0.53)	82.69 (53.83)	4.20 (0.69)	4145.51 (5465.12)	7.58 (1.35)
ULSAM	67	0	overall	71.05 (0.46)	163.81 (52.87)	5.05 (0.31)	n.a.	n.a.	11.37 (6.60)	2.25 (0.54)
			women	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
			men	71.05 (0.46)	163.81 (52.87)	5.05 (0.31)	n.a.	n.a.	11.37 (6.60)	2.25 (0.54)
YFS	1.827	55.12	overall	31.68 (4.99)	50.76 (23.36)	3.83 (0.44)	48.48 (32.34)	3.69 (0.62)	1010 (1497)	6.26 (1.18)
			women	31.68 (4.98)	50.6 (24.35)	3.82 (0.46)	50.05 (34.74)	3.70 (0.65)	1161 (1759)	6.37 (1.23)
			men	31.68 (4.99)	50.97 (22.1)	3.84 (0.42)	46.56 (29.03)	3.67 (0.58)	823.8 (1063)	6.13 (1.11)
Stage 1 discovery total		**28.700**								
STAGE 2 REPLICATION COHORTS										
CARLA	1.367	47.7	overall	62.74 (9.77)	30.55 (15.89)	3.30 (0.48)	23.25 (19.14)	2.94 (0.61)	291.60 (557.22)	4.88 (1.22)
			women	62.56 (9.59)	31.61 (16.18)	3.35 (0.45)	24.94 (19.35)	3.03 (0.58)	343.60 (623.83)	5.14 (1.14)
			men	62.91 (9.93)	29.58 (15.57)	3.27 (0.49)	21.70 (18.82)	2.86 (0.62)	244.00 (484.18)	4.65 (1.25)
FINCAVAS	542	44.83	overall	52.90 (12.94)	28.96 (15.35)	3.22 (0.57)	20.95 (12.15)	2.89 (0.56)	102.48 (190.76)	3.80 (1.29)
			women	54.51 (11.94)	27.29 (14.05)	3.16 (0.56)	19.98 (11.07)	2.85 (0.55)	91.89 (137.72)	3.76 (1.27)
			men	51.59 (13.57)	30.31 (16.23)	3.26 (0.58)	21.74 (12.92)	2.92 (0.57)	111.09 (224.71)	3.83 (1.32)
KORA S4	1.959	50.89	overall	44.98 (15.85)	42.40 (23.38)	3.62 (0.51)	33.76 (23.98)	3.30 (0.68)	105.48 (191.06)	3.80 (1.41)
			women	44.21 (15.41)	42.85 (24.47)	3.63 (0.51)	35.37 (25.40)	3.35 (0.67)	117.80 (220.36)	3.94 (1.37)
			men	45.78 (16.26)	41.94 (22.20)	3.61 (0.52)	32.09 (22.30)	3.25 (0.68)	92.71 (154.03)	3.65 (1.43)

Lifelines	12.101	58.59	overall	48.01 (11.14)	33.42 (24.22)	3.29 (0.66)	33.41 (28.13)	3.26 (0.71)	n.a.	n.a.
			women	48.00 (11.10)	33.84 (24.22)	3.31 (0.65)	34.87 (29.06)	3.30 (0.70)	n.a.	n.a.
			men	48.00 (11.21)	32.84 (24.22)	3.27 (0.68)	31.35 (26.62)	3.19 (0.72)	n.a.	n.a.
MRC NSHD	1.127	55.72	overall	63.3 (1.0)	32.3 (13.1)	3.4 (0.4)	21.2 (12.2)	2.9 (0.5)	227.3 (1.3)	4.7 (1.0)
			women	63.4 (1.0)	31.7 (12.3)	3.4 (0.4)	21.8 (12.2)	2.9 (0.5)	240.0 (4.7)	4.8 (1.0)
			men	63.3 (1.1)	33.1 (14.0)	3.4 (0.4)	20.5 (12.1)	2.9 (0.5)	162.9 (207.8)	4.6 (1.0)
NESDA	606	64.36	overall	42.33 (14.32)	77.01 (34.27)	4.25 (0.43)	45.34 (35.24)	3.57 (0.68)	47.78 (32.08)	3.68 (0.64)
			women	42.06 (14.17)	75.41 (31.94)	4.24 (0.42)	45.12 (33.08)	3.59 (0.65)	50.38 (32.43)	3.76 (0.60)
			men	42.82 (14.62)	79.90 (38.02)	4.28 (0.45)	45.76 (38.92)	3.54 (0.74)	43.08 (30.95)	3.54 (0.69)
NFBC 1966	1.941	54.97	overall	46.6 (0.6)	32.5 (14.3)	3.4 (0.4)	n.a.	n.a.	264.6 (407.9)	4.8 (1.3)
			women	46.6 (0.6)	32.3 (13.5)	3.4 (0.4)	n.a.	n.a.	304.8 (447.2)	5.0 (1.3)
			men	46.6 (0.6)	32.9 (15.3)	3.4 (0.5)	n.a.	n.a.	215.6 (348.2)	4.6 (1.3)
UCSD TWINS	230	76.52	overall	41.03 (16.53)	61.89 (32.41)	3.98 (0.6)	n.a.	n.a.	152.16 (364.59)	3.96 (1.60)
			women	41.27 (16.38)	61.52 (33.14)	3.98 (0.6)	n.a.	n.a.	165.10 (407.87)	4.02 (1.59)
			men	38.78 (16.96)	63.07 (30.18)	4.00 (0.63)	n.a.	n.a.	110.01 (150.62)	3.77 (1.60)
WHI CT–GARNET (controls)	1.648	100	overall	65.26 (6.77)	19.73 (15.63)	2.76 (0.65)	21.09 (19.74)	2.79 (0.68)	n.a.	n.a.
			women	65.26 (6.77)	19.73 (15.63)	2.76 (0.65)	21.09 (19.74)	2.79 (0.68)	n.a.	n.a.
			men	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
WHI CT–MOPMAP (controls)	1.198	100	overall	63.11 (6.61)	20.29 (15.93)	2.79 (0.65)	22.26 (20.86)	2.84 (0.69)	n.a.	n.a.
			women	63.11 (6.61)	20.29 (15.93)	2.79 (0.65)	22.26 (20.86)	2.84 (0.69)	n.a.	n.a.
			men	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
WHII	1.755	24.22	overall	60.30 (5.85)	38.73 (27.04)	3.53 (0.47)	n.a.	n.a.	382.48 (2618.92)	4.76 (1.20)
			women	60.64 (5.83)	40.81 (28.20)	3.52 (0.46)	n.a.	n.a.	484.38 (2851.76)	5.02 (1.22)
			men	60.19 (5.83)	38.06 (25.19)	3.56 (0.48)	n.a.	n.a.	349.92 (2697.58)	4.67 (1.19)
Stage 2 replication total		**24.474**								
STAGE 3 LOOKUP COHORTS OF OTHER ETHNICITY										
HCHS/SOL	10.830	59.41	overall	45.18 (13.61)	29.75 (22.53)	3.16 (0.69)	35.79 (29.25)	3.31 (0.73)	n.a.	n.a.
			women	45.91 (13.41)	29.89 (22.03)	3.18 (0.67)	36.98 (29.56)	3.36 (0.72)	n.a.	n.a.
			men	44.11 (13.83)	29.55 (23.23)	3.14 (0.71)	34.04 (28.70)	3.25 (0.75)	n.a.	n.a.
MRS	404	0	overall	22.51 (3.12)	62.90 (24.84)	4.06 (0.40)	58.57 (32.98)	3.93 (0.52)	4069.94 (4706.06)	7.82 (1.02)
			women	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
			men	22.51 (3.12)	62.90 (24.84)	4.06 (0.40)	58.57 (32.98)	3.93 (0.52)	4069.94 (4706.06)	7.82 (1.02)
Total HIS		**11.234**								
ARIC	1.582	63.15	overall	53.06 (5.76)	38.00 (19.77)	3.5 (0.52)	34.20 (24.54)	3.33 (0.63)	22.84 (37.44)	2.38 (1.31)
			women	52.94 (5.63)	36.93 (19.10)	3.48 (0.52)	34.51 (24.38)	3.34 (0.64)	23.98 (37.10)	2.45 (1.30)
			men	53.28 (5.98)	39.84 (20.77)	3.56 (0.51)	33.67 (24.82)	3.32 (0.62)	20.90 (38.00)	2.28 (1.33)
HANDLS	188	54.79	overall	47.32 (8.90)	32.79 (13.91)	3.40 (0.43)	34.04 (13.67)	3.45 (0.41)	483.87 (604.08)	5.68 (1.01)
			women	47.59 (9.17)	32.56 (12.55)	3.41 (0.40)	35.96 (13.79)	3.51 (0.92)	541.57 (566.36)	5.86 (0.97)
			men	47.04 (8.61)	33.06 (15.46)	3.39 (0.47)	31.68 (13.23)	3.37 (0.41)	413.94 (643.31)	5.47 (1.02)

MESA	1.480	54.73	overall	61.9 (10.0)	26.72 (19.78)	3.08 (0.64)	32.72 (27.80)	3.24 (0.70)	n.a.	n.a.
			women	61.84 (9.95)	26.71 (18.53)	3.09 (0.62)	33.33 (25.74)	3.27 (0.68)	n.a.	n.a.
			men	61.95 (10.14)	26.74 (20.99)	3.07 (0.65)	31.99 (30.11)	3.20 (0.72)	n.a.	n.a.
MRS	131	0	overall	23.74 (4.73)	59.83 (22.94)	4.03 (0.37)	58.76 (28.53)	3.96 (0.48)	3804.05 (4239.97)	7.87 (0.91)
			women	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
			men	23.74 (4.73)	59.83 (22.94)	4.03 (0.37)	58.76 (28.53)	3.96 (0.48)	3804.05 (4239.97)	7.87 (0.91)
WHI CT - SHARe	3.518	100	overall	60.84 (6.70)	22.03 (17.67)	2.86 (0.68)	26.79 (24.92)	3.01 (0.73)	n.a.	n.a.
			women	60.84 (6.70)	22.03 (17.67)	2.86 (0.68)	26.79 (24.92)	3.01 (0.73)	n.a.	n.a.
			men	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Total AfAm		**6.899**								
Stage 3 other ethnicity total		**18.133**								
POST-GWAS COHORTS										
ABCD	1.094	0.51	overall	5.51 (0.38)	73.10 (30.95)	4.2 (0.43)	80.87 (45.91)	4.23 (0.59)	121.39 (62.42)	4.66 (0.54)
			women	5.51 (0.39)	72.42 (30.95)	4.19 (0.43)	79.22 (46.19)	4.21 (0.59)	120.72 (62.65)	4.66 (0.53)
			men	5.50 (0.37)	73.80 (30.96)	4.21 (0.43)	82.58 (45.60)	4.25 (0.60)	122.09 (62.23)	4.67 (0.55)
OFS	1.326	56.03	overall	33.41 (15.73)	61.92 (32.46)	4.02 (0.45)	41.39 (22.66)	3.57 (0.56)	197.43 (2569.48)	10.58 (1.49)
			women	33.98 (14.90)	55.27 (29.54)	3.91 (0.44)	39.34 (23.34)	3.50 (0.59)	97.78 (233.66)	10.35 (1.54)
			men	32.68 (16.73)	70.24 (34.01)	4.16 (0.43)	43.96 (21.53)	3.67 (0.51)	318.79 (3816.42)	10.87 (1.38)

SD: standard deviation; n.a.: not available.

Supplementary Table 4: Technical information on the genotyping and imputation method of stage 1 discovery, stage 2 replication, stage 3 other ethnicity lookup, and post-GWAS cohorts used in the V_gHRV analyses.

Study acronym	Array(s)	Calling Algorithm	QC filters for exclusion of genotyped SNPs	QC filters for exclusion of individuals	Imputation software	Imputation reference panel (build)	Total SNPs after imputation	Statistical software	Genomic control lambda SDNN / RMSSD / pvRSA/HF
STAGE 1 DISCOVERY COHORTS									
ARIC	Affymetrix GeneChip SNP Array 6.0	Birdseed	call rate ≤ 95%; MAF <1%; pHWE< 1E-6	call rate <95%	MaCH v1.0.16	HapMap Phase II data in the CEU individuals release 22 (build36)	2.543.887	Probabel	1.015 / 1.011 / 1.012
CHS	Illumina 370CNV	BeadStudio	call rate <97%; MAF: n.a. - excluded SNPs with 0 heterozygotes; pHWE< 1E-5	call rate<=95% or if their genotype was discordant with known sex or prior genotyping	BIMBAM v0.99	HapMap Phase II data in the CEU individuals release 22 (build36)	2.318.082	R	1.011 / 1.009 / 1.016
FHS	Affymetrix 500K, Affymetrix 50K supplemental	BRLMM	call rate ≤ 97%; MAF: n.a.; pHWE< 1E-6	call rate <90%	Mach1 v1.0.15	HapMap Phase II data in the CEU individuals release 22 (build36)	2.466.720	PLINK, R	1.012 / 1.010 / 1.014
FINGESTURE	Affymetrix 6.0	Birdseed (as implemented in Affymetrix powertools 1.10.2 package)	call rate < 95%; MAF < 0.01; pHWE: n.a.	Sample genotyping rate ≤97%	MACH 1.0.15	HapMap Phase II data in the CEU individuals release 22 (build36)	2.543.888	MACH2QTL V1.0.4	0.997/ - / 1.006
FLEMENGHO-EPOGH	ILLUMINA 1M	ILLUMINA Genome Studio	call rate< 99%; MAF <0.01; pHWE< 1E-08	call rate <99%; sex mismatch; heterozygosity > 4SD from mean; non-Caucasians	MACH	HapMap Phase II data in the CEU individuals release 22 (build36)	2.581.944	PLINK, R	- / - / 1.003
GenR	Ilumina 610 Quad array	Genomestudio 2009 V.1.1.9	call rate <95%; MAF <1%; pHWE< 1E-6	Call rate <97,50%; Heterozygosity > 4SD from mean; Caucasian only, ethnic outliers excluded based on PCA	MACH	HapMap Phase II data in the CEU individuals release 22 (build36)	25.438.877	PLINK	- / - / 1.007

GTR	Illumina Cyto SNP12 v2	GenomeStudio	call rate < 95%; MAF <1%; pHWE< 1E-4	call rate <95%; sex mismatch; heterozygosity > 4SD from mean; non-Caucasians	IMPUTE v2	HapMap Phase II data in the CEU individuals release 22 (build36)	2.631.501	SNPTEST v2.2.0	1.013 / 1.022 / 1.017
KORA S4	Affymetrix 6.0	Birdseed v2	call rate< 93%; HapMap SNPs only	call rate< 93%; sex mismatch	MACH v1.0.15	HapMap Phase II data in the CEU individuals release 22 (build36)	2.543.887	MACH2QTL v1.0.8	1.022 / 1.012 / 1.007
MESA	Affymetrix 6.0	Birdseed v2	call rate < 95%; MAF: n.a.; pHWE: n.a; heterozygosity > 53%.	sex mismatches, expected and unexpected duplicates, call rate < 95%	IMPUTE v2.1.0	HapMap Phase I and II CEU individuals release 24 (build36)	3.854.661	R	0.994 / 0.998 / -
MRS	Illumina HumanOmniExpressExome array (HOEE 12v1.0)	Genome Studio	call rate< 95%; MAF: n.a.; pHWE< 5E-08	call rate <98%; heterozygosity <0.211 or >0.302; non-Caucasians	IMPUTEv2	1000Genomes global panel phase 1 release v3 (build 37)	32.179.008	SNPTEST v2.4.1	0.999 / 1.008 / 1.003
NESDA	Perlegen-Affymetrix 5.0; Affymetrix 6.0 907K	Proprietary	call rate ≤ 95%; MAF <0.01; pHWE: n.a.	Non-Caucasians, XO and XXY samples, and samples with a call rate <95%, high genome-wide homo- or heterozygosity, excess IBS	IMPUTE	HapMap Phase II data in the CEU individuals release 22 (build36)	2.135.543	SNPTEST v1.1.4	1.008 / 1.000 / 0.998
NTR	Affymetrix-Perlegen; Illumina 370, 660, 1M Omi; Affymetrix 6.0	Proprietary Affymetrix Calling Algorithms, Birdsuite 1 & 2.	callrate SNPs < 90%, MAF < 0.01, HWE < 0.00001	Callrate < 90%, Sex /IBD mismatch, F(st) > 0.10 or F < 0.10, Ethnicity based on PCs (deviating from maximum European PC values), Mendelian 2 error rate in relation to family member > 2%	IMPUTE	HapMap Phase II CEU B36 rel 24.	2.401.535	SNPTEST v1.1.4	0.998 / 1.002 / 1.015

PIVUS	Illumina OmniExpress + Metabochip	Proprietary	call rate< 95%; MAF< 0.01; pHWE< 1E-6	Call rate <95%; heterozygosity, gender check and relatedness	IMPUTE	HapMap Phase II data in the CEU individuals release 22 (build36)	2.616.874	PLINK	1.004 / - / 1.006
PREVEND	Illumina HumanCytoSNP12 v2 beadchip assay	Illumina Genome Studio software	call rate < 0.95; MAF <0.01; pHWE <E-5	call rate < 0.95; sex mismatch; non-Caucasians	BEAGLE v3.3.1	HapMap Phase II data in the CEU individuals release 22 (build36)	2.269.099	PLINK	1.014 / 1.000 / 1.001
RS1	Illumina, HumanHap550 - chip v3.0	BeadStudio	call rate < 98%; MAF <1%; pHWE< 1E-6	excess autosomal heterozygosity, sex mismatch or outlying identity-by-state clustering estimates	Mach1 v1.0.15	HapMap Phase II data in the CEU individuals release 22 (build36)	2.543.887	ProbABEL,R	1.006 / 1.011 / -
RS2	Illumina550K Duo, 610KQuad	GenomeStudio	call rate < 98%; MAF <1%; pHWE< 1E-6	excess autosomal heterozygosity, sex mismatch or outlying identity-by-state clustering estimates	Machv1.0.16	HapMap Phase II data in the CEU individuals release 22 (build36)	2.543.887	ProbABEL, R	0.995 / 1.007 / -
TRAILS-CC	Illumina Cyto SNP12 v2	GenomeStudio	call rate < 95%; MAF <1%; pHWE< 1E-4	call rate <95%; sex mismatch; heterozygosity > 4SD from mean; non-Caucasians	IMPUTE v2	HapMap Phase II data in the CEU individuals release 22 (build36)	2.631.501	SNPTEST v2.2.0	1.014 / 1.012 / 1.011
TRAILS-Pop	Illumina Cyto SNP12 v2	GenomeStudio	call rate < 95%; MAF <1%; pHWE< 1E-4	call rate <95%; sex mismatch; heterozygosity > 4SD from mean; non-Caucasians	IMPUTE v2	HapMap Phase II data in the CEU individuals release 22 (build36)	2.631.501	SNPTEST v2.2.0	0.994 / 0.994 / 0.992
ULSAM	Illumina OMNI2.5 + Metabochip	Proprietary	call rate< 99%%; MAF <0.01; pHWE: n.a.	Call rate <95%; heterozygosity, gender check and relatedness	IMPUTE v2	HapMap Phase II data in the CEU individuals release 22 (build36)	3.126.254	PLINK v1.07	1.005 / - / 1.007
YFS	Illumina 670k custom	Illuminus	call rate ≤ 95%; MAF<0.01; pHWE< 1E-6	Call rate <95%; heterozygosity, gender check and relatedness	MACH 1.0	HapMap Phase II data in the CEU individuals release 22 (build36)	2.543.887	ProbABEL v0.1-3	1.006 / 1.013 / 1.015

STAGE 2 REPLICATION COHORTS									
CARLA	TaqMan® (Applied Biosystems, Darmstadt, Germany)	Sequence Detection Software SDS 2.3	call rate< 95%; MAF <0.01; pHWE: n.a.	non-Caucasians	n.a.	n.a.	n.a.	R	-
FINCAVAS	Metabochip	GenomeStudio	call rate ≤ 95%; MAF <0.01; pHWE< 1E-6	Call rate <95%; heterozygosity, gender check and relatedness	SHAPEIT v2 / IMPUTE 2.3.0	1000Genomes global panel phase 1 release v3 (build 37)	38.943.535	SNPTEST v2.5	-
KORA S4	Affymetrix Axiom	Affymetrix Software	call rate ≤ 98%; MAF: <1%; pHWE< 1E-6	call rate ≤ 97%; sex mismatch; heterozygosity >5SD from mean; non-Caucasian; population outliers; participant in stage 1	SHAPEIT v2, IMPUTE v2.3.0	1000Genomes global panel phase 1 release v3 (build 37)	30.067.091	R	-
Lifelines	Illumina Cyto SNP12 v2	GenomeStudio	call rate < 98%; MAF <1%; pHWE< 1E-5	call rate <95%; sex mismatch; heterozygosity > 4SD from mean; non-Caucasians	MACH minimac	1000Genomes global panel phase 1 release v3 (build 37)	28.681.763	PLINK v1.07	-
MRC NSHD	LGC KASP	Proprietary	call rate < 90%; MAF <1%; pHWE< 0.05	call rate < 90%,	n.a.	n.a.	n.a.	SAS	-
NESDA	Affymetrix 6.0 907K	Birdseed	call rate< 95%; MAF <0.01; pHWE<1E-5.; unmapped; allele frequency difference with reference >20%; ambiguous SNPs with allele frequency>35%	call rate<90%; heterozygosity abs(PLINK F)>0.1; sex mismatch; unexpected relatedness	Minimac	1000Genomes global panel phase 1 release v3 (build 37)	3.000.779	SNPTEST v2.4.1	-

NFBC 1966	Illumina HumanCNV-370DUO Analysis BeadChip	Beadstudio	call rate < 95%; MAF <1%; pHWE< 1E-07	call rate <95%	IMPUTE v2	HapMap Phase II data in the CEU individuals release 22 (build36)	3.855.963	SNPTEST 2.4.1	-
UCSD TWINS	Illumina 610 Quad genotyping array	Proprietary	call rate< 95%; MAF <0.01; pHWE: n.a.	call rate <95%	MaCH	HapMap Phase II data in the CEU individuals release 22 (build36)	2.587.522	PLINK	-
WHI – GARNET	Human Omni1-Quad v1-0 B	BeadStudio v3.1.3.0	call rate ≤ 98%; MAF: n.a.; pHWE< 1E-4	call rate ≤ 98%	BEAGLE v3.3.1	1000Genomes global panel phase 1 release v3 (build 37)	8.905.697	lm.R	-
WHI – MOPMAP	Affymetrix Axiom Genome-Wide Human CEU I	Birdseed	call rate ≤ 90%; MAF <0.5%; pHWE< 1E-06	call rate ≤ 95%	MaCH minimac	HapMap Phase II data in the CEU individuals release 22 (build36)	2.543.886	lm.R	-
WHII	Illumina Metabochip	GenomeStudio	call rate< 0.95	call rate< 0.95, sex mismatch, duplicates	Minimac	1000 Genomes global panel phase 1 release v3 (build 37)	1.217.802	PLINK	-
STAGE 3 LOOKUP COHORTS OF OTHER ETHNICITY									
HCHS/SOL	Illumina HumanOmni 2.5-8v1-1 + custom content	GenomeStudio v2011.1	call rate: 98%; MAF: n.a.; pHWE< 1E-05	sex mismatch; unexpected duplicates	IMPUTEv2	1000Genomes global panel phase 1 release v3 (build 37)	25.568.744	R/Bioconductor GENESIS package	-
MRS	Illumina HumanOmni ExpressExome array (HOEE 12v1.0)	Genome Studio	call rate: 95%; MAF: n.a.; pHWE< 5E-08	call rate <98%; heterozygosity <0.211 or >0.302; non-Hispanics	IMPUTEv2	1000Genomes global panel phase 1 release v3 (build 37)	32.179.008	SNPTEST v2.4.1	-

ARIC	Affymetrix GeneChip SNP Array 6.0	Birdseed	call rate ≤ 90%; MAF <1%; pHWE: n.a.	call rate <95%	MaCH v1.0.16	HapMap Phase II data in the YRI+CEU individuals release 22 (build36)	2.653.878	Probabel	-
HANDLS	Illumina genotyping arrays including 1M and 1Mduo	Illumina BeadStudio	Genotype call rate <95%; HWE p-value < 1.0E-7; MAF < 0.01;	Sample call rate < 95%; excess heterozygosity > 3SD from the mean; Ethnic outliers, Cryptic relatedness; Sex mismatch	MACH/minimac	HapMap Phase II data in the YRI+CEU individuals release 22 (build36)	2.939.993	MACH2QTLv1.08	-
MESA	Affymetrix 6.0	Birdseed v2	call rate < 95%; MAF: n.a.; pHWE: n.a; heterozygosity > 53%.	sex mismatches, expected and unexpected duplicates, call rate < 95%	IMPUTE v2.2.2	1000Genomes global panel phase 1 release v3 (build 37)	39.295.080	R	-
MRS	Illumina HumanOmniExpressExome array (HOEE 12v1.0)	Genome Studio	call rate ≤ 95%; MAF: n.a.; pHWE< 5E-08	call rate <98%; heterozygosity <0.211 or >0.302; non-African-Americans	IMPUTEv2	1000Genomes global panel phase 1 release v3 (build 37)	32.179.008	SNPTEST v2.4.1	-
WHI – SHARe	Affymetrix GeneChip SNP Array 6.0	Birdseed	call rate ≤ 95%; MAF <1%; pHWE< 1E-06	call rate ≤ 95%	MaCH v1.0.16	HapMap Phase II data in the YRI+CEU individuals release 22 (build36)	2.203.608	lm.R	-
POST-GWAS COHORTS									
ABCD	Illumina HumanCoreExomeChip	GenomeStudio	call rate ≤ 95%; MAF <1%; pHWE< 1E-06	Call rate <95%; heterozygosity, gender check and relatedness	IMPUTE v2	1000Genomes global panel phase 1 release v3 (build 37)	27.448.454	GTOOL; PLINK	-

MAF: minor allele frequency; HWE: Hardy-Weinberg Equilibrium

Supplementary Table 5: Conditional analyses of the SNPs on chromosomes 14 and 15 for confirmation of independent secondary signals within these loci using (a) individual data from Lifelines Cohort Study (n=12,101) and (b) using the joint-and-conditional analysis in the Genome-wide Complex Trait Analysis software package of the stage 1 summary statistics.

(a)		conditioned on		SDNN		RMSSD	
SNP	locus	SNP	locus	PCondLL	POrigLL	PCondLL	POrigLL
rs2052015	14b	rs4899412	14b	1.2E-02	4.7E-05	2.1E-02	1.2E-04
rs2529471	14c	rs4899412	14b	2.5E-05	4.7E-06	2.3E-05	4.6E-06
rs36423	14a	rs4899412	14b	2.1E-03	1.3E-04	2.3E-02	2.4E-03
rs4899412	14b	rs2052015	14b	8.5E-01	1.3E-03	7.8E-01	1.9E-03
rs2529471	14c	rs2052015	14b	6.1E-05	4.7E-06	5.2E-05	4.6E-06
rs36423	14a	rs2052015	14b	1.4E-02	1.3E-04	9.0E-02	2.4E-03
rs4899412	14b	rs2529471	14c	7.8E-03	1.3E-03	1.1E-02	1.9E-03
rs2052015	14b	rs2529471	14c	6.3E-04	4.7E-05	1.4E-03	1.2E-04
rs36423	14a	rs2529471	14c	7.8E-04	1.3E-04	1.0E-02	2.4E-03
rs4899412	14b	rs36423	14a	2.4E-02	1.3E-03	1.8E-02	1.9E-03
rs2052015	14b	rs36423	14a	4.7E-03	4.7E-05	3.5E-03	1.2E-04
rs2529471	14c	rs36423	14a	2.7E-05	4.7E-06	1.8E-05	4.6E-06
rs2680344	15a	rs1812835	15b	4.0E-02	4.0E-03	1.9E-02	9.1E-04
rs1812835	15b	rs2680344	15a	2.7E-02	2.8E-03	8.7E-03	4.3E-04

PCondLL=*p*-value of the SNP in Lifelines when conditioned on other SNP;
POrigLL=original *p*-value of the SNP in Lifelines when not conditioned on any other SNP.

(b)		conditional p-value GCTA	
SNP	locus	SDNN	RMSSD
rs4899412	14b	3.1E-04	9.4E-03
rs2052015	14b	2.0E-03	4.6E-09[a]
rs2529471	14c	9.0E-07[b]	4.1E-05
rs36423	14a	1.0E-07[b]	2.7E-03
rs2680344	15a	1.1E-07[c]	7.6E-04
rs1812835	15b	1.3E-03	6.6E-07[d]

[a] rs2052015 was identified as the independent SNP associated with RMSSD in the locus on chromosome 14. The *p*-value given for this SNP is the unconditioned one. The *p*-values for the other three SNPs in this locus are *p*-values conditioned on rs2052015.
[b] rs2529471 and rs36423 were identified as independent SNP associated with SDNN in the locus on chromosome 14. The *p*-values given for these two SNPs are the *p*-values from the model that contained both SNPs. The *p*-values for the other two SNPs in this locus are *p*-values conditioned on rs2529471 and rs36423.
[c] rs2680344 was identified as independent SNP associated with SDNN in the locus on chromosome 15. The *p*-value given for this SNP is the unconditioned one. The *p*-value for the other SNP in this locus is the *p*-value conditioned on rs2680344.
[d] rs1812835 was identified as independent SNP associated with RMSSD in the locus on chromosome 15. The *p*-value given for this SNP is the unconditioned one. The *p*-value for the other SNP in this locus is the *p*-value conditioned on rs1812835.

Supplementary Table 6: Sex-stratified stage 1+2 meta-analysis results for SDNN, RMSSD, and pvRSA/HF for all SNPs that were genome-wide significant ($p<5x10^{-8}/3$) in the overall analysis. SNPs are sorted as in Table 1.

Locus	Chr	SNP	Position (bp) (b36)	Trait	Men					Women				
					N	EAF	β	SE	*p*-value	N	EAF	β	SE	*p*-value
1	19	rs12974991	5845584	RMSSD	18966	0.078	-0.121	0.012	**5.54E-23**	24172	0.078	-0.108	0.011	**2.69E-23**
		rs12974440	5845386	pvRSA/HF†	13625	0.074	-0.246	0.027	**1.87E-20**	15033	0.074	-0.241	0.029	**6.88E-20**
		rs12980262	5844058	SDNN	20270	0.076	-0.065	0.009	**4.79E-13**	25690	0.077	-0.055	0.008	**1.36E-11**
2	12	rs10842383	24663234	SDNN	21606	0.866	-0.048	0.006	**2.98E-14**	26116	0.865	-0.051	0.006	**1.35E-18**
				RMSSD	18979	0.865	-0.060	0.009	**3.90E-12**	24175	0.864	-0.068	0.008	**8.14E-19**
				pvRSA/HF†	14855	0.868	-0.105	0.019	**5.50E-09**	15362	0.869	-0.152	0.019	**1.04E-17**
3	6	rs236349	36928543	SDNN	22771	0.650	-0.036	0.005	**1.17E-14**	28524	0.653	-0.029	0.004	**8.53E-12**
				RMSSD	20144	0.655	-0.040	0.006	**8.13E-10**	26025	0.657	-0.033	0.006	**9.11E-09**
				pvRSA/HF†	16119	0.644	-0.074	0.013	**1.27E-10**	16666	0.648	-0.058	0.014	3.04E-05
4	12	rs7980799	33468257	RMSSD	18979	0.394	-0.041	0.006	**1.40E-10**	25229	0.391	-0.037	0.006	**2.44E-11**
		rs1351682	33490042	pvRSA/HF†	14702	0.436	-0.082	0.013	**5.67E-09**	15074	0.440	-0.063	0.014	1.48E-06
		rs1384598	33514166	SDNN	20223	0.432	-0.027	0.005	**2.64E-08**	27132	0.433	-0.020	0.004	1.81E-06
5	7	rs4262	93389364	SDNN	21606	0.391	-0.028	0.005	**1.81E-08**	27314	0.389	-0.028	0.004	**3.68E-10**
				pvRSA/HF†	14955	0.391	-0.067	0.014	9.44E-08	15457	0.387	-0.042	0.015	6.15E-05
		rs180238	93388383	RMSSD	18979	0.337	-0.037	0.006	**1.77E-08**	25373	0.335	-0.033	0.006	**5.12E-09**
6	14b	rs4899412	71534015	SDNN	20723	0.253	-0.024	0.005	5.80E-06	27443	0.252	-0.027	0.005	**1.54E-08**
		rs2052015	71556806	RMSSD	19526	0.166	-0.033	0.009	1.28E-04	25339	0.164	-0.038	0.008	9.30E-07
	14c	rs2529471	71883022	SDNN	21439	0.428	-0.024	0.005	9.07E-08	28095	0.428	-0.019	0.004	1.83E-06
	14a	rs36423	71422955	SDNN	20652	0.129	-0.032	0.007	4.93E-06	27445	0.130	-0.035	0.006	**1.05E-08**
				RMSSD	19422	0.127	-0.042	0.009	6.88E-06	25373	0.129	-0.039	0.008	2.15E-06
7	15a	rs2680344	71440538	SDNN	22768	0.777	-0.023	0.005	2.64E-05	28517	0.778	-0.025	0.005	4.42E-07
	15b	rs1812835	71294557	RMSSD	18979	0.416	-0.025	0.006	4.51E-05	25374	0.419	-0.026	0.005	1.53E-06
8	20	rs6123471	36273570	RMSSD	20144	0.532	-0.029	0.006	6.45E-06	26025	0.536	-0.019	0.006	5.10E-04

Chr: chromosome; bp: base pair position; N: sample size; EAF: effect allele frequency; β: beta/effect size; SE: standard error of β. †*p*-value, allele, EAF, N from *p*-value weighted meta-analysis of all cohorts using METAL and β, SE from inverse-variance meta-analysis of only HF cohorts using GWAMA.

Supplementary Table 7: Comparison of the meta-analysis results for the four stage 1 cohorts with ambulatory ECG measurements (CHS, FHS, FINGESTURE, ULSAM) versus those for the other stage 1 cohorts with HRV at rest in a laboratory setting for the 17 genome-wide significantly associated HRV SNPs identified in this study. SNPs are sorted as in Table 1.

Locus	Chr	SNP	Trait	Laboratory rest					Ambulatory					Difference in β's between laboratory rest and ambulatory	
				N	β	SE	*p*-value	I^2	N	β	SE	*p*-value	I^2	*p*-value	I^2
1	19	rs12974991	RMSSD	23646	-0.119	0.011	7.75E-27	40%	2703	-0.094	0.024	1.13E-04	**82%**	3.34E-01	0%
		rs12974440	pvRSA/HF†	20705	-0.241	0.024	7.64E-30	39%	2770	-0.272	0.045	2.73E-09	34%	5.40E-01	0%
		rs12980262	SDNN	24480	-0.055	0.008	2.08E-12	59%	2770	-0.037	0.016	2.46E-02	75%	2.92E-01	10%
2	12	rs10842383	SDNN	24487	-0.055	0.006	4.56E-22	15%	2770	-0.029	0.011	8.62E-03	54%	3.41E-02	**78%**
			RMSSD	23664	-0.074	0.008	9.57E-22	0%	2703	-0.041	0.016	1.25E-02	54%	6.24E-02	71%
			pvRSA/HF†	20705	-0.137	0.017	1.78E-20	48%	2770	-0.100	0.030	1.01E-03	5%	2.79E-01	15%
3	6	rs236349	SDNN	24487	-0.031	0.004	1.84E-12	17%	2770	-0.017	0.008	4.67E-02	0%	1.25E-01	57%
			RMSSD	23662	-0.034	0.007	1.48E-06	9%	2703	-0.013	0.015	3.93E-01	42%	2.05E-01	38%
			pvRSA/HF†	20705	-0.075	0.013	9.61E-11	33%	2770	-0.056	0.024	2.27E-02	0%	4.84E-01	0%
4	12	rs7980799	RMSSD	23453	-0.043	0.006	7.13E-14	0%	2703	-0.026	0.013	4.02E-02	0%	2.20E-01	34%
		rs1351682	pvRSA/HF†	20263	-0.072	0.012	1.19E-09	4%	2770	-0.075	0.023	3.52E-03	0%	8.88E-01	0%
		rs1384598	SDNN	22839	-0.025	0.004	2.97E-08	0%	2770	-0.021	0.008	1.10E-02	9%	7.17E-01	0%
5	7	rs4262	SDNN	24486	-0.030	0.004	9.91E-12	25%	2770	-0.020	0.009	2.71E-02	0%	2.89E-01	11%
			pvRSA/HF†	20704	-0.050	0.012	4.12E-08	14%	2770	-0.045	0.025	7.05E-02	0%	8.55E-01	0%
		rs180238	RMSSD	23663	-0.044	0.006	7.35E-14	25%	2703	-0.016	0.013	2.00E-01	0%	5.28E-02	73%
6	14b	rs4899412	SDNN	24485	-0.027	0.005	2.25E-08	46%	2770	-0.014	0.010	1.52E-01	0%	2.55E-01	23%
		rs2052015	RMSSD	23663	-0.046	0.008	1.57E-08	7%	1944	-0.033	0.022	1.32E-01	n.a.	5.77E-01	0%
	14c	rs2529471	SDNN	24487	-0.021	0.004	6.54E-07	6%	2770	-0.017	0.008	4.17E-02	0%	6.38E-01	0%
	14a	rs36423	SDNN	24482	-0.029	0.006	1.95E-06	54%	2703	-0.045	0.013	7.09E-04	0%	2.62E-01	20%
			RMSSD	23659	-0.042	0.008	4.14E-07	32%	2703	-0.025	0.020	2.13E-01	17%	4.37E-01	0%
7	15a	rs2680344	SDNN	24485	-0.028	0.005	5.39E-08	0%	2770	-0.010	0.010	3.25E-01	0%	1.10E-01	61%
	15b	rs1812835	RMSSD	23664	-0.027	0.006	1.30E-06	0%	2703	-0.016	0.012	1.97E-01	0%	4.12E-01	0%
8	20	rs6123471	RMSSD	23664	-0.024	0.006	3.75E-05	14%	2703	-0.048	0.012	6.49E-05	0%	7.09E-02	69%

Chr: chromosome; bp: base pair position; N: sample size; EAF: effect allele frequency; β: beta/effect size; SE: standard error of β. †*p*-value, allele, EAF, N from *p*-value weighted meta-analysis of all cohorts using METAL and β, SE from inverse-variance meta-analysis of only HF cohorts using GWAMA.

Supplementary Table 8: Analyses of the 17 HRV top SNPs on HRV traits corrected for heart rate.

We performed three corrections: (1) analytical construction of a meta-analysis of the log-transformed coefficients of variation (CV) of SDNN - ln ((SDNN/mean heart period)*100%) and RMSSD- ln ((RMSSD/mean heart period)*100%) using GWIS on the HRV and heart rate GWASs summary statistics; (2) meta-analysis of SNP associations with the log-transformed CV(SDNN) and CV(RMSSD) in Lifelines, NESDA, and TRAILS-Pop; (3) mediation analysis using Sobel's test testing whether heart rate mediates the SNP association with HRV in a meta-analysis of HRV traits and heart rate in LifeLines, NESDA, and TRAILS-Pop. For the mediation analysis for pvRSA/HF only data of NESDA and TRAILS-Pop were available. Significant *p*-values after Bonferroni correction for 11 independent SNPs (<0.0045) are shown in bold.

				HRV		**HR p-value**	**GWIS log-transformed CV(SDNN) and CV(RMSSD)**		**SNP associations log-transformed CV(SDNN) and CV(RMSSD)**		**Mediation analysis (HRV SNPs -> HR -> HRV traits)**			
									Meta-analysis of three cohorts[#]		**Meta-analysis of three cohorts[#]**			
Locus	**Chr**	**HRV SNP**	**HRV Trait**	**β**	***p*-value**		**β (SE)**	***p*-value**	**β (SE)**	***p*-value**	***p*-value**	***p*-value HR corrected**	**% mediated**	**Sobel *p*-value[*]**
		rs12974991	RMSSD	-0.116	**4.6E-46**	1.8E-01	-0.113 (0.010)	**9.5E-29**	-0.129 (0.012)	**6.8E-27**	**9.5E-22**	**4.4E-31**	1.0%	4.8E-01
1	19	rs12974440	pvRSA/HF	-0.244	**1.9E-41**	1.8E-01	n.a.		n.a.		**2.9E-13**	**1.2E-16**	-13.5%	2.9E-01
		rs12980262	SDNN	-0.060	**2.3E-23**	1.8E-01	-0.050 (0.007)	**4.8E-12**	-0.085 (0.011)	**1.3E-15**	**7.3E-14**	**8.1E-16**	4.9%	4.1E-01
			SDNN	-0.049	**9.3E-31**		-0.042 (0.005)	**1.3E-16**	-0.049 (0.008)	**1.3E-09**	**2.0E-11**	**1.7E-08**	26.1%	**3.7E-04**
2	12	rs10842383	RMSSD	-0.065	**2.5E-29**	**1.5E-10**	-0.061 (0.007)	**3.4E-18**	-0.060 (0.009)	**1.8E-10**	**4.5E-11**	**1.8E-08**	30.2%	**3.4E-04**
			pvRSA/HF	-0.124	**1.2E-25**		n.a.		n.a.		**1.3E-04**	5.1E-03	26.8%	**3.1E-03**
			SDNN	-0.033	**3.7E-25**		-0.025 (0.004)	**8.6E-10**	-0.031 (0.006)	**7.1E-07**	**2.0E-07**	**1.0E-06**	17.7%	1.4E-01
3	6	rs236349	RMSSD	-0.035	**9.1E-17**	**4.0E-05**	-0.027 (0.005)	**6.3E-07**	-0.039 (0.007)	**1.3E-07**	**2.8E-07**	**1.9E-07**	17.1%	9.3E-02
			pvRSA/HF	-0.069	**3.2E-15**		n.a.		n.a.		7.5E-02	1.7E-01	23.8%	2.8E-01
		rs7980799	RMSSD	-0.039	**3.2E-20**	**1.2E-12**	-0.034 (0.005)	**5.5E-11**	-0.032 (0.007)	**1.7E-06**	**6.1E-08**	**1.6E-04**	43.0%	**5.9E-06**
4	12	rs1351682	pvRSA/HF	-0.073	**5.7E-15**	**6.7E-09**	n.a.		n.a.		**8.4E-05**	**1.1E-04**	1.5%	1.0E-01
		rs1384598	SDNN	-0.023	**7.4E-13**	**5.5E-08**	-0.019 (0.004)	**8.8E-07**	-0.012 (0.006)	3.2E-02	**2.9E-03**	6.6E-02	45.7%	**9.8E-05**

5	7	rs4262	SDNN	-0.028	**4.3E-17**	**3.1E-05**	-0.025 (0.004)	**1.1E-09**	-0.025 (0.006)	**4.7E-05**	**5.2E-06**	**9.8E-04**	36.6%	**4.4E-04**
			pvRSA/HF	-0.05	**1.8E-11**		n.a.		n.a.		**2.9E-05**	**3.9E-03**	31.0%	**9.2E-04**
		rs180238	RMSSD	-0.034	**8.0E-16**	**5.1E-06**	-0.035 (0.005)	**4.9E-11**	-0.029 (0.007)	**3.7E-05**	**1.9E-05**	**1.3E-03**	38.5%	**1.8E-03**
6	14b	rs4899412	SDNN	-0.026	**3.1E-13**	1.7E-02	-0.022 (0.004)	**3.1E-07**	-0.017 (0.006)	9.0E-03	**1.7E-03**	1.5E-02	31.9%	9.4E-03
		rs2052015	RMSSD	-0.036	**3.6E-10**	7.2E-02	-0.042 (0.004)	**3.0E-08**	-0.031 (0.009)	**6.6E-04**	**1.4E-04**	1.2E-02	46.1%	**1.7E-03**
	14c	rs2529471	SDNN	-0.021	**1.9E-12**	1.3E-02	-0.018 (0.004)	**1.6E-06**	-0.022 (0.006)	**1.0E-04**	**5.5E-06**	**2.5E-04**	29.1%	**1.2E-03**
	14a	rs36423	SDNN	-0.033	**6.3E-13**	**3.5E-03**	-0.028 (0.006)	**4.0E-07**	-0.030 (0.009)	**1.0E-03**	**4.9E-04**	**3.2E-03**	25.5%	2.2E-01
			RMSSD	-0.04	**5.4E-11**		-0.036 (0.008)	**2.4E-06**	-0.028 (0.010)	7.0E-03	7.5E-03	2.6E-02	32.0%	2.2E-01
7	15a	rs2680344	SDNN	-0.024	**4.9E-11**	**3.8E-09**	-0.019 (0.005)	**4.2E-05**	-0.026 (0.007)	**1.7E-04**	**6.2E-05**	**3.4E-04**	21.5%	1.2E-02
	15b	rs1812835	RMSSD	-0.025	**5.2E-10**	**1.1E-06**	-0.021 (0.005)	**3.1E-05**	-0.024 (0.007)	**2.3E-04**	**2.2E-03**	1.3E-02	33.8%	1.4E-02
8	20	rs6123471	RMSSD	-0.024	**1.3E-08**	**1.6E-20**	-0.022 (0.005)	**4.2E-05**	-0.015 (0.008)	4.5E-02	6.3E-03	6.8E-01	87.8%	**2.2E-05**

Chr: chromosome; HR: heart rate; CV: coefficient of variation; SE: standard error.
[#]Lifelines (N=12,101), NESDA (N=2,118), TRAILS-Pop (N=1,191).
*Sobel *p*-values of the meta-analyses are calculated from meta z-scores that are determined using a sqrt(sample size) weighted *z*-score meta-analysis.

Supplementary Table 9: Association between HRV SNPs and heart rate.

Results in (a) are the look-up results of the 17 HRV SNPs identified in this study in the meta-analysis of heart rate (Den Hoed et al., 2013). Panels (b) and (c) present the explained variances in heart rate in the Lifelines (n=12,101), NESDA (n=2,118), TRAILS-Pop (n=1,191), and ABCD (n=1,094) cohorts by (b) the weighted multi-SNP genetic risk score based on the independent genome-wide significant HRV SNPs in the stage 1+2 meta-analysis, and (c) the optimal polygenic risk scores computed at the *p*-value threshold that explained the largest percentage of phenotypic variance. ΔR^2 is the difference in percentage of explained variance by the multi-SNP genetic or polygenic risk score between the models with and without the risk score while adjusting both for age, sex, and principal components. The maximum number of overlap between the HRV and heart rate meta-analysis was N=10,612.

a) Look-up results

Identified heart rate variability SNPs					**Heart rate lookup**			
Locus	**Chr**	**HRV SNP**	**Position (bp)**	**HRV Trait**	**β#**	**SE**	**N**	***p*-value***
1	19	rs12974991	5845584	RMSSD	0.1521	0.1072	80077.3	1.77E-01
		rs12974440	5845386	pvRSA/HF	0.1505	0.1072	80091.2	1.77E-01
		rs12980262	5844058	SDNN	0.1486	0.1072	80091.3	1.77E-01
2	12	rs10842383	24663234	SDNN, RMSSD, pvRSA/HF	0.4922	0.0731	87263.7	**1.52E-10**
3	6	rs236349	36928543	SDNN, RMSSD, pvRSA/HF	0.2583	0.0598	67947	**3.95E-05**
4	12	rs7980799	33468257	RMSSD	0.4052	0.0542	85488.7	**1.19E-12**
		rs1351682	33490042	pvRSA/HF	0.3136	0.0514	93068.9	**6.72E-09**
		rs1384598	33514166	SDNN	0.305	0.0534	87057.3	**5.52E-08**
5	7	rs4262	93389364	SDNN, pvRSA/HF	0.2514	0.0574	82597.3	**3.13E-05**
		rs180238	93388383	RMSSD	0.2788	0.0581	86814.9	**5.08E-06**
6	14b	rs4899412	71534015	SDNN	0.1485	0.0594	89581.2	1.73E-02
		rs2052015	71556806	RMSSD	0.1473	0.0778	86557.5	7.19E-02
	14c	rs2529471	71883022	SDNN	0.1325	0.0505	93152.6	1.26E-02
	14a	rs36423	71422955	SDNN, RMSSD	0.2357	0.0767	90388.5	**3.49E-03**
7	15a	rs2680344	71440538	SDNN	0.3787	0.0611	91249.2	**3.80E-09**
	15b	rs1812835	71294557	RMSSD	0.2745	0.0536	84616.4	**1.09E-06**
8	20	rs6123471	36273570	RMSSD	0.5012	0.0513	89348.2	**1.56E-20**

Chr: chromosome; bp: base pair position based on build 36 (hg18); SE: standard error of β; N: sample size.

Effect size is shown for the allele increasing the risk of low levels of HRV.

* *P*-values from the discovery stage using only Europeans. Significant *p*-values <0.05/11 (independent SNPs) are shown in bold.

b) Multi-SNP genetic risk scores

		Multi-SNP genetic risk score*											
		Lifelines			NESDA			TRAILS-Pop			ABCD		
trait	risk score	N SNPs	*p*-value	ΔR^2	N SNPs	*p*-value	ΔR^2	N SNPs	*p*-value	ΔR^2	N SNPs	*p*-value	ΔR^2
Heart rate	SDNN	9	3.70E-08	0.23%	9	1.40E-03	0.39%	10	1.40E-02	0.55%	10	2.70E-01	0.11%
Heart rate	RMSSD	7	1.90E-05	0.14%	11	1.60E-03	0.38%	11	3.50E-04	1.07%	11	1.90E-01	0.16%
Heart rate	pvRSA/HF	5	5.20E-04	0.09%	5	4.20E-02	0.13%	5	2.90E-04	1.13%	4	3.10E-01	0.10%

n.a.=not available.

* For Lifelines, NESDA, and TRAILS-Pop the weights (i.e. effects sizes) and number of genome-wide significant SNPs included in the risk score were adjusted by analytically extracting the cohort's effect size and standard error from the meta effect size and standard error, respectively, and recalculating the *p*-value based on these adjusted effect sizes and standard errors, since these cohorts were included in stage 1 and/or 2.

c) Polygenic risk scores

	Polygenic risk score*															
	Lifelines				NESDA				TRAILS-Pop				ABCD			
risk score	*p* cutoff	N SNPs	*p*-value	ΔR^2	*p* cutoff	N SNPs	*p*-value	ΔR^2	*p* cutoff	N SNPs	*p*-value	ΔR^2	*p* cutoff	N SNPs	*p*-value	ΔR^2
SDNN	<5E-3	3186	2.30E-08	0.25%	<5E-8	6	9.00E-03	0.29%	<5E-8	7	5.60E-03	0.69%	<5E-5	71	1.80E-02	0.51%
RMSSD	<5E-5	80	1.30E-10	0.34%	<5E-3	3341	1.70E-03	0.43%	<5E-7	11	5.80E-04	0.99%	<5E-4	466	5.80E-02	0.33%
pvRSA/HF	<0.05	20747	5.90E-06	0.17%	<5E-4	399	8.10E-04	0.50%	<5E-7	6	5.80E-03	0.68%	<5E-5	67	6.50E-02	0.31%

* Weighted polygenic risk score were determined based on independent SNPs in the stage 1 meta-analysis. For NESDA and TRAILS-Pop the weights (i.e. effects sizes) and *p*-values were adjusted by analytically extracting the cohort's effect size and standard error from the meta effect size and standard error, respectively, and recalculating the *p*-value based on these adjusted effect size and standard error, since these cohorts were included in stage 1.

Supplementary Table 10: Association between heart rate SNPs and the three HRV traits. Look-up of the 21 heart rate SNPs identified in the GWAS meta-analysis of heart rate by Den Hoed et al.[14] in the current study's meta-analyses results for the three HRV traits (a). The effects of the genetic risk score for the heart rate SNPs on SDNN, RMSSD, and pvRSA/HF (b). Explained variance in HRV traits in the Lifelines (n=12,101), NESDA (n=2,118), TRAILS-Pop (n=1,191), and ABCD (n=1,094) cohorts by the weighted multi-SNP genetic risk score based on the 21 heart rate SNPs (c), and the optimal polygenic risk scores for heart rate computed at the *p*-value threshold that explained the largest percentage of phenotypic variance in the HRV traits (d).

(a) Look-up of previously identified heart rate loci				**HRV look-up**				
Locus	**Chr**	**HR SNP**	**Position (bp)**	**HRV Trait**	**β#**	**SE**	**N**	**p-value***
1	14	rs365990	22931651	SDNN	-0.0132	0.0039	27257	**8.08E-04**
				RMSSD	-0.0153	0.0053	26367	4.28E-03
				pvRSA/HF†	-0.0189	0.0109	20660	2.18E-01
2	6	rs1015451	122173184	pvRSA/HF†	0.0353	0.0180	18020	8.27E-02
				SDNN	-0.0041	0.0059	24617	4.92E-01
				RMSSD	-0.0116	0.0086	23727	1.83E-01
3	7	rs13245899	100335067	SDNN	-0.0116	0.0046	27256	1.23E-02
				pvRSA/HF†	0.0158	0.0131	20659	8.31E-02
				RMSSD	0.0011	0.0063	26366	8.58E-01
4	1	rs11118555	206007476	SDNN	-0.0130	0.0057	25873	2.46E-02
				RMSSD	-0.0091	0.0081	24984	2.62E-01
				pvRSA/HF†	-0.0099	0.0167	19277	9.06E-01
5	11	rs174549	61327958	pvRSA/HF†	-0.0204	0.0114	20660	8.18E-02
				RMSSD	-0.0052	0.0055	26367	3.49E-01
				SDNN	-0.0024	0.0040	27257	5.54E-01
6	6	rs11153730	118774215	pvRSA/HF†	0.0226	0.0108	19277	4.10E-03
				SDNN	0.0019	0.0039	25041	6.29E-01
				RMSSD	0.0015	0.0052	24984	7.78E-01
7	12	rs17287293	24662145	pvRSA/HF†	-0.1263	0.0152	19081	**3.59E-21**
				SDNN	-0.0488	0.0052	25873	**5.46E-21**
				RMSSD	-0.0670	0.0071	24984	**1.25E-20**
8	20	rs6127471	36277452	RMSSD	-0.0283	0.0052	26367	**5.83E-08**
				SDNN	-0.0160	0.0038	27257	**2.58E-05**
				pvRSA/HF†	-0.0299	0.0107	20660	**6.84E-04**
9	2	rs17362588	179429291	RMSSD	-0.0376	0.0089	24981	**2.75E-05**
				pvRSA/HF†	-0.0560	0.0184	19274	**7.16E-05**
				SDNN	-0.0233	0.0062	25871	**1.91E-04**
10	12	rs7980799	33468257	RMSSD	-0.0402	0.0052	26156	**1.92E-14**
				pvRSA/HF†	-0.0719	0.0111	20464	**1.29E-11**
				SDNN	-0.0232	0.0038	26992	**1.80E-09**
11	15	rs4489968	71452559	SDNN	-0.0242	0.0050	27256	**1.94E-06**
				RMSSD	-0.0309	0.0071	26366	**1.47E-05**
				pvRSA/HF†	-0.0469	0.0144	20659	**3.97E-04**
12	3	rs7612445	180655673	SDNN	-0.0233	0.0048	27251	**1.60E-06**
				RMSSD	-0.0295	0.0067	26366	**1.13E-05**
				pvRSA/HF†	-0.0333	0.0136	20659	5.68E-03

				RMSSD	-0.0121	0.0055	26153	3.00E-02
13	14	rs17796783	84879664	SDNN	-0.0046	0.0041	26989	2.63E-01
				pvRSA/HF†	0.0042	0.0113	20657	6.39E-01
				pvRSA/HF†	-0.0387	0.0189	17983	5.57E-03
14	7	rs2350782	136293174	RMSSD	-0.0156	0.0095	23691	1.04E-01
				SDNN	-0.0100	0.0066	24514	1.28E-01
				pvRSA/HF†	0.0209	0.0134	18716	3.14E-02
15	5	rs6882776	172596769	RMSSD	0.0054	0.0065	24212	4.10E-01
				SDNN	0.0031	0.0047	25048	5.09E-01
				RMSSD	-0.0417	0.0061	22345	**1.44E-11**
16	7	rs180242	93387532	SDNN	-0.0284	0.0044	23235	**1.27E-10**
				pvRSA/HF†	-0.0499	0.0127	16638	**1.56E-07**
				RMSSD	-0.0034	0.0057	26363	5.54E-01
17	2	rs13030174	231979528	pvRSA/HF†	-0.0028	0.0118	20656	8.29E-01
				SDNN	0.0004	0.0042	27253	9.28E-01
				SDNN	-0.0073	0.0041	25874	7.54E-02
18	3	rs9647379	173267862	RMSSD	-0.0094	0.0057	24984	9.92E-02
				pvRSA/HF†	-0.0071	0.0116	19277	1.69E-01
				SDNN	0.0024	0.0038	25874	5.31E-01
19	12	rs2067615	105673552	RMSSD	-0.0010	0.0051	24984	8.45E-01
				pvRSA/HF†	-0.0018	0.0110	19081	8.88E-01
				pvRSA/HF†	-0.0470	0.0108	20464	**2.84E-09**
20	12	rs826838	37392998	RMSSD	-0.0290	0.0050	26367	**1.04E-08**
				SDNN	-0.0172	0.0037	27257	**3.36E-06**
				pvRSA/HF†	0.0109	0.0115	20660	1.56E-01
21	2	rs4140885	188041309	SDNN	-0.0005	0.0040	27257	9.00E-01
				RMSSD	-0.0001	0.0056	26367	9.82E-01

Chr: chromosome; bp: base pair position based on build 36 (hg18); HR: heart rate; SE: standard error of β; N: sample size.
Effect size is shown for the allele increasing the heart rate.
**P*-values from the discovery stage using only Europeans. Significant *p*-values <0.05/21 are shown in bold.

(b) effect of the multi-SNP genetic risk score of heart rate composed of all genome-wide significant SNPs based on summary statistics

			genetic risk score heart rate	
HRV parameter	**Sample size**	**N SNPs**	**Effect size (95% CI)**	***p*-value***
SDNN	27257	20	-0.032 (-0.04,-0.03)	**1.7E-37**
RMSSD	26367	21	-0.043 (-0.05,-0.04)	**1.0E-38**
pvRSA/HF	20660	21	-0.05 (-0.06,-0.04)	**1.6E-13**

†*p*-value, allele, EAF, N from *p*-value weighted meta-analysis of all cohorts using METAL and β, SE from inverse-variance meta-analysis of only HF cohorts using GWAMA.

(c) effects of the multi-SNP genetic risk score of heart rate based on individual level data

	Multi-SNP genetic risk score*											
	Lifelines			NESDA			TRAILS-Pop			ABCD		
Trait	N SNPs	*p*-value	ΔR^2	N SNPs	*p*-value	ΔR^2	N SNPs	*p*-value	ΔR^2	N SNPs	*p*-value	ΔR^2
SDNN	15	3.30E-09	0.24%	21	4.60E-07	0.90%	21	6.00E-02	0.29%	20	4.90E-02	0.35%
RMSSD	15	3.20E-15	0.44%	21	2.70E-07	0.90%	21	1.50E-02	0.49%	20	1.70E-02	0.52%
pvRSA/HF	15	n.a.	n.a.	21	2.10E-06	0.64%	21	5.40E-02	0.31%	20	6.70E-02	0.31%
heart rate	15	3.30E-41	1.44%	21	2.50E-10	1.69%	21	7.30E-05	1.25%	20	4.60E-03	0.73%

* For Lifelines, NESDA, and TRAILS-Pop the weights (i.e. effects sizes) and number of genome-wide significant SNPs included in the risk score were adjusted by analytically extracting the cohort's effect size and standard error from the meta effect size and standard error, respectively, and recalculating the *p*-value based on these adjusted effect sizes and standard errors, since these cohorts were included in stage 1 and/or 2.

(d) effects of the polygenic risk score of heart rate based on individuals level data

	Polygenic risk score*															
	Lifelines				NESDA				TRAILS-Pop				ABCD			
Trait	*p* cut-off	N SNPs	*p*-value	ΔR^2	*p* cut-off	N SNPs	*p*-value	ΔR^2	*p* cut-off	N SNPs	*p*-value	ΔR^2	*p* cut-off	N SNPs	*p*-value	ΔR^2
SDNN	<5E-8	19	1.20E-09	0.26%	<5E-4	430	5.00E-06	0.78%	<5E-5	97	1.80E-02	0.46%	<5E-4	438	1.30E-02	0.57%
RMSSD	<5E-8	19	3.50E-16	0.48%	<5E-4	430	1.90E-06	0.82%	<5E-8	18	8.20E-03	0.58%	<5E-4	438	3.80E-02	0.39%
pvRSA/HF	n.a.	n.a.	n.a.	n.a.	<5E-4	430	1.50E-06	0.70%	<5E-5	97	2.70E-02	0.41%	<5E-2	18305	1.50E-01	0.19%
heart rate	<5E-7	28	3.50E-42	1.50%	<5E-8	21	8.10E-10	1.66%	<5E-8	18	3.10E-06	1.73%	<5E-4	438	1.40E-04	1.32%

* Weighted polygenic risk score were determined based on independent SNPs in the stage 1 meta-analysis. For NESDA and TRAILS-Pop the weights (i.e. effects sizes) and *p*-values were adjusted by analytically extracting the cohort's effect size and standard error from the meta effect size and standard error, respectively, and recalculating the *p*-value based on these adjusted effect size and standard error, since these cohorts were included in stage 1.

Supplementary Table 11: Look-up results of genome-wide association consortia targeting cardiometabolic outcomes.
In panel (a) results are shown for the associations with the genetic risk scores composed of the HRV SNPs identified in this study. Panel (b) shows the genetic correlations with the HRV traits are shown as computed using LD Score regression based on GWAS summary statistics.

(a)			SDNN		RMSSD		pvRSA/HF*	
Trait or disease	**Reference**	**Sample size**	**Effect size# (95% CI)**	***p*-value**	**Effect size# (95% CI)**	***p*-value**	**Effect size# (95% CI)**	***p*-value**
Heart rate	Den Hoed et al., 2013[14]	93153	8.65 (7.35,9.94)	**3.7E-39**	6.93 (6.02,7.83)	**1.5E-50**	2.66 (2.10,3.23)	**2.2E-20**
Systolic blood pressure	Ehret et al., 2011[15]	69616	1.64 (-0.79,4.08)	1.9E-01	0.94 (-0.78,2.65)	2.8E-01	0.16 (-0.90,1.22)	7.7E-01
Diastolic blood pressure	Ehret et al., 2011[15]	69604	-0.37 (-1.91,1.17)	6.4E-01	-0.83 (-1.91,0.25)	1.3E-01	-0.22 (-0.89,0.45)	5.2E-01
Heart failure	Smith et al., 2010[16]	20926	0.58 (0.27,1.23)	1.6E-01	0.69 (0.41,1.16)	1.6E-01	0.90 (0.66,1.25)	5.2E-01
Coronary Artery Disease	Deloukas et al., 2013[17]	83174	1.04 (0.72,1.52)	8.3E-01	1.08 (0.83,1.37)	6.0E-01	0.96 (0.82,1.12)	6.4E-01
Atrial fibrillation	Ellinor et al., 2012[18]	59133	0.62 (0.38,1.02)	5.8E-02	0.72 (0.51,1.02)	6.7E-02	1.15 (0.93,1.43)	2.1E-01
Sudden cardiac death	SCD consortium, unpublished	29928	1.10 (0.52,2.33)	8.0E-01	1.37 (0.81,4.55)	2.4E-01	0.95 (0.68,1.32)	7.6E-01
Type 2 diabetes	Morris et al., 2012[19]	69033	0.99 (0.86,1.14)	9.1E-01	1.01 (0.90,1.11)	9.2E-01	0.96 (0.83,1.11)	6.1E-01
Body mass index	Locke et al., 2015[20]	233947	-0.0052 (-0.10,0.09)	9.1E-01	0.01 (-0.05,0.07)	7.3E-01	-0.0074 (-0.035,0.03)	7.2E-01
eGFR for creatinin	Pattaro et al., 2016[21]	74354	0.02 (-0.01,0.05)	1.7E-01	0.005 (-0.02,0.03)	6.3E-01	0.001 (-0.01,0.01)	9.4E-01
Urinary albumin excretion(UACR)	Teumer et al., 2016[22]	31077	0.11 (-0.13,0.34)	4.2E-01	0.10 (-0.06,0.27)	2.2E-01	0.08 (-0.03,0.18)	1.4E-01

* *p*-value from *z*-score weighted meta-analysis of all cohorts using METAL and β and standard error from inverse-variance meta-analysis of only HF cohorts using GWAMA were used
# Effect size is either the incremental change in the phenotype for quantitative traits or the odds ratio for binary traits when the multi-SNP genetic risk score of HRV decreases by one unit.

(b) Trait or disease	Reference	Sample size	SDNN genetic correlation (SE)	SDNN *p*-value	RMSSD genetic correlation (SE)	RMSSD *p*-value	pvRSA/HF* genetic correlation (SE)	pvRSA/HF* *p*-value
Heart rate	Den Hoed et al., 2013[14]	93153	-0.656 (0.09)	**1.0E-12**	-0.738 (0.10)	**1.3E-14**	-0.548 (0.11)	**2.2E-07**
Systolic blood pressure	Ehret et al., 2011[15]	69616	-0.204 (0.08)	8.4E-03	-0.230 (0.08)	6.4E-03	-0.314 (0.10)	**2.1E-03**
Diastolic blood pressure	Ehret et al., 2011[15]	69604	-0.267 (0.08)	**4.0E-04**	-0.271 (0.08)	**4.0E-04**	-0.349 (0.10)	**3.0E-04**
Heart failure	Smith et al., 2010[16]	20926	-0.291 (0.20)	1.5E-01	-0.095 (0.19)	6.1E-01	-0.281 (0.20)	1.5E-01
Coronary Artery Disease	Deloukas et al., 2013[17]	83174	-0.133 (0.09)	1.2E-01	-0.113 (0.09)	2.3E-01	-0.116 (0.10)	2.3E-01
Atrial fibrillation	Ellinor et al., 2012[18]	59133	0.050 (0.13)	7.0E-01	0.058 (0.13)	1.3E-01	-0.019 (0.12)	8.8E-01
Sudden cardiac death	SCD consortium, unpublished	29928	-0.123(0.23)	5.9E-01	0.080(0.22)	7.2E-01	-0.100 (0.26)	7.0E-01
Type 2 diabetes	Morris et al., 2012[19]	69033	-0.263 (0.11)	1.2E-02	-0.209 (0.13)	1.2E-01	-0.160 (0.13)	2.1E-01
Body mass index	Locke et al., 2015[20]	233947	-0.085 (0.04)	4.5E-02	0.042 (0.05)	3.6E-01	-0.036 (0.05)	4.5E-01
eGFR for creatinin	Pattaro et al., 2016[21]	175579	-0.011 (0.07)	8.7E-01	-0.017 (0.07)	8.1E-01	-0.031 (0.07)	6.6E-01
Urinary albumin excretion(UACR)	Teumer et al., 2016[22]	51886	0.002 (0.13)	9.9E-01	-0.055 (0.14)	6.9E-01	-0.183 (0.15)	2.1E-01

* *p*-value from *z*-score weighted meta-analysis of all cohorts using METAL and β and standard error from inverse-variance meta-analysis of only HF cohorts using GWAMA were used.

Supplementary Table 12: Effects of the HRV SNPs identified in this study on atrial fibrillation (N=59,133).

Odds ratios (OR) with confidence intervals (95%CI) are given for the HRV decreasing allele. Significantly associated SNPs (*p*-value <0.0045) are shown in bold.

Locus	Chr	SNP	Position (bp)	HRV trait	OR (95%CI)	*p*-value
1	19	rs12974440	5845584	RMSSD	1.00 (0.92-1.07)	9.2E-01
		rs12974991	5845386	pvRSA/HF	1.00 (0.92-1.07)	9.2E-01
		rs12980262	5844058	SDNN	1.00 (0.92-1.07)	9.3E-01
2	12	rs10842383	24663234	SDNN, RMSSD, pvRSA/HF	**1.16 (1.10-1.23)**	**3.5E-07**
3	6	rs236349	36928543	SDNN, RMSSD, pvRSA/HF	0.94 (0.91-0.98)	5.3E-03
4	12	rs1351682	33468257	pvRSA/HF	0.98 (0.95-1.03)	4.5E-01
		rs1384598	33490042	SDNN	0.99 (0.95-1.03)	6.4E-01
		rs7980799	33514166	RMSSD	0.99 (0.95-1.03)	6.5E-01
5	7	rs180238	93389364	RMSSD	1.00 (0.96-1.05)	8.7E-01
		rs4262	93388383	SDNN, pvRSA/HF	1.02 (0.98-1.06)	3.8E-01
6	14b	rs2052015	71534015	RMSSD	1.00 (0.94-1.07)	9.7E-01
		rs2529471	71556806	SDNN, RMSSD	0.96 (0.92-0.99)	2.3E-02
	14c	rs36423	71883022	SDNN, RMSSD	0.94 (0.88-1.00)	4.2E-02
	14a	rs4899412	71422955	SDNN	0.98 (0.94-1.03)	5.0E-01
7	15a	rs1812835	71440538	SDNN, RMSSD	0.95 (0.92-0.99)	2.2E-02
	15b	rs2680344	71294557	SDNN, RMSSD	**0.89 (0.84-0.93)**	**4.3E-07**
8	20	rs6123471	36273570	RMSSD	0.99 (0.95-1.02)	4.5E-01

Chr: chromosome; bp: base pair position (build 36).

Supplementary Table 13: RegulomeDB main results of functional variant analysis.

HRV SNP	Chr	Position (bp; b37)	RegulomeDB score
rs12980262	19	5893057	TF binding + any motif + DNase Footprint + DNase peak
rs12974440	19	5894385	TF binding + any motif + DNase peak
rs12974991	19	5894583	TF binding + DNase peak
rs236349	6	36820564	TF binding + DNase peak
rs180238	7	93550446	TF binding + DNase peak
rs4262	7	93551427	TF binding + DNase peak
rs36423	14	72353201	TF binding or DNase peak
rs2529471	14	72813268	TF binding or DNase peak
rs2680344	15	73653484	TF binding or DNase peak
rs6123471	20	36840155	TF binding or DNase peak
rs7980799	12	33576989	other
rs1351682	12	33598774	other
rs1384598	12	33622898	other
rs2052015	14	72487052	other
rs1812835	15	73507503	other
rs10842383	12	24771966	NA
rs4899412	14	72464261	NA

TF binding: the variant is located at a site where transcription factors are anticipated to bind.

Supplementary Table 14: eQTL analysis. (a) The 17 HRV SNPs were assessed in the NESDA-NTR eQTL database[23, 24]. Only SNPs with a significant *cis* effect on gene expression are shown. All HRV SNPs with significant results in the NESDA/NTR eQTL database[23, 24] were looked up in several other eQTL databases for multiple tissues: (b) a second [25] and (c) third independent whole blood eQTL database [26], (d) lymphoblastoid cell lines[27], (e) eQTLs for ten different regions of the brain [28], (f) GTex, and (g) a heart eQTL database [29].

(a)			Association between HRV SNP and gene expression in NESDA/NTR			Top eQTL with strongest association with gene expression			*p*-value HRV SNP conditional on top eQTL
HRV SNP	**Gene**	**chr:bp_allele ; Probeset_id**	***p*-value**	**FDR**	**Beta**	**SNP & Allele**	***p*-value**	**LD HRV SNP**	
rs4262	*GNG11*	7:93551428_C ; 11722379_at	2.35E-07	1.75E-03	-0.100	7:93540958_C	1.48E-08	0.797	>0.05
rs180238	*GNG11*	7:93550447_C ; 11722379_at	6.00E-08	5.26E-04	-0.109	7:93540958_C	1.48E-08	0.971	>0.05
rs4899412	*RGS6*	14:72464262_T ; 11752774_a_at	2.71E-06	1.60E-02	0.097	14:72465028_C	2.35E-06	0.999	>0.05
rs4899412	*RGS6*	14:72464262_T ; 11750930_a_at	4.63E-07	3.50E-03	0.108	14:72425522_T	1.86E-07	0.896	>0.05
rs1812835	*NEO1*	15:73507504_A ; 11717210_a_at	1.65E-07	1.37E-03	-0.106	15:73420724_A	3.12E-09	0.775	>0.05
rs1812835	*NEO1*	15:73507504_A ; 11717212_a_at	5.91E-11	<1.34e-05	-0.131	15:73377436:G_GT_I	2.89E-13	0.743	>0.05

(b)			Association between HRV SNP and expression in whole blood		Top eQTL in BIOS whole blood		
HRV SNP	**Gene**	**chr:bp_allele**	***p*-value**	***Z*-score**	**SNP**	***p*-value**	**LD HRV SNP**
rs4262	*GNG11*	7:93551428_C	NS	NS	rs180279	1.45E-19	0.002
rs180238	*GNG11*	7:93550447_C	NS	NS	rs180279	1.45E-19	0.001
rs4899412 *(by its proxy rs1987722 [r^2=0.99])*	*RGS6*	14:72465028_C	1.27E-36	12.64	rs1987722	1.27E-36	0.99
rs1812835	*NEO1*	15:73507504_A	4.93E-80	-18.944	rs1038137	1.72E-119	0.75

(c)			Association between HRV SNP and expression in whole blood		Top eQTL in Westra et al. whole blood		
HRV SNP	**Gene**	**chr:bp_allele**	***p*-value**	***Z*-score**	**SNP**	***p*-value**	**LD HRV SNP**
rs4262 *(by its proxy rs180236 r^2=0.77)*	*GNG11*	7:93391277_A	1.12E-04	3.86	rs180275	7.16E-09	0.002
rs180238 *(by its proxy rs180236 r^2=0.32)*	*GNG11*	7:93391277_A	1.12E-04	3.86	rs180275	7.16E-09	0.001
rs4899412	*RGS6*	n.a.	n.a.	n.a.	rs8008967	6.83E-04	0.002
rs1812835	*NEO1*	15:73507504_A	5.60E-04	-3.45	rs3784801	1.63E-04	0.92

(d)			Association between HRV SNP and expression in Geuvadis		Top eQTL in Geuvadis		
HRV SNP	**Gene**	**chr:bp_allele**	***p*-value**	**Beta**	**SNP**	***p*-value**	**LD HRV SNP**
rs4262	*GNG11*	7:93551428_C	NS	NS	NS	NS	NS
rs180238	*GNG11*	7:93550447_C	NS	NS	NS	NS	NS
rs4899412	*RGS6*	14:72464262_T	NS	NS	rs1548687	3.44E-07	0.002
rs1812835	*NEO1*	*15:73507504_A*	1.39E-17	n.a.	rs62016808	4.18E-25	0.75

(e)			Association between HRV SNP and expression in Braineac (tissue)		Top eQTL in Braineac		
HRV SNP	**Gene**	**chr:bp_allele**	***p*-value**	**Beta**	**SNP**	***p*-value**	**LD HRV SNP**
rs4262	*GNG11*	7:93551428_C	2.8E-04 (medulla)	n.a.	rs4266	1.20E-05	1
rs180238	*GNG11*	7:93550447_C	0.0013 (medulla)	n.a.	rs4266	1.20E-05	0.047
rs4899412	*RGS6*	14:72464262_T	NS	n.a.	NS		
rs1812835	*NEO1*	15:73507504_A	NS	n.a.	NS		

(f)			Association between HRV SNP and expression in GTEx (tissue)		Top eQTL in GTEx		
HRV SNP	**Gene**	**chr:bp_allele**	***p*-value**	**Beta**	**SNP**	***p*-value**	**LD HRV SNP**
rs4262	*GNG11*	7:93551428_C	8.10E-09 (Tibial artery)	-0.21	rs4262	8.10E-09	1
rs180238	*GNG11*	7:93550447_C	NS	NS	rs4262	8.10E-09	0.81
rs4899412	*RGS6*	14:72464262_T	NS	NS	rs2108469	4.10E-10	0.006
rs1812835	*NEO1*	15:73507504_A	4.4E-06 (EBV- lympho-blastoid cell line)	-0.62	rs2169951	NS	0.84

(g)			Association between HRV SNP and expression in Koopmans Heart eQTL database		Top eQTL in Heart eQTL database		
HRV SNP	**Gene**	**chr:bp_allele**	***p*-value**	**Beta**	**SNP**	***p*-value**	**LD HRV SNP**
rs4262	*GNG11*	7:93551428_C	NS	NS		NS	
rs180238	*GNG11*	7:93550447_C	NS	NS		NS	
rs4899412	*RGS6*	14:72464262_T	NS	NS		NS	
rs1812835	*NEO1*	15:73507504_A	NS	NS		NS	

chr: chromosome; bp: base pair position (build 37); FDR: false discovery rate; LD: linkage disequilibrium (r^2)**.**

Supplementary Table 15: mQTL analysis using the BIOS (*cis*) mQTL database[30] to assess the effect of the 17 HRV SNPs on methylation in nearby genes.

			mQTL							Top mQTL		
HRV SNP	**Chr:bp**	**Effect allele**	**Methylation Site**	**Gene(s)**	**Position methylation site (bp)**	**Location in gene region**	**Relation to UCSC CpG Island**	***p*-value**	**Beta**	**SNP**	***p*-value**	**LD HRV SNP**
rs12974440	19:5894386	A	cg22854549	*VMAC; NDUFA11*	5904785	TSS200; TSS1500	Island	5.72E-82	-19.18	rs55660714	1.71E-103	0.73
rs12974440	19:5894386	A	cg03715305	*NDUFA11*	5894715	3'UTR	S_Shelf	1.63E-25	-10.44	rs17271904	3.89E-26	0.98
rs12974440	19:5894386	A	cg19211619	*CAPS*	5913923	TSS1500	N_Shore	8.07E-07	4.93	rs12982903	1.76E-07	0.95
rs12974991	19:5894584	A	cg22854549	*VMAC; NDUFA11*	5904785	TSS200; TSS1500	Island	8.38E-83	-19.28	rs55660714	1.71E-103	0.71
rs12974991	19:5894584	A	cg03715305	*NDUFA11*	5894715	3'UTR	S_Shelf	4.21E-26	-10.57	rs17271904	3.89E-26	1.00
rs12974991	19:5894584	A	cg19211619	*CAPS*	5913923	TSS1500	N_Shore	3.47E-07	5.10	rs12982903	1.76E-07	0.97
rs12980262	19:5893058	A	cg22854549	*VMAC; NDUFA11*	5904785	TSS200; TSS1500	Island	9.98E-82	-19.15	rs55660714	1.71E-103	0.73
rs12980262	19:5893058	A	cg03715305	*NDUFA11*	5894715	3'UTR	S_Shelf	1.52E-25	-10.45	rs17271904	3.89E-26	0.98
rs12980262	19:5893058	A	cg19211619	*CAPS*	5913923	TSS1500	N_Shore	7.94E-07	4.94	rs12982903	1.76E-07	0.95
rs7980799	12:33576990	A	cg21043657	*SYT10*	33590837	Body	N_Shore	1.72E-23	9.99	rs6488162	2.92E-25	0.90
rs1351682	12:33598775	G	cg21043657	*SYT10*	33590837	Body	N_Shore	5.52E-21	9.40	rs6488162	2.92E-25	0.80
rs1384598	12:33622899	T	cg21043657	*SYT10*	33590837	Body	N_Shore	9.71E-21	9.34	rs6488162	2.92E-25	0.80
rs4262	7:93551428	C	cg08038054	*GNG11*	93550781	TSS1500		5.22E-51	-15.02	rs180236	2.60E-55	0.87
rs4262	7:93551428	C	cg06439941	*GNG11*	93550756	TSS1500		3.63E-30	-11.41	rs180236	9.11E-36	0.87
rs2529471	14:72813269	C	cg17922283	*RGS6*	72799938	Body		2.84E-14	7.61	rs2090737	3.74E-19	0.71
rs4899412	14:72464262	T	cg19493789	*RGS6*	72396233		N_Shelf	NS	NS	rs2238280	4.16E-07	0.88
rs1812835	15:73507504	A	cg11357013	*NEO1*	73588054	Body		6.18E-30	-11.37	rs62016851	1.15E-31	0.93
rs1812835	15:73507504	A	cg19281068	*NEO1*	73345607	Body	S_Shore	3.88E-17	8.42	rs4609810	7.37E-22	0.78
rs1812835	15:73507504	A	cg11552023	*NEO1*	73595120	3'UTR		7.39E-10	-6.16	rs4132536	2.28E-17	0.77
rs1812835	15:73507504	A	cg17150474	*NEO1*	73343980	TSS1500	Island	3.29E-09	5.92	rs1023924	1.31E-11	0.83

Chr: chromosome; bp: base pair position (build 37); LD: linkage disequilibrium (r^2). TSS200= methylation site is located within 200 bp of a transcription start site; TSS1500= methylation site is located within 1500 bp of a transcription start site; 3'UTR= methylation site is located in the 3'untranslated region; Body= methylation site is located within a gene (gene body); Island= methylation site is located in a CpG island; S_Shelf= methylation site is located in the south shelf of a CpG island; N_Shelf= methylation site is located in the north shelf of a CpG island; S_Shore= methylation site is located in the south shore of a CpG island; N_Shore= methylation site is located in the north shore of a CpG island

Supplementary Table 16: DEPICT tissue enrichment analysis summarized by tissue or cell type.
Genes were considered to be enriched for certain tissues or cell-types if they showed a Z-score for enrichment > 2.0 in Supplementary Data 3.

	Enriched tissue or cell type												
Gene	**Blood**	**Central Nervous System**	**Respiratory**	**Salivary/ mucosa**	**Immuno logical**	**Stem cells**	**Heart**	**Muscle**	**Sensory**	**Epithelium**	**Endo crine**	**(Embryonic) membrane**	**Vasculature**
C6orf89	x				x	x		x					
CAPS			x	x					x	x			
CPNE5	x	x	x		x								
FUT5	x			x				x					
GNG11	x									x		x	x
GNGT1	x			x					x			x	
HCN4	x		x	x		x	x						
KIAA1755		x					x						
LINC00477	x		x		x			x					
NDUFA11							x		x		x		
NEO1		x											
PPIL1	x				x	x							
RANBP3		x									x		
RGS6	x	x				x							
Sum	9	5	4	4	4	4	3	3	3	2	2	2	1

Supplementary Table 17: Results of VEGAS showing gene-based significance based on the stage 1 GWAS meta-analyses results for SDNN, RMSSD, and pvRSA/HF. Genes are shown for which the i-value was significant at the multiple testing correction level of $2.5x10^{-6}$ (bolded) for at least one of the HRV traits. Genes in red italics were newly detected compared to the SNP-based analysis.

		SDNN				**RMSSD**				**pvRSA/HF**			
Chr	Gene	nSNPs	*p*-value	Best-SNP	SNP-*p*-value	nSNPs	*p*-value	Best-SNP	SNP-*p*-value	nSNPs	*p*-value	Best-SNP	SNP-*p*-value
2	*CCDC141*	149	2.01E-03	rs17362588	0.000191	149	**<1.0E-06**	rs13004438	1.58E-07	149	5.41E-04	rs12693173	1.05E-05
7	*TFPI2*	133	**<1.0E-06**	rs4262	1.56E-12	110	**<1.0E-06**	rs180238	2.24E-13	134	**<1.0E-06**	rs4262	7.41E-09
7	*GNGT1*	120	**<1.0E-06**	rs4262	1.56E-12	102	**<1.0E-06**	rs180238	2.24E-13	121	**<1.0E-06**	rs4262	7.41E-09
7	*GNG11*	120	**<1.0E-06**	rs4262	1.56E-12	106	**<1.0E-06**	rs180238	2.24E-13	121	**<1.0E-06**	rs4262	7.41E-09
12	*C12orf67*	98	**<1.0E-06**	rs10842383	1.51E-22	95	**<1.0E-06**	rs10842383	2.61E-22	98	**<1.0E-06**	rs10842383	7.61E-23
12	*SYT10*	181	**<1.0E-06**	rs1384598	1.20E-09	176	**<1.0E-06**	rs7980799	1.92E-14	176	**<1.0E-06**	rs6488162	2.42E-12
12	*ALG10*	80	1.74E-03	rs1705748	0.000109	78	3.00E-06	rs1705748	6.80E-09	79	**2.00E-06**	rs4001713	4.52E-09
12	*ALG10B*	101	4.45E-04	rs11183514	4.69E-05	90	**<1.0E-06**	rs4575342	1.34E-08	101	**<1.0E-06**	rs4575342	9.55E-10
12	*CPNE8*	289	1.27E-04	rs826879	2.74E-06	272	**<1.0E-06**	rs826879	8.41E-09	284	**<1.0E-06**	rs11168761	6.08E-10
14	*RGS6*	1243	**1.00E-06**	rs36423	1.03E-08	1216	4.00E-06	rs2052015	5.97E-09	1236	1.85E-02	rs17108294	0.0001619
15	*HCN4*	110	**<1.0E-06**	rs2680344	1.26E-07	109	**<1.0E-06**	rs7173389	1.16E-06	110	3.60E-05	rs2680344	4.23E-06
19	*NRTN*	56	6.00E-05	rs8108862	4.03E-12	54	**<1.0E-06**	rs8108862	2.77E-28	55	**<1.0E-06**	rs8108862	1.81E-37
19	*FUT6*	58	1.10E-05	rs8108862	4.03E-12	57	**<1.0E-06**	rs8108862	2.77E-28	57	**<1.0E-06**	rs8108862	1.81E-37
19	*FUT3*	74	**<1.0E-06**	rs12980262	3.00E-13	72	**<1.0E-06**	rs12974991	7.80E-30	73	**<1.0E-06**	rs12974440	9.09E-38
19	*FUT5*	75	**2.00E-06**	rs12980262	3.00E-13	73	**<1.0E-06**	rs12974991	7.80E-30	74	**<1.0E-06**	rs12974440	9.09E-38
19	*NDUFA11*	72	**1.00E-06**	rs12980262	3.00E-13	68	**<1.0E-06**	rs12974991	7.80E-30	70	**<1.0E-06**	rs12974440	9.09E-38
19	*VMAC*	71	3.00E-06	rs12980262	3.00E-13	66	**<1.0E-06**	rs12974991	7.80E-30	69	**<1.0E-06**	rs12974440	9.09E-38
19	*CAPS*	72	**1.00E-06**	rs12980262	3.00E-13	67	**<1.0E-06**	rs12974991	7.80E-30	70	**<1.0E-06**	rs12974440	9.09E-38
19	*RANBP3*	94	3.00E-06	rs12980262	3.00E-13	89	**<1.0E-06**	rs12974991	7.80E-30	93	**<1.0E-06**	rs12974440	9.09E-38
19	*RFX2*	101	2.82E-03	rs7258475	2.11E-12	98	5.00E-06	rs7258475	3.50E-26	102	**2.00E-06**	rs7258475	3.46E-30
20	*KIAA1755*	147	9.00E-06	rs6127466	1.53E-05	144	**<1.0E-06**	rs6123471	5.15E-08	143	2.85E-03	rs6123471	0.0003297

Supplementary Table 18: Common SNP and narrow-sense heritabilities of the HRV traits and heart rate as calculated (a) by genomic restricted maximum likelihood analysis in 9,571 unrelated individuals from the Lifelines Cohort Study, (b) by LD Score regression on the stage 1 meta-analysis results and (c) from classical biometrical modeling in the Oman Family Study.
For (a): Common SNP heritabilities are shown on the diagonal, genetic correlations above the diagonal. For (b): Narrow-sense heritabilities are shown on the diagonal, genetic correlations above the diagonal. For (c): Narrow-sense heritabilities are shown on the diagonal, genetic correlations above the diagonal, and environmental correlations below the diagonal. The *p*-values (between brackets) indicate whether the genetic correlation is different from 1.

(a)	SDNN	RMSSD	pvRSA/HF
SDNN	0.1076	0.98 (n.s.)	n.a.
RMSSD		0.1319	n.a.
pvRSA/HF			n.a.

n.a.: not applicable.

(b)	SDNN	RMSSD	pvRSA/HF
SDNN	0.1112	1.00 (n.s.)	1.00 (n.s.)
RMSSD		0.1125	1.00 (n.s.)
pvRSA/HF			0.1177

(c)	SDNN	RMSSD	HF
SDNN	0.1521	0.71 (2.5E-06)	0.73 (1.7E-05)
RMSSD	0.74	0.1989	0.90 (n.s.)
HF	0.74	0.80	0.1723
Heart Rate	-0.61	-0.69	-0.64

n.s.: not significant.

Supplementary Table 19: Prioritization of genes in HRV GWAS-identified loci (±40 kb of lead SNPs) using MetaRanker, ToppGene, Endeavour, and DEPICT.

HRV SNP	Chr	Gene	*p*-value for the gene being causal*						Prioritized for functional follow-up
			MetaRanker	ToppGene	Endeavour	DEPICT#			
						SDNN	RMSSD	pvRSA/HF	
rs10842383	12	*LINC00477*	6.2E-13	-	-	0.53	0.43	0.07	1
rs12974991, rs12974440, rs12980262	19	*AC024592.9*	-	0.06	-	-	-	-	0
rs12974991, rs12974440, rs12980262	19	*CAPS*	7.5E-13	-	-	0.30	0.64	0.58	1
rs12974991, rs12974440, rs12980262	19	*FUT5*	3.2E-13	0.07	0.56	0.78	0.35	0.52	1
rs12974991, rs12974440, rs12980262	19	*NDUFA11*	7.7E-14	0.06	-	0.52	0.54	0.42	1
rs12974991, rs12974440, rs12980262	19	*RANBP3*	-	-	-	0.86	0.76	0.71	1
rs12974991, rs12974440, rs12980262	19	*VMAC*	7.7E-13	-	-	-	-	-	0
rs236349	6	*C6orf89*	2.1E-13	0.14	0.78	0.97	0.81	0.45	1
rs236349	6	*CPNE5*	2.5E-13	-	-	0.42	0.14	0.17	1
rs236349	6	*PPIL1*	1.1E-13	-	-	0.12	0.26	0.78	1
rs2680344, rs1812835	15	*HCN4*	4.9E-15	-	-	0.45	0.09	0.09	1
rs2680344, rs1812835	15	*NEO1*	-	-	-	0.58	0.21	-	1
rs4262, rs180238	7	*GNG11*	4.5E-13	-	-	0.74	0.30	0.24	1
rs4262, rs180238	7	*GNGT1*	1.5E-13	2.3E-03	0.19	0.84	0.32	0.51	1
rs4262, rs180238	7	*TFPI2*	5.7E-13	-	-	-	-	-	0
rs4899412, rs2052015, rs2529471, rs36423	14	*RGS6*	-	-	-	0.69	0.43		1
rs6123471	20	*KIAA1755*	-	-	-		0.71	-	1
rs7980799	12	*SYT10*	2.7E-13	0.02	0.16	-	-	-	1

***** Caution should be exercised when using the *p*-value as a measure of confidence that the highlighted genes are indeed causal for the associations identified by GWAS, or as a means to compare evidence across tools. Each tool uses different databases and algorithms to generate these *p*-values and as such, *p*-values cannot necessarily be compared across tools. We advise to use the presence of a *p*-value as an indication that the respective tool identifies a gene as potentially being causal.

DEPICT is considered to be the most advanced tool since it uses the most up-to-date information to derive which genes are likely to be causal. For DEPICT, only identified genes that were located within ±40 kb of GWAS lead SNPs are reported.

Supplementary Table 20: Network and functional enrichment analyses.
Gene ontology (GO) terms related to cell membrane signal transduction are highlighted in yellow; GO terms related to cellular anabolic, catabolic, and respiratory processes are highlighted in green. Significant false discovery rate *q*-values are shown in red font, suggestive ones in purple.

GO id	Description	*q*-value	Occurrences in Sample	Occurrences in Genome
GO:0071377	cellular response to glucagon stimulus	3.01E-13	10	33
GO:0033762	response to glucagon	3.01E-13	10	33
GO:0036065	fucosylation	1.90E-11	7	11
GO:0008417	fucosyltransferase activity	7.35E-11	7	13
GO:0006004	fucose metabolic process	1.67E-08	6	13
GO:0006112	energy reserve metabolic process	5.55E-07	10	143
GO:0015980	energy derivation by oxidation of organic compounds	3.00E-05	11	285
GO:0071375	cellular response to peptide hormone stimulus	7.32E-05	10	244
GO:1901653	cellular response to peptide	7.32E-05	10	247
GO:0043434	response to peptide hormone	8.89E-05	10	255
GO:1901652	response to peptide	9.70E-05	10	260
GO:0043413	macromolecule glycosylation	1.65E-04	9	209
GO:0070085	glycosylation	2.02E-04	9	216
GO:0016758	transferase activity, transferring hexosyl groups	3.49E-04	7	113
GO:0019320	hexose catabolic process	4.23E-04	6	72
GO:0046365	monosaccharide catabolic process	4.67E-04	6	74
GO:0003924	GTPase activity	1.01E-03	7	136
GO:0006486	protein glycosylation	1.39E-03	8	208
GO:0008277	regulation of G-protein coupled receptor protein signaling pathway	1.53E-03	6	93
GO:0044724	single-organism carbohydrate catabolic process	1.86E-03	6	97
GO:0016757	transferase activity, transferring glycosyl groups	2.15E-03	7	157
GO:0016052	carbohydrate catabolic process	2.27E-03	6	102
GO:0009101	glycoprotein biosynthetic process	6.01E-03	8	262
GO:0031305	integral component of mitochondrial inner membrane	1.21E-02	3	13
GO:0031304	intrinsic component of mitochondrial inner membrane	1.47E-02	3	14
GO:0006626	protein targeting to mitochondrion	2.74E-02	4	49
GO:0070585	protein localization to mitochondrion	3.09E-02	4	51
GO:0019318	hexose metabolic process	4.84E-02	6	182
GO:0005834	heterotrimeric G-protein complex	5.25E-02	3	22
GO:0005996	monosaccharide metabolic process	9.49E-02	6	209
GO:0030695	GTPase regulator activity	9.49E-02	5	132

SUPPLEMENTARY NOTES

1. Gene-based GWAS (VEGAS)

The results of the VEGAS gene-based GWAS analysis corroborate those of the SNP-based analysis (Supplementary Table 17). In seven of our eight loci with genome-wide significant SNPs (Table 1) the closest gene or other nearby genes also emerged from VEGAS as significantly associated with at least one of the HRV traits. Only the loci on chromosome 6 (*PPIL*) and 15 (*NEO1*) were not represented. In addition, a few new genes close to the top hits in our SNP-based analyses emerged: *TFPI2* and *GNGT1* (both on chromosome 7), *ALG10*, *ALG10B*, and *CPNE8* (all on chromosome 12), and *NRTN*, *FUT6*, *FUT3*, *FUT5*, *VMAC*, *CAPS*, *RANBP3*, and *RFX2* (all on chromosome 19). Only one gene in a new locus was identified: *CCDC141* (on chromosome 2, next to *TTN* coding for the titin protein, a key determinant of myocardial passive stiffness). *CCDC141* and *CPNE8* have previously been reported to be associated in a GWAS for heart rate[14].

2. Heritability and genetic correlations

The common SNP heritability estimated by Genomic Restricted Maximum Likelihood (GREML) analysis was 10.8% and 13.2% for SDNN and RMSSD in the Lifelines cohort (Supplementary Table 18, panel a). Applying LD score regression analysis on the summary statistics of the stage 1 meta-analysis suggested a common SNP heritability of 11.1% for SDNN, 11.2% for RMSSD, and 11.8% for pvRSA/HF (Supplementary Table 18, panel b). Classical modeling on family data from the Oman Family Study yielded heritability estimates between 15.2 and 20.0%.

Previous studies showed high phenotypic correlation between pvRSA/HF and RMSSD[31, 32] and suggested a large overlap in their genetic architecture[33, 34]. We confirmed the phenotypic correlations in the TRAILS-Pop, NESDA, and Lifelines cohorts where they ranged from 0.70 (SDNN-pvRSA/HF) to 0.96 (RMSSD-pvRSA/HF). Bivariate analysis using GREML and LD score regression analysis further showed genetic correlations of unity between pvRSA/HF and RMSSD, but also with SDNN, indicating complete overlap between the genetic variants influencing all three HRV traits (Supplementary Table 18, panel a+b). Modeling of the family data from the Oman Family Study confirmed high genetic correlation between the HRV traits, including SDNN (0.71-0.90) (Supplementary Table 18, panel c). This shows that SDNN, notwithstanding its different etiology, is influenced by sets of genetic variants that also influence the RMSSD and pvRSA/HF traits.

We observed large negative genetic correlations (between -0.74 and -0.55) of SDNN, RMSSD, and pvRSA/HF with heart rate (Supplementary Table 11, panel b). High genetic overlap between heart rate and HRV fits the notion that HRV increases with a net increase of the tonic vagal effects on the sinoatrial node, which in turn reduces average heart rate. However, we note that the full relationship between HRV and heart rate is more complex because heart rate is under parallel sympathetic control, changes in which are not captured well by the HRV measures used [35, 36] and because changes in heart rate can lead to lower HRV, even at unchanged vagal input [37].

3. Association between heart rate SNPs and HRV

After establishing the effects of HRV SNPs on the variance in heart rate we also examined the reverse question, i.e. whether SNPs known to be associated with heart rate accounted for part of the variance in HRV. The reverse association of the 21 heart rate SNPs identified by a GWAS

study for heart rate[14] with the three HRV traits showed that nine heart rate SNPs were associated with HRV, all in the expected direction, after correction for multiple testing (Supplementary Table 10, panel a)). Using *gtx*, a multi-SNP genetic risk scores for heart rate was associated with the three HRV traits (Supplementary Table 10, panel b).

In the Lifelines, NESDA, TRAILS-Pop, and ABCD cohorts, a multi-SNP genetic risk score for heart rate explained 0.2 to 0.9% of HRV variance in the four cohorts (Supplementary Table 10, panel c). The full polygenic risk score for heart rate explained a similar amount of the variance in the three HRV traits: 0.2 to 0.8% (Supplementary Table 10, panel d).

4. Network and functional enrichment analyses

Based on our 17 HRV SNPs, we selected candidate genes as input for the interaction network analysis with the GeneMANIA algorithm to prioritize potentially causal genes [38] with additional input from the VEGAS analyses (Supplementary Table 17) and four publicly available bioinformatics tools (Supplementary Table 19). As one of the genes (*LINC00477*) could not be found by GeneMANIA, 24 query genes were used as input. Functional network and enrichment analysis on these genes resulted in 31 significantly enriched gene ontology terms of which 23 had false discovery rate ≤ 0.01 (Supplementary Table 20). These 23 gene ontology terms showed that the genes near our hits were broadly related to two categories of significantly enriched biological processes, namely: (1) cellular signaling and cellular responses, including G-protein coupled receptor protein signaling and the responses to glucagon and peptides, and (2) metabolic processes in the cell (e.g. fucosylation and glycosylation) (Supplementary Fig. 10).

5. Tissue and gene-set enrichment analyses

In silico tissue enrichment analysis using DEPICT[6] highlights a role for hormones in HRV regulation, with enrichment for the adrenal cortex, endocrine glands, gonads, gastrointestinal tract and female reproductive organs (Supplementary Fig. 8a,c,e). Gene-set enrichment analysis using DEPICT highlights the importance of cardiac development (Supplementary Fig. 8b,d,f).

6. Known biological functions of the genes closest to the HRV SNPs

Here we describe the known biological functions of the genes closest to the eight HRV lead SNPs identified in the two-stage meta-analysis.

NDUFA11 (Chr 19, full name: NADH dehydrogenase (ubiquinone) 1 alpha sub-complex, 11, also known as B14.7 and Cl-B14.7) encodes a subunit of the membrane-bound mitochondrial complex I involved in mitochondrial respiration and electron transport. NDUFA11's cDNA was cloned and sequenced from human heart mitochondria. The non-synonymous lead SNP in our meta-analysis induces a change in the structure of this complex with as yet unknown functional effects. Clinically, five phenotypes are frequently seen with homozygous mutations of this gene: severe neonatal lactic acidosis, cardiomyopathy-encephalopathy, hepatopathy-tubulopathy, leukodystrophy with macrocephaly, and Leigh's and Leigh-like neurodegenerative disorder, which are the most common presentations of complex I deficiency at a young age [39, 40].

LINC00477 (C12orf67) (Chr 12, full name: long intergenic non-protein coding RNSA 477) is of unknown biological function but the locus has also been associated with resting heart rate [14] and PR interval [41].

PPIL1 (Chr 6, full name: peptidylprolyl isomerase (cyclophilin)-like 1) is a member of the cyclophilin family. After being recruited by Ski interaction protein (SKIP) PPIL1 participates in the activation of spliceosomes and thereby facilitates the folding of proteins in the spliceosomes [42, 43]. No relation with heart rate or HRV has been described but *PPIL1* has been reported to be one of two key driver genes involved in coronary artery disease networks [44].
SYT10 (Chr 12, full name: synaptotagmin-10) has a role in the regulation of calcium dependent exocytosis, including calcium regulated release of neurotransmitter from presynaptic nerve terminals [45, 46]. *SYT10* has been previously identified as being associated with heart rate [14]. Synaptotagmin-1 plays a known role in calcium-triggered acetylcholine release from the neuromuscular junction [47]. Our meta-analyses results hint at a possible role for synaptotagmin-10 in sinoatrial acetylcholine release but this remains to be tested.
GNG11 (Chr 7, full name: guanine nucleotide binding protein (G protein, gamma 11) which is also a previously identified heart rate gene [14] encodes the γ11 subunit of Gαβγ heterotrimers. *GNG11* is one of 12 genes encoding the Gγ subunits that all undergo post-translational isoprenylation of their C termini, in case of γ11 by a farnesyl [48]. Because the γ subunits have lower amino acid sequence homology then the β subunits, they are thought to be the main determinant of signaling diversity and fidelity of the Gβγ components [49, 50]. γ11 shows a unique pattern of expression compared to the other 11 γ subunits in that it is abundantly expressed in the heart but poorly in the brain[50].
RGS6 (Chr 14, full name: regulator of G-protein signaling 6) is a large gene coding one of the RGS superfamily that act as GTPase-activating proteins (GAPs) for the α subunit of Gαβγ heterotrimers by accelerating their intrinsic GTPase activity. This ends both Gα and Gβγ mediated cellular signaling. RGS6 exhibits a uniquely robust expression in the heart [10]. RGS6 is known to reduce parasympathetic signaling through the M_2R in the sinoatrial node as well as $G\alpha_o$ coupled adenosine A1 receptors [10, 51, 52].
HCN4 (Chr 15, full name: hyperpolarization activated cyclic nucleotide gated potassium channel 4) codes for a non-GIRK potassium channel in the sinoatrial node that is a core part of the 'clock circuit' generating the pacemaker potential. *HCN4* is expressed abundantly in the heart from the early embryonic phase onward [12]. *HCN4* was previously found to be associated with heart rate [14]. Depending on total load and severity, loss-of-function mutations in *HCN4* can lead to asymptomatic bradycardia, Brugada syndrome and Sick Sinus Syndrome, atrial fibrillation and possibly left ventricular noncompaction cardiomyopathy [53, 54].
NEO1 (Chr 15, full name: neogenin 1, aka *HsT16534*, *IGDCC2*, *NGN* or *NTN1R2*) is a multifunctional transmembrane receptor closely related to the immunoglobulin (Ig) superfamily and is a netrin receptor [55]. Neogenin plays a role in early cerebellar neurite out-growth and projection in chicken and quail [56, 57] and is known to be a regulator of axonal guidance in the nervous system [58]. No role in HRV or heart rate has been described previously.
KIAA1755 (Chr 20, no full name in Ensembl or at NCBI) which has also been associated with heart rate [14]. Effects of the non-synonymous lead SNP (rs6123471) is unclear as the function of this gene remains unknown.

7. Software and internet databases used

Annovar	URL: http://annovar.openbioinformatics.org/en/latest/
DEPICT	URL: http://www.broadinstitute.org/mpg/depict/
Endeavour	URL: http://homes.esat.kuleuven.be/~bioiuser/endeavour/index.php
GeneMANIA	URL: http://genemania.org/
Genome-wide Complex Trait Analysis (GCTA) version 1.24.4	URL: http://cnsgenomics.com/software/gcta/
gtx (R package)	URL: https://cran.r-project.org/web/packages/gtx/index.html
GWAMA	URL: http://www.well.ox.ac.uk/gwama/
LD score regression	URL: https://github.com/bulik/ldsc
LocusZoom	URL: http://locuszoom.sph.umich.edu/locuszoom/
METAL	URL: http://csg.sph.umich.edu//abecasis/Metal/index.html
MetaRanker	URL: http://www.cbs.dtu.dk/services/MetaRanker/
PriorityPruner version 0.1.1	URL: http://prioritypruner.sourceforge.net/
PLINK version 1.07	URL: http://pngu.mgh.harvard.edu/purcell/plink/
PolyPhen	URL: http://genetics.bwh.harvard.edu/pph2/
RegulomeDB	URL: http://regulomedb.org/
SIFT	URL: http://sift.jcvi.org/
SNP annotation and proxy search (SNAP)	URL: https://www.broadinstitute.org/mpg/snap/
SOLAR	URL: http://solar-eclipse-genetics.org/
ToppGene	URL: https://toppgene.cchmc.org/

8. Acknowledgments, study consent, and funding

Stage 1 cohorts
ARIC: We thank the staff and participants of the ARIC study for their important contributions. The Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts (HHSN268201100005C, HHSN268201100006C, HHSN268201100007C, HHSN268201100008C, HHSN268201100009C, HHSN268201100010C, HHSN268201100011C, and HHSN268201100012C), R01HL087641, R01HL59367 and R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN268200625226C. Infrastructure was partly supported by Grant Number UL1RR025005, a component of the National Institutes of Health and NIH Roadmap for Medical Research.
The Atherosclerosis Risk in Communities (ARIC) Study (#11-0734) was approved by the Biomedical Institutional Review Board (IRB) at the University of North Carolina (Chapel Hill, NC).

CHS: We thank the contributing CHS investigators and institutions, a list of principal contributors can be found at CHS-NHLBI.org. CHS research was supported by NHLBI contracts

HHSN268201200036C, HHSN268200800007C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086; and NHLBI grants U01HL080295, R01HL087652, R01HL105756, R01HL103612, R01HL120393, R01HL062181, and R01HL130114, with additional contribution from the National Institute of Neurological Disorders and Stroke (NINDS). Additional support was provided through R01AG023629 from the National Institute on Aging (NIA). The provision of genotyping data was supported in part by the National Center for Advancing Translational Sciences, CTSI grant UL1TR000124, and the National Institute of Diabetes and Digestive and Kidney Disease Diabetes Research Center (DRC) grant DK063491 to the Southern California Diabetes Endocrinology Research Center. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.
Written informed consent was obtained from all Cardiovascular Health Study participants. The study was approved by the Institutional Review Boards of the University of Washington, Seattle, WA, USA; the University of California, Davis, CA, USA; the Johns Hopkins School of Public Health, Baltimore, MD, USA; the University of Pittsburgh, Pittsburgh, PA, USA; the Institutional Review Board in the Office of Research, Wake Forest University, Winston-Salem, NC, USA; and the Committee on Human Subjects of the University of Vermont, Burlington, VT, USA. Current approval at the University of Washington IRB runs through 12/12/17.

FHS: The National Heart, Lung and Blood Institute's Framingham Heart Study is supported by contract N01-HC-25195 and HHSN268201500001I.
The FHS study protocol was approved by the Institutional Review Board of the Boston University Medical Center and all participants gave written informed consent.

FINGESTURE: Is supported by the Sigrid Juselius Foundation and the Finnish Foundation for Cardiovascular Research, Helsinki, Finland. The genotyping of this cohort was supported by the Montreal Heart Institute Foundation.
Written informed consent was obtained from all FINGESTURE participants. The study was approved by the Regional Ethics Committee of the Northern Ostrobothnia Hospital District, Oulu, Finland (IRB number 21/2007).

FLEMENGHO-EPOGH: Nuclear families were recruited in the framework of the European Project On Genes in Hypertension, which was supported by the European Union (contract numbers IC15-CT98-0329-EPOGH and QLGI-CT-2000-01137-EURNETGEN). The study was also supported by research grants G.0174.97, G.0291.98, and G.0424.03 from the Fonds voor Wetenschappelijk Onderzoek Vlaanderen (Brussels, Belgium); by a special research grant (Onderzoekstoelage OT/99/28) from the Katholieke Universiteit Leuven (Leuven, Belgium); by research grants (OK 375 and OK 376) from the Czech Ministry of Education; and by the International Scientific Collaboration between Poland and Flanders (contract number BIL 00/18).
Both the FLEMENGHO and EPOGH studies were conducted according to the principles outlined in the Helsinki Declaration for Investigation of Human Participants. Each local institutional review board approved the study protocol. Participants provided written informed consent.

GenR: We gratefully acknowledge the contribution of general practitioners, hospitals, midwives and pharmacies in Rotterdam to The Generation R Study. GenR is conducted by the Erasmus Medical Center Rotterdam in close collaboration with the Faculty of Social Sciences of the Erasmus University Rotterdam, the Municipal Health Service Rotterdam area, the Rotterdam

Homecare Foundation and the Stichting Trombosedienst & Artsenlaboratorium Rijnmond (STAR), Rotterdam.
Written informed consent was obtained from mothers and fathers of all participants. The Medical Ethical Committee of the Erasmus Medical Centre, Rotterdam (MEC-2007-413-NL21545.078; MEC 198.782.2001.31; MEC 217.595/2002/202; MEC-2007-413, MEC-2012-165) approved the study protocol.
GTR: The sample for the Twin Interdisciplinary Neuroticism Study (TWINS) was selected from the Groningen Twin Register (GTR). TWINS was supported by the Netherlands Organization for Health Research and Development (ZonMw 904-57-130), and the UK-Netherlands Partnership Program in Science (BR 56-481 and BR 96-229), which is jointly run and financed by the British Council and the Netherlands Organization for Scientific Research (NWO).
The GTR study was approved by the Ethics Committee of the University Medical Center Groningen (METc 2000/060e), and all subjects gave written consent prior to participation.

KORA: The KORA study was initiated and financed by the Helmholtz Zentrum München – German Research Center for Environmental Health, which is funded by the German Federal Ministry of Education and Research (BMBF) and by the State of Bavaria. Furthermore, KORA research was supported within the Munich Center of Health Sciences (MC-Health), Ludwig-Maximilians-Universität, as part of LMUinnovativ. Statistical analyses were carried out on the Genetic Cluster Computer (http://www.geneticcluster.org) hosted by SURFsara and financially supported by the Netherlands Scientific Organization (NWO 480-05-003) along with a supplement from the Dutch Brain Foundation and the VU University Amsterdam.
All participants of KORA S4 provided written informed consent for participation in the study, which was approved by the Ethics Committee of the Bavarian Medical Association (#99186).

MESA: We thank the other investigators, the staff, and the participants of the MESA study for their valuable contributions. A full list of participating MESA investigators and institutions can be found at http://www.mesa-nhlbi.org. MESA and the MESA SHARe project are conducted and supported by the National Heart, Lung, and Blood Institute (NHLBI) in collaboration with MESA investigators. Support for MESA is provided by contracts HHSN268201500003I, N01-HC-95159, N01-HC-95160, N01-HC-95161, N01-HC-95162, N01-HC-95163, N01-HC-95164, N01-HC-95165, N01-HC-95166, N01-HC-95167, N01-HC-95168, N01-HC-95169, UL1-TR-001079, UL1-TR-000040, and DK063491. Funding for SHARe genotyping was provided by NHLBI Contract N02-HL-64278. Genotyping was performed at Affymetrix (Santa Clara, California, USA) and the Broad Institute of Harvard and MIT (Boston, Massachusetts, USA) using the Affymetrix Genome-Wide Human SNP Array 6.0. This publication was developed under a STAR research assistance agreement, No. RD831697 (MESA Air), awarded by the U.S Environmental protection Agency. It has not been formally reviewed by the EPA. The views expressed in this document are solely those of the authors and the EPA does not endorse any products or commercial services mentioned in this publication.
Written informed consent was obtained from all MESA participants. The study was approved by the Institutional Review Board at each field center and the data coordinating center. Each Institutional Review Board is certified by the U.S. Office of Human Research Protections.

MRS: We thank the MRS study team and the 1st Marine Division and Navy Medicine at 29 Palms and at Camp Pendleton. Funding for this study was provided by NIH grant 1 R01MH093500 (to CMN). The Marine Corps and Navy Bureau of Medicine and Surgery

(BUMED) and VA Health Services and Development (HSR&D) provided funding for MRS-I and MRS-II (DGB, VBR, CMN, AM, MAG, AXM).
Written informed consent was obtained from all MRS participants. The study was approved by the University of California-San Diego Institutional Review Board (IRB number 150563).
NESDA: Funding was obtained from the Netherlands Organization for Scientific Research (Geestkracht program grant 10-000-1002); the Center for Medical Systems Biology (CSMB, NOW Genomics), Biobanking and Biomolecular Resources Research Infrastructure (BBMRI-NL), VU University's Institutes for Health and Care Research (EMGO+) and Neuroscience Campus Amsterdam, University Medical Center Groningen, Leiden University Medical Center, National Institutes of Health (NIH, R01D0042157-01A, MH081802, Grand Opportunity grants 1RC2 MH089951 and 1RC2 MH089995). Part of the genotyping and analyses were funded by the Genetic Association Information Network (GAIN) of the Foundation for the National Institutes of Health. Computing was supported by BiG Grid, the Dutch e-Science Grid, which is financially supported by NWO. Part of the statistical analyses were carried out on the Genetic Cluster Computer (http://www.geneticcluster.org) hosted by SURFsara and financially supported by the Netherlands Scientific Organization (NWO 480-05-003) along with a supplement from the Dutch Brain Foundation and the VU University Amsterdam.
Written informed consent was obtained from all NESDA participants. The study was approved by the Central Ethics Committee on Research Involving Human Subjects of the VU University Medical Centre, Amsterdam, an Institutional Review Board certified by the U.S. Office of Human Research Protections (IRB number IRB00002991 under Federal-wide Assurance-FWA00017598; IRB/institute code 03-183).

NTR, Netherland Twin Register: Funding was obtained from the Netherlands Organization for Scientific Research (NWO) and The Netherlands Organization for Health Research and Development (ZonMW) grants 904-61-090, 985-10-002, 912-10-020, 904-61-193,480-04-004, 463-06-001, 451-04-034, 400-05-717, Addiction-31160008, Middelgroot-911-09-032, Spinozapremie 56-464-14192, Biobanking and Biomolecular Resources Research Infrastructure (BBMRI –NL, 184.021.007). VU Institute for Health and Care Research (EMGO+); the European Community's Seventh Framework Program (FP7/2007-2013), ENGAGE (HEALTH-F4-2007-201413); the European Research Council (ERC Advanced, 230374, ERC Starting grant 284167), Rutgers University Cell and DNA Repository (NIMH U24 MH068457-06), the Avera Institute, Sioux Falls, South Dakota (USA) and the National Institutes of Health (NIH, R01D0042157-01A, MH081802; R01 DK092127-04, Grand Opportunity grants 1RC2 MH089951 and 1RC2 MH089995). Part of the genotyping and analyses were funded by the Genetic Association Information Network (GAIN) of the Foundation for the National Institutes of Health. Computing was supported by BiG Grid, the Dutch e-Science Grid, which is financially supported by NWO.
Written informed consent was obtained from all NTR participants. NTR studies were approved by the Central Ethics Committee on Research Involving Human Subjects of the VU University Medical Centre, Amsterdam, an Institutional Review Board certified by the U.S. Office of Human Research Protections (IRB number IRB00002991 under Federal-wide Assurance-FWA00017598; IRB/institute codes, NTR 03-180).

PIVUS: We thank the SNP&SEQ Technology Platform (www.genotyping.se), which is part of the National Genomics Infrastructure hosted by Science for Life laboratory at Uppsala University for excellent genotyping. This project was supported by Knut and Alice Wallenberg Foundation

(Wallenberg Academy Fellow), European Research Council (ERC Starting Grant), Swedish Diabetes Foundation (2013-024), Swedish Research Council (2012-1397, 2012-1727, and 2012-2215), Marianne and Marcus Wallenberg Foundation, County Council of Dalarna, Dalarna University, and Swedish Heart-Lung Foundation (20120197). The computations were performed on resources provided by SNIC through Uppsala Multidisciplinary Center for Advanced Computational Science (UPPMAX) under Project b2011036. Genotyping was funded by the Wellcome Trust under award WT064890. Analysis of genetic data was funded by the Wellcome Trust under awards WT098017 and WT090532.
Written informed consent was obtained from all participants of the PIVUS study. The protocols were approved by the Ethics Committee of Uppsala University.

PREVEND: PREVEND genetics is supported by the Dutch Kidney Foundation (Grant E033), the EU project grant GENECURE (FP-6 LSHM CT 2006 037697), the National Institutes of Health (grant 2R01LM010098), The Netherlands organization for health research and development (NWO-Groot grant 175.010.2007.006, NWO VENI grant 916.761.70, ZonMw grant 90.700.441), and the Dutch Inter University Cardiology Institute Netherlands (ICIN).
All participants of the PREVEND study provided informed consent. This study has been approved by the review board of the University Medical Center Groningen. This study adheres to the principles expressed in the Declaration of Helsinki.

RS: We are grateful to the study participants, the staff from the Rotterdam Study and the participating general practitioners and pharmacists. Furthermore, we thank Pascal Arp, Mila Jhamai, Marijn Verkerk, Lizbeth Herrera and Marjolein Peters, MSc, and Carolina Medina-Gomez, MSc, for their help in creating the GWAS database, and Karol Estrada, PhD, Yurii Aulchenko, PhD, and Carolina Medina-Gomez, MSc, for the creation and analysis of imputed data. The generation and management of GWAS genotype data for the Rotterdam Study (RS I, RS II) was executed by the Human Genotyping Facility of the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC, Rotterdam, The Netherlands. The GWAS datasets are supported by the Netherlands Organisation of Scientific Research NWO Investments (nr. 175.010.2005.011, 911-03-012), the Genetic Laboratory of the Department of Internal Medicine, Erasmus MC, the Research Institute for Diseases in the Elderly (014-93-015; RIDE2), the Netherlands Genomics Initiative (NGI)/Netherlands Organization for Scientific Research (NWO) Netherlands Consortium for Healthy Aging (NCHA), project nr. 050-060-810. The Rotterdam Study is funded by Erasmus Medical Center and Erasmus University, Rotterdam, Netherlands Organization for the Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam.
The Rotterdam study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands, on the basis of the Wet Bevolkingsonderzoek ERGO. All participants provided written informed consent.

TRAILS: This research is part of the TRacking Adolescents' Individual Lives Survey (TRAILS), which includes the TRAILS population cohort (TRAILS-Pop) and the TRAILS clinical cohort (TRAILS-CC). Participating centers of TRAILS include the University Medical Center and University of Groningen, the Erasmus University Medical Center Rotterdam, the University of

Utrecht, the Radboud Medical Center Nijmegen, and the Parnassia Bavo group, all in the Netherlands. We are grateful to everyone who participated in this research or worked on this project to make it possible. TRAILS has been financially supported by various grants from the Netherlands Organization for Scientific Research NWO (Medical Research Council program grant GB-MW 940-38-011; ZonMW Brainpower grant 100-001-004; ZonMw Risk Behavior and Dependence grants 60-60600-97-118; ZonMw Culture and Health grant 261-98-710; Social Sciences Council medium-sized investment grants GB-MaGW 480-01-006 and GB-MaGW 480-07-001; Social Sciences Council project grants GB-MaGW 452-04-314 and GB-MaGW 452-06-004; NWO large-sized investment grant 175.010.2003.005; NWO Longitudinal Survey and Panel Funding 481-08-013 and 481-11-001), the Dutch Ministry of Justice (WODC), the European Science Foundation (EuroSTRESS project FP-006), Biobanking and Biomolecular Resources Research Infrastructure BBMRI-NL (CP 32), and the participating universities. Part of statistical analyses were carried out on the Genetic Cluster Computer (http://www.geneticcluster.org) hosted by SURFsara and financially supported by the Netherlands Scientific Organization (NWO 480-05-003) along with a supplement from the Dutch Brain Foundation and the VU University Amsterdam.
TRAILS was approved by Dutch Central Committee on Research Involving Human Subjects (CCMO); www.ccmo.nl. If both parents and children agreed to participate, parental written informed consent was obtained after the procedures had been fully explained.

ULSAM: We thank the SNP&SEQ Technology Platform (www.genotyping.se), which is part of the National Genomics Infrastructure hosted by Science for Life laboratory at Uppsala University for excellent genotyping. This project was supported by Knut and Alice Wallenberg Foundation (Wallenberg Academy Fellow), European Research Council (ERC Starting Grant), Swedish Diabetes Foundation (2013-024), Swedish Research Council (2012-1397, 2012-1727, and 2012-2215), Marianne and Marcus Wallenberg Foundation, County Council of Dalarna, Dalarna University, and Swedish Heart-Lung Foundation (20120197). The computations were performed on resources provided by SNIC through Uppsala Multidisciplinary Center for Advanced Computational Science (UPPMAX) under Project b2011036. Genotyping was funded by the Wellcome Trust under award WT064890. Analysis of genetic data was funded by the Wellcome Trust under awards WT098017 and WT090532. Andrew P Morris is a Wellcome Trust Senior Fellow in Basic Biomedical Science (award WT098017).
Written informed consent was obtained from all ULSAM participants. The study was approved by the ethics committee at Uppsala University (IRB numbers 251/90 and 2013/350).

YFS: We gratefully acknowledge the expert technical assistance in the statistical analyses by Irina Lisinen. The Young Finns Study has been financially supported by the Academy of Finland: grants 286284, 134309 (Eye), 126925, 121584, 124282, 129378 (Salve), 117787 (Gendi), and 41071 (Skidi); the Social Insurance Institution of Finland; Kuopio, Tampere and Turku University Hospital Medical Funds (grant X51001); Juho Vainio Foundation; Paavo Nurmi Foundation; Finnish Foundation of Cardiovascular Research ; Finnish Cultural Foundation; Tampere Tuberculosis Foundation ; Emil Aaltonen Foundation ; and Yrjö Jahnsson Foundation.
The Young Finns Study was approved by the local ethics committees (University Hospitals of Helsinki, Turku, Tampere, Kuopio and Oulu) and was conducted following the guidelines of the Declaration of Helsinki. All participants gave their written informed consent.

Stage 2 cohorts

CARLA: This study was funded by a grant from the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) as part of the Collaborative Research Center 598 ‘‘Heart failure in the elderly–cellular mechanisms and therapy’’ at the Medical Faculty of the Martin-Luther-University Halle-Wittenberg; by a grant of the Wilhelm-Roux Programme of the Martin-Luther-University Halle-Wittenberg; by the Federal Employment Office; and by the Ministry of Education and Cultural Affairs of Saxony-Anhalt.
Written informed consent was obtained from all CARLA participants. The study was approved by the Ethics Committee of The Medical Faculty of the Martin-Luther University Halle-Wittenberg and by the State Data Privacy Commissioner of Saxony-Anhalt, Germany.

FINCAVAS: We thank the staff of the Department of Clinical Physiology for collecting the exercise test data. The Finnish Cardiovascular Study (FINCAVAS) has been financially supported by the Competitive Research Funding of the Tampere University Hospital (Grant 9M048 and 9N035), the Finnish Cultural Foundation, the Finnish Foundation for Cardiovascular Research, the Emil Aaltonen Foundation, Finland, and the Tampere Tuberculosis Foundation.
The FINCAVAS study protocol was approved by the Ethical Committee of the Hospital District of Pirkanmaa, Finland, and all patients have given informed consent prior to the interview and measurements as stipulated in the Declaration of Helsinki.

HANDLS: The Healthy Aging in Neighborhoods of Diversity across the Life Span (HANDLS) study was supported by the Intramural Research Program of the NIH, National Institute on Aging and the National Center on Minority Health and Health Disparities (project # Z01-AG000513 and human subject’s protocol number 09-AG-N248). We thank the HANDLS study participants, staff and field workers for their contribution to this work. Data analyses for the HANDLS study utilized the high-performance computational resources of the Biowulf Linux cluster at the National Institutes of Health, Bethesda, MD, (http://hpc.nih.gov).
HANDLS is reviewed and approved by the National Institute of Environmental Health Sciences Institutional Review Board at NIH

HCHS/SOL: We thank the staff and participants of HCHS/SOL for their important contributions. A complete list of staff and investigators is available on the study website http://www.cscc.unc.edu/hchs/. The Hispanic Community Health Study/Study of Latinos was carried out as a collaborative study supported by contracts from the National Heart, Lung, and Blood Institute (NHLBI) to the University of North Carolina (N01-HC65233), University of Miami (N01-HC65234), Albert Einstein College of Medicine (N01-HC65235), Northwestern University (N01-HC65236), and San Diego State University (N01-HC65237). The following Institutes/Centers/Offices contribute to the HCHS/SOL through a transfer of funds to the NHLBI: National Center on Minority Health and Health Disparities, the National Institute of Deafness and Other Communications Disorders, the National Institute of Dental and Craniofacial Research, the National Institute of Diabetes and Digestive and Kidney Diseases, the National Institute of Neurological Disorders and Stroke, and the Office of Dietary Supplements. The Genetic Analysis Center at University of Washington was supported by NHLBI and NIDCR contracts (HHSN268201300005C AM03 and MOD03). Genotyping efforts were supported by NHLBI HSN 26220/20054C, NCATS CTSI grant UL1TR000124, and NIDDK Diabetes Research Center (DRC) grant DK063491.

Written informed consent was provided by all HCHS/SOL participants; IRB approvals were obtained by all field sites recruiting participants: Bronx Site at Albert Einstein College of Medicine, Yeshiva University (FWA00000140) and Montifiore Medical Center (FWA0000258); Chicago Site at University of Illinois, Chicago (FWA00000083, IRB00000117 IRB#3); San Diego Site at San Diego State University: (IORG0000333, IRB00000576); Miami site at University of Miami, Florida (FWA00002247).

Lifelines: We wish to acknowledge the services of the Lifelines Cohort Study, the contributing research centers delivering data to Lifelines, and all the study participants. The Lifelines Cohort Study, and generation and management of GWAS genotype data for the Lifelines Cohort Study is supported by the Netherlands Organization of Scientific Research NWO (grant 175.010.2007.006), the Economic Structure Enhancing Fund (FES) of the Dutch government, the Ministry of Economic Affairs, the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the Northern Netherlands Collaboration of Provinces (SNN), the Province of Groningen, University Medical Center Groningen, the University of Groningen, Dutch Kidney Foundation and Dutch Diabetes Research Foundation. Part of the statistical analyses were carried out on the Genetic Cluster Computer (http://www.geneticcluster.org) hosted by SURFsara and financially supported by the Netherlands Scientific Organization (NWO 480-05-003) along with a supplement from the Dutch Brain Foundation and the VU University Amsterdam.
All participants signed an informed consent form before they received an invitation for the physical examination. The LifeLines Cohort Study is conducted according to the principles of the Declaration of Helsinki and in accordance with research code University Medical Center Groningen (UMCG). The LifeLines study is approved by the medical ethical committee of the UMCG, the Netherlands (METc 2007/152).

MRC NSHD: We are very grateful to the members of this birth cohort for their continuing interest and participation in the study. We would like to acknowledge the Swallow group, UCL, who performed the DNA extractions (Rousseau, et al 2006). DOI: 10.1111/j.1469-1809.2006.00250.x MRC NSHD was funded by the Medical Research Council (MC_UU_12019/1); doi: 10.5522/NSHD/Q102.
Written informed consent was obtained from all NSHD study members. Ethical approval was given by the Central Manchester Research Ethics Committee (07/H1008/168, 07/H1008/245) and the Scottish A Research Ethics Committee (08/MRE00/12).

NFBC 1966: We thank the late Professor Paula Rantakallio (launch of NFBCs), and Ms Outi Tornwall, Ms Minttu Jussila (DNA biobanking), Ms Nelli Perkiömäki (HRV analysis), the participants in the 46y study and the NFBC project center. The authors would like to acknowledge the contribution of the late Academian of Science Leena Peltonen. The DNA extractions, sample quality controls, biobank up-keeping and aliquotting was performed in the National Public Health Institute, Biomedicum Helsinki, Finland and supported financially by the Academy of Finland and Biocentrum Helsinki. NFBC1966 received financial support from the Academy of Finland (project grants 104781, 120315, 129269, 1114194, 24300796, 267435, 285547 Center of Excellence in Complex Disease Genetics and SALVE), University of Oulu, Oulu University Hospital and Biocenter, Oulu, Finland [grant numbers 24301140, 24000692, 75617], European Regional Development Fund [grant number 539/2010 A31592], NHLBI grant 5R01HL087679-02 through the STAMPEED program (1RL1MH083268-01), NIH/NIMH

(5R01MH63706:02), ENGAGE project and grant agreement HEALTH-F4-2007-201413, EU FP7 EurHEALTHAgeing -277849, the Medical Research Council, UK (G0500539, G0600705, G1002319, PrevMetSyn/SALVE), the MRC, Centenary Early Career Award, the Sigrid Juselius Foundation and the Finnish Foundation for Cardiovascular Research. The program is currently being funded by the H2020-633595 DynaHEALTH action and the Academy of Finland EGEA-project.
Written informed consent was obtained from all NFBC1966 participants. The study was approved by the Regional Ethics Committee of the Northern Ostrobothnia Hospital District, Oulu, Finland (IRB number 94/2011).

UCSD TWINS: We wish to dedicate this work to the memory Daniel T. O'Connor, whose body of work, particularly with the University of California San Diego Twins cohort, and passion for new knowledge were unprecedented. This study was supported by P30 DK079337 and U01 HL69758-01.
Written informed consent was obtained from all UCSD twin participants. The study was approved by the University of California-San Diego Institutional Review Board (IRB number 120582).

WHI: We thank all contributors to WHI science, which are listed at https://www.whi.org/researchers/Documents%20%20Write%20a%20Paper/WHI%20Investigator%20Long%20List.pdf.
The Women's Health Initiative study was approved by the human subjects review committee at each participating institution and all participants provided written informed consent.

WHI CT – GARNET: The Women's Health Initiative clinical trials were funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, U.S. Department of Health and Human Services through contracts HHSN268201100046C, HHSN268201100001C, HHSN268201100002C, HHSN268201100003C, HHSN268201100004C, and HHSN271201100004C. Within the Genomics and Randomized Trials Network, a GWAS of Hormone Treatment and CVD and Metabolic Outcomes in the WHI was funded by the National Human Genome Research Institute, National Institutes of Health, U.S. Department of Health and Human Services through cooperative agreement U01HG005152 (Reiner).
The Women's Health Initiative study was approved by the human subjects review committee at each participating institution and all participants provided written informed consent.

WHI CT – MOPMAP: The Women's Health Initiative clinical trials were funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, U.S. Department of Health and Human Services through contracts HHSN268201100046C, HHSN268201100001C, HHSN268201100002C, HHSN268201100003C, HHSN268201100004C, and HHSN271201100004C. The Modification of PM-Mediated Arrhythmogenesis in Populations was funded by the National Institute of Environmental Health Sciences, National Institutes of Health, U.S. Department of Health and Human Services through grant R01ES017794 (Whitsel).
The Women's Health Initiative study was approved by the human subjects review committee at each participating institution and all participants provided written informed consent.

WHI - SHARe: The Women's Health Initiative clinical trials were funded by the National Heart, Lung, and Blood Institute, National Institutes of Health, U.S. Department of Health and Human

Services through contracts HHSN268201100046C, HHSN268201100001C, HHSN268201100002C, HHSN268201100003C, HHSN268201100004C, and HHSN271201100004C. The SNP Health Association Resource project was funded by the National Heart, Lung and Blood Institute, National Institutes of Health, U.S. Department of Health and Human Services through contract N02HL64278 (Kooperberg).
The Women's Health Initiative study was approved by the human subjects review committee at each participating institution and all participants provided written informed consent.

WHII: The Whitehall II study is supported by grants from the Medical Research Council (G0902037), British Heart Foundation (RG/07/008/23674), Stroke Association, National Heart Lung and Blood Institute (5RO1 HL036310) and National Institute on Aging (5RO1AG13196 and 5RO1AG034454).
Ethical approval for the Whitehall II study was obtained from University College London Medical School committee on the ethics of human research (reference number 85/0938), and the London-Harrow and Scotland A Research Ethics Committees on Ethics of Human Research. All participants provided written informed consent.

Post-GWAS cohorts and consortia performing post-GWAS analyses and lookups
ABCD: We thank all participating hospitals, obstetric clinics, general practitioners and primary schools for their assistance in implementing the ABCD study. We also gratefully acknowledge all the women and children who participated in this study for their cooperation. The ABCD study has been supported by grants from The Netherlands Organisation for Health Research and Development (ZonMW) and The Netherlands Heart Foundation. Genotyping was funded by the BBMRI-NL grant CP2013-50. Dr M.H. Zafarmand was supported by BBMRI-NL (CP2013-50). Dr. T.G.M. Vrijkotte was supported by ZonMW (TOP 40–00812–98–11010).
All ABCD participants gave written informed consent for themselves and their children. Approval was obtained from the Central Ethics Committee on Research Involving Human Subjects of the Academic Medical Centre (AMC), Amsterdam and the Registration Committee of Amsterdam (IRB number: MEC 02/039#07.17.1039) .

AFGen consortium: See PMID 22544366 for contributors and funding information. Dr. Lubitz is further supported by NIH K23HL114724 and a Doris Duke Charitable Foundation Clinical Scientist Development Award 2014105.

BIOS (Biobank-based Integrative Omics Study) consortium: eQTL and mQTL results were provided by the Biobank-based Integrative Omics Study (BIOS) Consortium, which is funded by the Biobanking and Biomolecular Research Infrastructure (BBMRI-NL, NWO project 184.021.007).
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CHARGE-HF consortium: For a full list of CHARGE - Heart Failure Working Group members contributing to this work funding sources, and further acknowledgements, please see PMID 20445134.

CHARGE-SCD consortium: Foram N. Ashar, Christine M. Albert, Christopher Newton-Cheh, Jennifer A. Brody, Martina Muller-Nurasyid, Anna Moes, Angel Mak, Heikki Huikuri, M Juhani Junttila, Philippe Goyette, Sara L. Pulit, Aravinda Chakravarti, Georg Ehret, Josef Coresh, Man Li, Ronald J. Prineas, Catherine O. Johnson, Oscar H. Franco, Pui-Yan Kwok, Thomas Lumley, Florence Dumas, Barbara McKnight, Jerome I. Rotter, Rozenn N. Lemaitre, Susan Heckbert, Christopher J. O'Donnell, Shih-Jen Hwang, Jean-Claude Tardif, Marja-Leena Kortelainen, Martin VanDenBurgh, Andre G Uitterlinden, Albert Hofman, Bruno H. C. Stricker, Thomas Meitinger, Mark Eijgelsheim, John D. Rioux, Ken Rice, Xavier Jouven, Stefan Kaab, Bruce M. Psaty, David S. Siscovick, Dan E. Arking, Nona Sotoodehnia, for the SCD working group of the CHARGE Consortium.

CKDGen consortium: We thank all members of the CKDGen Consortium for the sharing of summary statistics.

Lifelines Cohort Study: Behrooz Z. Alizadeh, H. Marike Boezen, Lude Franke, Pim van der Harst, Gerjan Navis, Marianne Rots, Harold Snieder, Morris Swertz, Bruce H.R. Wolffenbuttel, Cisca Wijmenga.

OFS: We thank the people of the Taimsa, Birkat AI-Moz, Radat Al-Busaidi, Karsha, and Farq villages for their continuous encouragement and participation. We thank the Ministry of Health and the Wali (Governor) of Nizwa District and the H. M. Sultan Qaboos Strategic Research Trust Fund (Grant SR/MED/PHYS/ O4/01) for supporting this work.
The Oman Family Study (OFS) was approved by the Research and Ethics Committee of College of Medicine and Health Sciences at Sultan Qaboos University, Muscat Oman under the title "Hypertension and the metabolic syndrome in Oman intervention clinical trial and mapping of genes" (SR/MED/PHYS/01/01 and SR/MED/PHYS/04/01). All participants fully consented to the study by signing the written consent form or by thumb stamping for those who were illiterate.

N. Sotoodehnia was supported by grants R01HL116747 and R01HL111089.
M. den Hoed was supported by the Swedish research Council (2015-03657) and Swedish Heart-Lung Foundation (20140543).

S.W. van der Laan was funded through grants from the Netherlands CardioVascular Research Initiative (“GENIUS”, CVON2011-19), the Interuniversity Cardiology Institute of the Netherlands (ICIN, 09.001), and through the FP7 EU project CVgenes@target (HEALTH-F2-2013-601456)

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