

# Identification of genomic loci associated with resting heart rate and shared genetic predictors with all-cause mortality

Ruben N. Eppinga<sup>1</sup>, Yanick Hagemeijer<sup>1</sup>, Stephen Burgess<sup>2</sup>, David A. Hinds<sup>3</sup>, Kari Stefansson<sup>4</sup>, Daniel F. Gudbjartsson<sup>5</sup>, Dirk J. van Veldhuisen<sup>1</sup>, Patricia B. Munroe<sup>6,7</sup>, Niek Verweij<sup>1</sup>, Pim van der Harst<sup>1,8,9</sup>

- (1.) University of Groningen, University Medical Center Groningen, Department of Cardiology, 9700 RB Groningen, the Netherlands
- (2.) Department of Public Health and Primary Care, University of Cambridge, Cambridge CB1 8RN, United Kingdom
- (3.) 23andMe, Inc., Mountain View, California, USA.
- (4.) deCODE Genetics/Amgen, Inc., Reykjavik, Iceland, Faculty of Medicine, University of Iceland, Reykjavik, Iceland.
- (5.) deCODE Genetics/Amgen, Inc., Reykjavik, School of Engineering and Natural Sciences, University of Iceland, 101 Reykjavik, Iceland.
- (6.) Department of Clinical Pharmacology, William Harvey Research Institute, Barts and The London, Queen Mary University of London, London, EC1M 6BQ, United Kingdom
- (7.) NIHR Barts Cardiovascular Biomedical Research Unit, Queen Mary University of London, London EC1M 6BQ, United Kingdom
- (8.) University of Groningen, University Medical Center Groningen, Department of Genetics, 9700 RB Groningen, the Netherlands
- (9.) Durrer Center for Cardiogenetic Research, ICIN - Netherlands Heart Institute, 3511GC Utrecht, the Netherlands

*Corresponding author:*

Pim van der Harst

University of Groningen, University Medical Center Groningen,  
Department of Cardiology & Department of Genetics  
Hanzeplein 1, 9700RB Groningen, the Netherlands

Email: [p.van.der.harst@umcg.nl](mailto:p.van.der.harst@umcg.nl)

## ABSTRACT

Resting heart rate is a heritable trait correlated with lifespan. Little is known about the genetic contribution of resting heart rate and its relationship with mortality. We performed a genome-wide association discovery and replication analysis starting with 19.9 million genetic variants and studying up to 265,046 individuals to identify 64 loci associated with resting heart rate ( $P < 5 \times 10^{-8}$ ), 46 of these were novel. We then used the identified genetic variants as an instrument to study the association between resting heart rate and all-cause mortality. We observed that a genetically predicted resting heart rate of 5 beats per minute was associated with a 20% increased mortality risk (hazard ratio 1.20, 95% CI of 1.11-1.28,  $P = 8.20 \times 10^{-7}$ ) translating to a 2.9 years reduction in life expectancy for males and 2.6 years for females. Our findings provide novel evidence for shared genetic predictors of resting heart rate and all-cause mortality.

Among mammals, there exists an inverse semi-logarithmic relation between resting heart rate and life expectancy with only the human species deviating from this line<sup>1,2</sup>. In humans, resting heart rate is a well-established predictor of overall mortality in the general population<sup>3-8</sup>, as well as in patients with hypertension<sup>9</sup>, coronary artery disease (CAD)<sup>10</sup>, and heart failure<sup>11</sup>. The association of heart rate with life expectancy or risk does not provide sufficient evidence for a shared or causal relationship. Heart rate is regulated by complex interactions of biological systems, including the autonomous nervous and hormonal systems<sup>12</sup>. In addition, resting heart rate is associated with many other cardiovascular risk factors, including blood pressure, smoking, glucose metabolism, lipids, C-reactive protein, metabolic syndrome, body mass index, and diabetes mellitus<sup>13-16</sup>. In some conditions, including heart failure, reduction of heart rate has been directly demonstrated to lead to event reduction providing evidence that heart rate is indeed a modifiable causal risk factor and not just a risk marker or a reflection of comorbidities<sup>11</sup>. However, in patients with CAD and hypertension,  $\beta$ -adrenergic receptor-blocking agent (beta-blockers) were not associated with lower risk of cardiovascular events beyond its effect on blood pressure<sup>17,18</sup>, in patients with permanent atrial fibrillation, lenient rate control is as effective as strict rate control<sup>19</sup>, and heart rate reduction with ivabradine did not improve outcomes in patients with CAD<sup>20</sup> but in patients with heart failure it did<sup>21</sup>. A mechanistic explanation linking higher resting heart rate with increased mortality remains enigmatic. To further our knowledge on genes influencing resting heart rate we performed a genome-wide association study on 134,251 participants from UK Biobank<sup>22</sup> and replicated our findings in 130,795 additional individuals. Using the identified genetic variants as instrumental variables we explored the relationship between resting heart rate with cardiovascular risk factors, comorbidities and fatal and non-fatal outcomes. Bioinformatic analyses of associated variants were also undertaken to identify potential biological pathways and mechanisms.

We studied 134,251 individuals participating in UK Biobank. The average age was 56.6 years (interquartile range [IQR] 50 to 63), and 47.2% of the participants were male. Baseline characteristics are presented in **Table 1 and Supplementary Table 1**. The median duration of follow-up for mortality was 4.9 years (IQR 4.3 to 5.5 years) and there were 2,364 mortality events in total. Incidence rate 3.6 events (95% CI 3.4 to 3.7 events) per 1000 person-years of follow-up.

In UK Biobank we identified genetic variants at 76 loci associated with resting heart rate at  $P < 5 \times 10^{-8}$  (**Figure 1, Table 2, Supplementary Table 2, Supplementary Figures 1-3**). 64 of these loci replicated in 130,795 individuals derived from 4 cohorts and 46 loci were novel<sup>23</sup>. The genetic variants at the 64 loci were well imputed with an info  $> 0.9$  except one (rs11183443) which had an information measure of 0.30. At 11 loci we found evidence for multiple independent associations with resting heart rate in conditional analyses (**Supplementary Table 3**). As expected, the magnitudes of the associations were small and ranged from 0.2 to 1.1 bpm per effect allele. Collectively, the total variance explained by the 64 loci for resting heart rate was 2.5%.

We studied the potential modifying effect of gender, beta-blockers and calcium-channel blockers on the association of genetic variants on resting heart rate but did not observe any significant interactions (**Supplementary Table 4**).

We summed the number of resting heart rate increasing alleles weighted for the strength of the association in the replication dataset to create a weighted GRS for each individual, and evaluated associations with cardiovascular measures. Genetically determined higher resting heart rate was associated with higher body-mass index, systolic and diastolic blood pressure and higher odds of having hypertension, active smoking behavior, experiencing supraventricular tachycardias, and lower odds of device implantation (all  $P < 0.05$ ; **Table 3**). Shared heritability estimates are presented in **Supplementary Table 5** and indicate correlations of resting heart rate with body-mass index, blood pressure, hypertension, diabetes, active smoking behavior, and myocardial infarction.

In a random-effects meta-analysis of the genetic variant-specific  $\beta_3$  (the putative association between resting heart rate and outcome mediated through that variant) of all hypothesis generating loci ( $P < 1 \times 10^{-5}$ ) we observed a significant association between genetic variants associated with resting heart rate and all-cause mortality translating to a relative increase of 20% in all-cause mortality risk per 5 bpm increase of resting heart rate (**Table 4, Supplementary Figure 4**). When restricting the number of genetic variants stepwise from  $P < 1 \times 10^{-5}$  to  $P < 5 \times 10^{-8}$  the hazard ratio reduced, but remained significant (**Table 4**). Next we calculated weighted and

unweighted GRS and found similar associations with all-cause mortality (**Table 4**). The Kaplan-Meier failure curves for all-cause mortality are shown in **Supplementary Figure 5**. There was no specific cause of death driving the association (**Supplementary Table 6**). We extrapolated a relative risk of 1.20 to life expectancy using the National Life Tables of the United Kingdom and estimated a reduction of 2.9 years for males and 2.6 years for females per 5 bpm increase in resting heart rate.

A conceptual figure of the potential explanations of the observed association between genetic variants of heart rate and outcome is provided as **Supplementary Figure 6**. We performed several analyses to test for pleiotropic effects, identify confounders and mediators. First, we ruled out the possibility that extreme associations drive the genetic association with all-cause mortality by repeating the meta-analysis without the 12 genetic variants that each showed an association with mortality at  $P < 0.05$  (**Table 4**). Second, we adjusted for resting heart rate in the Cox regression model predicting all-cause mortality. The association of the genetic variants with all-cause mortality was abolished suggesting the genetic association is mediated via resting heart rate (**Table 4**). Next, we adjusted for covariates observed to be associated with identified genetic variants in UK Biobank (**Table 4**). Introducing baseline body-mass index, diastolic blood pressure, hypertension, diabetes, active smoking, history of heart failure, supraventricular tachycardias, myocardial infarction, device implantation, beta-blockers and calcium channel-blockers did not affect the association between the genetic variants for heart rate and all-cause mortality (**Table 4**). Also when we excluded all genetic variants that individually showed nominal association ( $P < 0.05$ ) with any of the significant variables in **Table 3**, the association between the genetic variants for heart rate and all-cause mortality remained significant. Next we considered potential confounders of variables not currently available in the UK Biobank cohort and performed multivariable Mendelian randomization to adjust for lipids (LDL, HDL, Total Cholesterol, Triglycerides) and red blood cell (RBC, PCV, MCV, and Hb) variables. The adjustments did not attenuate the association of the heart rate genetic variants with all-cause mortality (**Table 4**). The results of the MR-Egger method confirmed the absence of evidence for directional (unbalanced) pleiotropy (**Table 4**). Also when using genetic variant coefficients derived from the associations with resting heart rate when restricted to

healthy individuals (**Table 1**) the prediction of all-cause mortality remained similar (**Table 4**) further supporting the notion that underlying diseases or heart-rate lowering medication has not confounded our observation. Also when using genetic variant coefficients estimated in in the replication sample the association with all-cause mortality persisted. When extrapolating the estimates from our sensitivity analyses, (ranging from 1.11 to 1.29 (**Table 4**)), this would translate to a reduction in life expectancy for males between 1.9 up to 4.1 years and females 1.8 up to 3.7 years per 5 bpm increase in resting heart rate.

At 19 of our 64 loci the sentinel genetic variant or a genetic variant in LD ( $r^2 > 0.8$ ) have reported GWAS associations. These include: lipid, metabolic and blood pressure related traits (**Supplementary Table 7**). Our 64 loci were highly enriched for deoxyribonuclease I (DNase I) hypersensitive sites, marking transcriptionally active regions of the genome in human fetal heart tissue (**Figure 2a**). Enrichment testing of expression in 209 tissue and cell types identified cardiovascular tissues and the adrenal gland to be the most relevant for our association findings (**Figure 2b, Supplementary Table 8**). Across the 64 loci, 1,668 annotated genes are located within 1 Mb of all the sentinel genetic variants. Based on proximity, the presence of non-synonymous genetic variants in high linkage disequilibrium (LD), cis-expression quantitative trait loci (eQTL) and Data-driven Expression-Prioritized Integration for Complex Traits (DEPICT)<sup>24</sup> analyses we prioritized 102 potential candidate genes at our 64 loci (**Supplementary Note, Supplementary Tables 9-11**). A systematic search of our 102 candidate genes in Online Mendelian Inheritance in Man (OMIM) identified several Mendelian diseases with cardiac phenotypes. These were related to cardiomyopathies (*TTN*, *DES*, *SCN5A*, , *PLN*, *MYH6*, *MYH7*, *SPEG*), Brugada syndrome (*SCN5A*, *CACNA1C*, *HCN4*), Long QT (*SCN5A*, *KCNJ5*, *ALG10*), arrhythmias (*SCN5A*, *HCN4*, *CACNA1D*, *MYH6*) and congenital heart disease (*NKX2-5*, *PLN*, *TBX20*, *MYH6*, *MYH7*). The DEPICT tool identified 622 significantly (FDR<5%) enriched gene sets (**Supplementary Tables 12-13**). We clustered them on the basis of the correlation between scores for all genes (**Supplementary Note**) resulting in 74 gene sets relevant to cardiac biology (**Supplementary Figure 7**).

This work highlights the unprecedented opportunities provided by large scale projects such as UK Biobank, the 100,000 genomes<sup>25</sup>, and the Precision Medicine Initiative<sup>26</sup> to discover novel genetic associations and to study links with outcomes and mortality. In this GWAS and replication study, performed in 265,046 individuals, we found 46 novel genetic loci associated with resting heart rate increasing the total number of heart rate loci to 67<sup>23</sup>. Several epidemiologic studies have reported an association between higher resting heart rate and increased mortality from both cardiovascular and non-cardiovascular causes<sup>3-8</sup>. In all of these studies, this association is potentially confounded by differences in demographics and physiological characteristics such as body-mass index, smoking, alcohol consumption and blood pressure. Also data from intervention trials do not provide a consistent link between heart rate reduction and improvement of clinical outcomes. Selective sinus-node inhibition with ivabradine has beneficial effects on outcomes in patients with chronic heart failure<sup>21</sup> but did not improve outcomes in patients with CAD<sup>20</sup>.

In the present work we show that genetic variants associated with higher resting heart rate confer a risk for all-cause mortality. We studied the strength of these genetic variants with mortality and studied the role of heart rate in comparison of other, potential confounding variables closely associated with heart rate. The identified genetic variants associated with heart rate were also associated with potential measured (body mass index, systolic and diastolic blood pressure, hypertension, smoking, supraventricular tachycardia, device implantation,) and unmeasured confounders. However, also our analyses adjusting for covariates, allowing genetic variants to have pleiotropic effects, removing genetic variants associated with other traits, or using estimates derived from healthy participants and 130,795 independent participants consistently suggest that heart rate is linked to mortality, and by extension life-expectancy. Indeed, only heart rate itself attenuated the association of the genetic variants with the outcome to the null. This leaves two likely possibilities. Either the genetic variants exert their effect on mortality directly via heart rate as a mediator or, alternatively, the genetic variants share underlying biology resulting in both increased heart rate as well as increased mortality risk. While direct specific intervention (sinus node inhibition) on heart rate does not consistently result in reduction in mortality<sup>20,21,27</sup> we hypothesize the association originates from a shared biology not targeted by sinus node inhibition. This could

involve basic cellular biology behind heart rate and possibly involve vulnerability to cardiac arrhythmias causing (sudden) death which might contribute to all classifications of death and might eventually be relevant for a plethora of also non-cardiac diseases and conditions. This theory can be supported by the identification of predominant cardiac candidate genes at the identified loci and the co-localization of DNase hypersensitivity sites in cardiac tissue. However, also alternative speculations involving basic metabolic rate, energetics, free radicals, could result in cumulative general damage and affect life span<sup>28</sup>.

In addition to an interpretation of causation, there are several other limitations of our study that are important to acknowledge. Although recent studies<sup>29,30</sup> and empirical estimates on the UK10K<sup>31</sup> and 1000 Genomes project<sup>32</sup> support the use of a genome-wide significant threshold at the level of  $P < 5.0 \times 10^{-8}$ , the adequacy of this value for UK Biobank has not been fully investigated. In addition, among the loci identified, a number of candidate genes have a known function relevant for cardiac conditions however, for none of the genes have we proven it is the mechanism for the association with heart rate. Our findings are based on statistical analyses of large datasets and do not include experimental validation of each locus to identify the underlying biological mechanisms. As with all bioinformatics analyses, the results should be interpreted as hypothesis-generating and requiring wet lab validation. In addition, the candidate gene list only provides a first interpretation using arbitrarily defined guidelines used in the GWAS community to suggest genes for further evaluation. Also heart rate is a complex trait and the principal reason for genes to be associated does not necessarily imply a role via the cardiac pacemaker or sinus node function. Due to the relative short follow-up currently available and limited number of events our analyses were focused on all-cause mortality and a crude subdivision according to ICD-10 chapters. Based on gene and pathway analyses differences in death due to the ICD chapter "circulatory system" might be expected but this was not observed. The reason remains speculative but it might be due to heterogenic causes of death within each chapter and also deaths in other chapters might be influenced by the heart but not attributed to it. When more subjects are genotyped and long-term follow-up becomes available, future analyses may allow further differentiation within each ICD-10 chapter to study associations of resting heart rate with specific causes of deaths.



In conclusion, in this GWAS, we have identified 46 novel loci associated with resting heart rate. The identified loci influencing resting heart rate are also implicated in overall mortality (and consequently life expectancy) and therefore warrant further research into the underlying mechanisms.

### **Data Availability Statement**

The GWAS discovery data that support the findings of this study are available at, <http://www.cardiomics.net>.

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### **Author Contributions**

Participant recruitment, characterization and data generation: D.A.H., K.S., D.F.G., D.J.v.V., P.v.d.H., Data quality control and analysis: (UKBiobank) R.N.E., Y.H., (23andMe) D.A.H.; (deCODE) K.S., D.F.G.; (LifeLines) R.N.E., N.V., P.v.d.H.; (Prevend) R.N.E., N.V., P.v.d.H., Statistical analysis review: R.N.E., S.B., D.F.G., P.B.M., N.V., P.v.d.H., Central data analysis: R.N.E., Y.H., N.V., Supervision of the project: P.v.d.H., Draft of first version of the manuscript: R.N.E., P.v.d.H.. All authors critically reviewed and approved the final version of the manuscript.

### **Conflict of Interest**

None declared.

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## Figure legends

### **Figure 1. Genomewide $-\log_{10}$ *P*-value plot and effects for significant loci**

Genomewide  $-\log_{10}$  *P*-value plots are shown for heart rate. Genetic variants within loci reaching genomewide significance are labeled in blue when previously identified and red when novel ( $\pm 1$ Mb of lowest *P*-value). The dashed line indicates the genomewide significance threshold ( $P=5 \times 10^{-8}$ ). Candidate genes have been identified by one or multiple strategies; n=nearest; c=coding, non-synonymous variant; e=eQTL; d=DEPICT tool.

**Figure 2. Biological insights (a)** The 64 genomewide associated variants were enriched within DHSs of fetal heart tissue (N=8) specifically, suggesting that functionality of regulatory DNA elements may underlie some of the associations. **(b)** DEPICT identified statistically significant enrichment for 9 tissue annotations of which cardiovascular tissues were the most relevant for the heart rate associated loci.

**Table 1. Baseline characteristics of participants**

	All (N=134,251)	SD or percentage (%)	Healthy Individuals (N=11,405)	SD or percentage (%)
Age	56.6	8.0	53.7	7.6
Sex (Male)	63,349	47.2%	5,993	52.5%
Body-mass index	27.5	4.8	26.3	4.0
Resting heart rate	69.5	11.1	68.3	10.5
Blood pressure				
Systolic	138.0	18.6	135.5	17.7
Diastolic	82.3	10.1	81.6	9.8
Ethnicity				
Asian/ Chinese	2,478	1.8%	248	2.2%
Black	1,734	1.3%	173	1.5%
Mixed	684	0.5%	52	0.5%
White	127,919	95.3%	10,797	94.7%
Other/ undefined	1,436	1.1%	135	1.2%
Smoking current	16,708	12.4%	1,390	8.3%
Medical History				
Hypertension	38,339	28.6%	0	0%
Diabetes	7,419	5.5%	0	0%
Myocardial Infarction	3,395	2.5%	0	0%
Heart failure	720	0.5%	0	0%
Atrial fibrillation / flutter	2,048	1.5%	0	0%
Supraventricular tachycardia	425	0.3%	0	0%
Device implantation	399	0.3%	0	0%
Medication			0	0%
Beta-blockers	9,526	7.8%	0	0%
Calcium channel-blockers	9,797	8.0%	0	0%

Abbreviations; SD, Standard Deviation.

**Table 2. Results of the newly identified loci that showed association with heart rate at genome-wide significance ( $P < 5 \times 10^{-8}$ )**

Variant	Chr.	Pos. <sup>a</sup>	Alleles		EAF	Discovery (UK Biobank)			Replication			Meta-analysis			N	Candidate Genes
			Non-coded	Coded		$\beta$	SE	P	$\beta$	SE	P	$\beta$	SE	P		
rs145358377	1	6272136	G	GA	0.36	-0.29	0.04	1.69x $10^{-10}$	-0.182	0.077	1.78x $10^{-2}$	-0.259	0.039	1.94x $10^{-11}$	265,046	RNF207 <sup>nc</sup> ; ICMT <sup>n</sup>
rs272564	1	45012273	A	C	0.28	0.41	0.05	5.02x $10^{-17}$	0.271	0.058	3.30x $10^{-6}$	0.351	0.037	4.51x $10^{-21}$	265,046	RNF220 <sup>n</sup>
rs2152735	1	87893132	G	A	0.33	-0.32	0.05	6.48x $10^{-12}$	-0.291	0.056	2.06x $10^{-7}$	-0.306	0.036	7.23x $10^{-18}$	265,046	LMO4 <sup>n</sup>
rs11454451	1	217722890	C	CT	0.26	0.28	0.05	8.81x $10^{-9}$	0.216	0.059	2.45x $10^{-4}$	0.256	0.038	1.29x $10^{-11}$	265,046	GPATCH2 <sup>n</sup>
rs1260326	2	27730940	T	C	0.39	-0.29	0.04	4.54x $10^{-11}$	-0.256	0.054	1.75x $10^{-6}$	-0.275	0.034	4.29x $10^{-16}$	265,046	GCKR <sup>nc</sup>
rs12713404	2	60006705	G	T	0.38	-0.26	0.05	1.75x $10^{-8}$	-0.120	0.053	2.42x $10^{-2}$	-0.199	0.035	9.33x $10^{-9}$	265,046	BCL11A <sup>n</sup>
rs564190295	2	175547672	G	GCCGCC GCCCCC	0.15	-0.36	0.06	1.00x $10^{-8}$	-0.344	0.142	1.57x $10^{-2}$	-0.355	0.057	4.95x $10^{-10}$	197,184	WIPF1 <sup>n</sup>
rs907683	2	220299541	G	T	0.43	-0.35	0.04	1.27x $10^{-15}$	-0.296	0.061	1.10x $10^{-6}$	-0.334	0.036	1.02x $10^{-20}$	265,046	SPEG <sup>nd</sup> ; DES <sup>n</sup>
rs4608502	2	228134155	T	C	0.33	0.27	0.05	5.44x $10^{-9}$	0.221	0.055	6.02x $10^{-5}$	0.249	0.035	1.85x $10^{-12}$	265,046	COL4A3 <sup>n</sup>
rs7641050	3	48762507	T	C	0.22	0.31	0.05	2.39x $10^{-9}$	0.186	0.062	2.57x $10^{-3}$	0.257	0.040	7.15x $10^{-11}$	265,046	IP6K2 <sup>n</sup> ; DALRD3 <sup>c</sup> ; KLHDC8B <sup>ed</sup> ; P4HTM <sup>e</sup> ;

																	AMT <sup>e</sup> ; QRICH1 <sup>ed</sup>
rs3749237	3	49770032	G	A	0.32	0.33	0.05	5.18x 10 <sup>-13</sup>	0.150	0.056	7.40x 10 <sup>-3</sup>	0.258	0.035	3.09x 10 <sup>-13</sup>	265,046	IP6K1 <sup>n</sup> ; GMPPB <sup>n</sup> ; FAM212A <sup>d</sup> ; DAG1 <sup>d</sup> ; KLHDC8B <sup>ed</sup> ; LAMB2 <sup>d</sup> ; PRKAR2A <sup>d</sup> ; QRICH1 <sup>ed</sup>	
rs2358740	3	53455569	G	T	0.32	-0.26	0.05	9.24x 10 <sup>-9</sup>	-0.128	0.055	2.03x 10 <sup>-2</sup>	-0.208	0.035	3.58x 10 <sup>-9</sup>	265,046	CACNA1D <sup>n</sup>	
rs1483890	3	69410725	A	G	0.30	0.29	0.05	3.56x 10 <sup>-10</sup>	0.272	0.056	1.38x 10 <sup>-6</sup>	0.284	0.036	2.54x 10 <sup>-15</sup>	265,046	FRMD4B <sup>n</sup>	
rs11920570	3	122090102	G	A	0.26	0.37	0.05	3.91x 10 <sup>-14</sup>	0.127	0.058	2.75x 10 <sup>-2</sup>	0.268	0.037	5.18x 10 <sup>-13</sup>	265,046	CCDC58 <sup>n</sup>	
rs12501032	4	23951018	C	G	0.31	0.29	0.05	3.65x 10 <sup>-10</sup>	0.278	0.057	9.80x 10 <sup>-7</sup>	0.288	0.036	1.83x 10 <sup>-15</sup>	265,046	PPARGC1A <sup>n</sup>	
rs6845865	4	148974602	T	C	0.16	-0.38	0.06	3.16x 10 <sup>-11</sup>	-0.281	0.072	9.07x 10 <sup>-5</sup>	-0.342	0.045	2.25x 10 <sup>-14</sup>	265,046	ARHGAP10 <sup>nd</sup> ; EDNRA <sup>d</sup>	
rs13165531	5	30888583	A	T	0.42	-0.26	0.04	2.75x 10 <sup>-9</sup>	-0.166	0.053	1.65x 10 <sup>-3</sup>	-0.221	0.034	4.31x 10 <sup>-11</sup>	265,046	CDH6 <sup>n</sup>	
rs1468333 <sup>#</sup>	5	137552970	T	C	0.63	-0.27	0.04	1.23x 10 <sup>-9</sup>	-0.233	0.054	1.52x 10 <sup>-5</sup>	-0.255	0.034	9.53x 10 <sup>-14</sup>	265,046	CDC23 <sup>n</sup>	
rs236349	6	36820565	A	G	0.34	0.29	0.05	2.46x 10 <sup>-10</sup>	0.273	0.055	8.11x 10 <sup>-7</sup>	0.281	0.035	1.01x 10 <sup>-15</sup>	265,046	PPIL1 <sup>ne</sup>	
rs58437978	7	35258277	T	C	0.50	-0.27	0.04	2.26x 10 <sup>-10</sup>	-0.183	0.057	1.32x 10 <sup>-3</sup>	-0.240	0.034	2.61x 10 <sup>-12</sup>	265,046	TBX20 <sup>n</sup>	
rs41748	7	116446573	T	G	0.45	-0.24	0.04	1.90x	-0.120	0.052	2.23x	-0.193	0.033	7.14x	265,046	MET <sup>n</sup>	

rs11563648	7	126970046	G	C	0.27	-0.31	0.05	1.79x 10 <sup>-10</sup>	-0.121	0.058	3.74x 10 <sup>-2</sup>	-0.231	0.037	4.42x 10 <sup>-10</sup>	265,046	ZNF800 <sup>n</sup>
rs138186803	7	130965408	AT	A	0.41	-0.30	0.04	7.81x 10 <sup>-12</sup>	-0.550	0.107	2.70x 10 <sup>-7</sup>	-0.333	0.040	1.27x 10 <sup>-16</sup>	197,184	MKLN1 <sup>n</sup>
rs56233017	8	144981488	G	A	0.04	-0.68	0.11	8.41x 10 <sup>-11</sup>	-0.637	0.135	2.49x 10 <sup>-6</sup>	-0.666	0.083	1.09x 10 <sup>-15</sup>	265,046	PLEC <sup>n</sup>
rs10739663	9	128278739	A	G	0.45	-0.29	0.04	1.20x 10 <sup>-11</sup>	-0.229	0.052	1.05x 10 <sup>-5</sup>	-0.266	0.033	9.62x 10 <sup>-16</sup>	265,046	MAPKAP1 <sup>ne</sup>
rs12576326	11	44980383	A	G	0.34	0.27	0.05	1.40x 10 <sup>-9</sup>	0.219	0.058	1.57x 10 <sup>-4</sup>	0.253	0.036	1.20x 10 <sup>-12</sup>	265,046	TP53I11 <sup>n</sup>
rs75190942	11	128764571	C	A	0.09	-0.50	0.08	4.72x 10 <sup>-11</sup>	-0.498	0.099	4.90x 10 <sup>-7</sup>	-0.496	0.060	1.19x 10 <sup>-16</sup>	265,046	KCNJ5 <sup>nd</sup> ; C11orf45 <sup>n</sup>
rs2283274	12	2184466	G	C	0.18	-0.43	0.06	6.53x 10 <sup>-14</sup>	-0.371	0.071	1.58x 10 <sup>-7</sup>	-0.405	0.044	7.21x 10 <sup>-20</sup>	265,046	CACNA1C <sup>n</sup>
rs10841486	12	20472202	T	C	0.22	-0.30	0.05	8.65x 10 <sup>-9</sup>	-0.148	0.063	1.89x 10 <sup>-2</sup>	-0.238	0.040	2.98x 10 <sup>-9</sup>	265,046	PDE3A <sup>nd</sup>
rs1050288	12	27955296	C	T	0.34	-0.26	0.05	1.70x 10 <sup>-8</sup>	-0.142	0.057	1.36x 10 <sup>-2</sup>	-0.213	0.036	2.74x 10 <sup>-9</sup>	265,046	KLHL42 <sup>n</sup>
rs10880689 <sup>s</sup>	12	37930102	A	G	0.60	0.20	0.04	4.65x 10 <sup>-6</sup>	0.221	0.054	3.91x 10 <sup>-5</sup>	0.208	0.034	8.10x 10 <sup>-10</sup>	265,046	ALG10B <sup>n</sup>
rs867400	12	64976850	T	C	0.43	0.30	0.04	7.80x 10 <sup>-12</sup>	0.301	0.053	1.05x 10 <sup>-8</sup>	0.298	0.033	4.58x 10 <sup>-19</sup>	265,046	RASSF3 <sup>nd</sup>
rs12579753	12	82219376	C	T	0.23	-0.28	0.05	3.92x 10 <sup>-8</sup>	-0.193	0.062	1.74x 10 <sup>-3</sup>	-0.246	0.039	4.81x 10 <sup>-10</sup>	265,046	PPFIA2 <sup>ne</sup>
rs12889267	14	21542766	A	G	0.16	0.41	0.06	7.78x 10 <sup>-13</sup>	0.421	0.073	7.89x 10 <sup>-9</sup>	0.416	0.045	3.61x 10 <sup>-20</sup>	265,046	NDRG2 <sup>n</sup> ; ARHGEF40 <sup>ncd</sup> ; ZNF219 <sup>d</sup>
rs17180489	14	72885471	G	C	0.14	-0.52	0.06	3.14x	-0.370	0.132	5.01x	-0.490	0.055	9.15x	214,007	RGS6 <sup>n</sup>



rs1549118	14	78379684	C	T	0.28	0.26	0.05	4.59x 10 <sup>-17</sup>	0.113	0.057	4.80x 10 <sup>-3</sup>	0.200	0.037	4.67x 10 <sup>-19</sup>	265,046	ADCK1 <sup>n</sup>
rs4900069	14	91583373	A	C	0.37	0.25	0.04	1.55x 10 <sup>-8</sup>	0.125	0.054	2.14x 10 <sup>-2</sup>	0.200	0.034	5.38x 10 <sup>-8</sup>	265,046	C14orf159 <sup>n</sup>
rs3915499	16	15910743	G	A	0.32	0.32	0.05	5.94x 10 <sup>-12</sup>	0.284	0.056	3.72x 10 <sup>-7</sup>	0.303	0.035	1.24x 10 <sup>-17</sup>	265,046	MYH11 <sup>nd</sup>
rs7194801	16	65286870	T	C	0.43	-0.33	0.04	6.78x 10 <sup>-14</sup>	-0.240	0.052	4.49x 10 <sup>-6</sup>	-0.291	0.033	3.58x 10 <sup>-18</sup>	265,046	CDH11 <sup>n</sup>
rs79121763	17	15195279	C	T	0.09	-0.52	0.08	1.53x 10 <sup>-11</sup>	-0.376	0.110	6.59x 10 <sup>-4</sup>	-0.471	0.063	7.17x 10 <sup>-14</sup>	265,046	TEKT3 <sup>n</sup> ; PMP22 <sup>d</sup>
rs11083258	18	25766218	A	C	0.17	-0.33	0.06	7.25x 10 <sup>-9</sup>	-0.192	0.071	6.96x 10 <sup>-3</sup>	-0.276	0.045	5.51x 10 <sup>-10</sup>	265,046	CDH2 <sup>nd</sup>
rs61735998	18	34289285	G	T	0.02	-0.98	0.14	1.39x 10 <sup>-12</sup>	-0.593	0.176	7.74x 10 <sup>-4</sup>	-0.834	0.109	2.06x 10 <sup>-14</sup>	265,046	FHOD3 <sup>ncd</sup>
rs16974196	19	40833470	G	A	0.32	0.26	0.05	1.36x 10 <sup>-8</sup>	0.217	0.057	1.55x 10 <sup>-4</sup>	0.244	0.036	1.11x 10 <sup>-11</sup>	265,046	C19orf47 <sup>nd</sup> ; MAP3K10 <sup>e</sup>
rs12721051	19	45422160	C	G	0.18	-0.32	0.06	1.40x 10 <sup>-8</sup>	-0.241	0.071	6.45x 10 <sup>-4</sup>	-0.287	0.044	5.23x 10 <sup>-11</sup>	265,046	APOE <sup>n</sup> ; APOC1 <sup>n</sup> ; PVRL2 <sup>d</sup>
rs17265513	20	39832628	T	C	0.19	0.30	0.05	2.36x 10 <sup>-8</sup>	0.146	0.066	2.78x 10 <sup>-2</sup>	0.240	0.042	1.12x 10 <sup>-8</sup>	265,046	ZHX3 <sup>nc</sup> ; EMILIN3 <sup>d</sup>
rs2076028	22	39150450	G	A	0.29	-0.36	0.05	1.81x 10 <sup>-14</sup>	-0.197	0.057	5.49x 10 <sup>-4</sup>	-0.295	0.036	5.45x 10 <sup>-16</sup>	265,046	SUN2 <sup>n</sup> ; CBY1 <sup>e</sup> ; FAM227A <sup>e</sup> ; JOSD1 <sup>e</sup> ; TOMM22 <sup>e</sup> ; DDX17 <sup>d</sup> ; GTPBP1 <sup>d</sup>

Abbreviations; EAF, Effect Allele Frequency; Chr, Chromosome; Pos, Position;  $\beta$ , Beta; SE, Standard Error.  
<sup>a</sup>Positions are according to 1000 Genomes Phase 3, and allele coding is based on the positive strand.  
Candidate genes have been identified by one or multiple strategies; n=nearest; c=coding, non-synonymous variant; e=eQTL; d=DEPICT tool. #Proxy of rs35284930,  $R^2=0.85$ . \$Proxy of rs11183443,  $R^2=0.92$ .

**Table 3. Association between genetically determined heart rate and cardiovascular profile using a weighted GRS**

	Participants (N= 134,251)	Percentage (%)	Estimated Association*	95% CI	P value
Body-mass index	134,251	100.0%	0.14	0.08 to 0.20	2.24×10 <sup>-6</sup>
Blood pressure					
Systolic	134,217	99.0%	-0.51	-0.30 to -0.72	2.55×10 <sup>-6</sup>
Diastolic	134,217	99.0%	0.78	0.66 to 0.90	1.32×10 <sup>-36</sup>
Hypertension	39,996	29.8%	1.04	1.01 to 1.07	4.41×10 <sup>-3</sup>
Diabetes	7,857	5.9%	1.04	0.99 to 1.09	0.16
Smoking current	16,708	12.4%	1.07	1.03 to 1.11	2.98×10 <sup>-4</sup>
Myocardial Infarction	3,848	2.9%	0.99	0.92 to 1.07	0.80
Heart failure	1,131	0.8%	1.14	0.99 to 1.31	0.06
Atrial fibrillation / flutter	2,780	2.1%	1.01	0.93 to 1.10	0.79
Supraventricular tachycardia	546	0.4%	1.28	1.05 to 1.56	0.02
Device implantation	482	0.4%	0.80	0.66 to 0.96	0.02
Medication					
Beta-blockers	9,526	7.8%	1.04	0.99 to 1.09	0.10
Calcium channel-blockers	9,797	8.0%	1.02	0.98 to 1.07	0.34

\* The effect estimates with 95% Confidence Interval (CI) estimated using weighted GRS (per 5 bpm increase in resting heart rate) are shown as odds ratios for categorical variables (hypertension, diabetes, smoking current, myocardial infarction, heart failure, atrial fibrillation / flutter, supraventricular tachycardia, device implantation, beta-blockers and calcium-channel blockers) and  $\beta$  estimates for quantitative variables (body-mass index, systolic and diastolic blood pressure).

**Table 4. Association between genetically determined resting heart rate and all-cause mortality**

Association with mortality	Number of GVs	Estimated Association HR*	95% CI	P value
Standard MR with all				
GVs ( $P < 10^{-2}$ )	1980	1.19	1.14 to 1.23	$3.77 \times 10^{-19}$
GVs ( $P < 10^{-3}$ )	1739	1.19	1.14 to 1.23	$5.91 \times 10^{-19}$
GVs ( $P < 10^{-4}$ )	848	1.19	1.13 to 1.24	$1.13 \times 10^{-11}$
GVs ( $P < 10^{-5}$ )	272	1.20	1.11 to 1.28	$8.20 \times 10^{-7}$
GVs ( $P < 10^{-6}$ )	121	1.14	1.05 to 1.25	$3.33 \times 10^{-3}$
GVs ( $P < 10^{-7}$ )	82	1.13	1.02 to 1.25	$1.46 \times 10^{-2}$
GVs ( $P < 5 \times 10^{-8}$ )	76	1.11	1.00 to 1.22	$5.01 \times 10^{-2}$
GVs ( $P < 10^{-5}$ ) excluding those associated ( $P < 0.05$ ) mortality	260	1.15	1.07 to 1.24	$1.53 \times 10^{-4}$
GVs ( $P < 10^{-5}$ ) with adj. for resting heart rate	272	1.02	0.95 to 1.09	0.65
GVs ( $P < 10^{-5}$ ) with adj. for covariates <sup>#</sup>	272	1.18	1.10 to 1.27	$4.69 \times 10^{-6}$
GVs ( $P < 10^{-5}$ ) excluding those associated ( $P < 0.05$ ) with variable <sup>#</sup>	55	1.29	1.09 to 1.53	$3.66 \times 10^{-3}$
GVs ( $P < 10^{-5}$ ) betas estimated on 11,405 healthy individuals	272	1.14	1.07 to 1.23	$6.85 \times 10^{-5}$
GVs ( $P < 10^{-5}$ ) betas estimated on 130,795 individuals from replication	269	1.11	1.01 to 1.22	$2.70 \times 10^{-2}$
GRS weighted GVs ( $P < 10^{-5}$ )	272	1.18	1.10 to 1.26	$3.22 \times 10^{-6}$
GRS unweighted GVs ( $P < 10^{-5}$ )	272	1.05	1.03 to 1.08	$4.37 \times 10^{-5}$
Multivariable MR with adj. for covariates <sup>#</sup>	272	1.26	1.13 to 1.42	$8.03 \times 10^{-5}$
Multivariable MR with adj. for lipid covariates <sup>\$</sup>	209	1.18	1.09 to 1.27	$1.99 \times 10^{-5}$
Multivariable MR with adj. for red blood cell covariates <sup>@</sup>	173	1.18	1.09 to 1.28	$4.53 \times 10^{-5}$

MR-Egger method ( $P < 10^{-5}$ )	272	1.21	1.05 to 1.40	$8.00 \times 10^{-3}$
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\*Hazard ratio (HR) with 95% Confidence Interval (CI) estimated with standard Mendelian Randomization (MR) and weighted Genetic Risk Score (GRS) per 5 bpm and for unweighted GRS per 5 summed risk alleles; Genetic Variants (GVs); Adjustment (adj.); #Baseline body-mass index, systolic and diastolic blood pressure, hypertension, diabetes, active smoking, and a history of myocardial infarction, heart failure, atrial fibrillation / flutter, supraventricular tachycardias, myocardial infarction, device implantation, beta-blockers and calcium channel-blockers; §Lipid covariates including; Low Density Lipoprotein (LDL), High Density Lipoproteins (HDL) Total Cholesterol and Triglycerides; @Red blood cell covariates including; Red Blood Cell Count (RBC), Packed Cell Volume (PCV), Mean Corpuscular Volume (MCV) and Hemoglobin count (Hb).

## Online Methods

### Populations.

*Discovery:* To identify genetic variants associated with resting heart rate we analyzed 134,251 participants from the UK Biobank. The UK Biobank recruited persons aged 40 - 69 years who were registered with a general medical practitioner within the UK National Health Service (NHS). In total, the study recruited 503,325 individuals between 2006 and 2010. The study has approval from the North West Multi-centre Research Ethics Committee, and all participants provided informed consent. Detailed methods used by UK Biobank have been described elsewhere<sup>22</sup>. For sensitivity analyses we defined a subgroup of healthy individuals which were free of any (prevalent or incident) disease(s) and diagnosis and confirmed they were not using heart rate modifying medication (beta-blockers, and calcium-channel blockers drugs (N=11,405)).

*Replication:* Replication of genome wide significant lead SNPs was undertaken in the meta-analysed data of 130,795 individuals derived from 23andMe, deCODE, PREVEND and LifeLines sample collections (**Supplementary Table 14**).

### Ascertainment of resting heart rate.

As detailed in the **Supplementary Note**, resting heart rate in UK Biobank was assessed by two methods: an automated reading during blood pressure measurement (in 501,340 participants) and during arterial stiffness measurement using the pulse waveform obtained of the finger with an infrared sensor (in 170,790 participants). Multiple available measurements for one individual were averaged.

### Ascertainment of cardiovascular events and mortality.

The prevalence and incidence of cardiovascular risk factors (**Supplementary Table 15**), conditions and events in UK Biobank were captured through data collected at the Assessment Centre in-patient Health Episode Statistics (HES) as detailed in the **Supplementary Note**. Information on the cause of death was obtained via the National Health Service (NHS) Information Centre for participants from England and Wales, and from the NHS Central Register, Scotland for participants from Scotland. All-cause

mortality included all deaths occurring before February 17<sup>th</sup> 2014 (or December 31<sup>st</sup> 2012, for the participants enrolled in Scotland).

### **Genotyping and Imputation.**

Genotype imputation data in UK Biobank was available for 152,249 (25%) individuals as of May 2015 [Interim Data Release]. In 49,923 individuals genotyping was performed as part of the UK Biobank Lung Exome Variant Evaluation (UK BiLEVE; 807,411 variants) project and in an additional 102,326 individuals genotyping was performed on the UK Biobank Axiom array (Affymetrix; 820,967 variants). Imputed genotype data was provided by UK Biobank based on merged UK10K and 1000 Genomes Phase 3 panel produced by the Wellcome Trust Centre for Human Genetics resulting in 72,355,667 single nucleotide polymorphisms, short indels and large structural variants. Quality control for genotyping has been performed prior to analysis and described in detail elsewhere<sup>33</sup>. We excluded variants with minor allele frequency of <0.001, and information measure <0.3 leaving 19,941,912 variants for the current analyses. Samples were excluded from our analyses if they had at least one related sample (N=17,308) based on genetic relatedness factor data, and high missingness or excess heterozygosity (N=480). A flow diagram of samples sizes after exclusion of participants is provided in **Supplementary Figure 8**.

### **Statistical Analysis.**

A genome-wide association study (GWAS) was performed using SNPTTEST with 19,941,912 genotyped or imputed genetic variants and resting heart rate in 134,251 individuals of UK Biobank using linear regression assuming an additive genetic model. Covariates included in the model were: age, age<sup>2</sup>, sex, the first 10 principal components, and genotyping array. Independent genetic loci were defined as 1Mb at either side of the genetic variant that showed the strongest association in a given locus and pair-wise LD  $r^2 < 0.1$ . The strongest associated variant (lowest *P*-value) within a locus with at least one genetic variant at  $P < 5 \times 10^{-8}$  was designated the sentinel genetic variant. Replication of these variants was undertaken in the 23andMe, deCODE, Prevend and LifeLines cohorts using fixed-effects meta-analysis by inverse variance weighting (**Supplementary Table 14**). An association was considered replicated if (1)

the direction of effect was concordant, (2) the replication- $P < 0.025$  (one-way), and (3) meta- $P < 5 \times 10^{-8}$ . For detecting secondary associations not explained by the sentinel genetic variant at each locus, we repeated the GWAS while including all sentinel genetic variants ( $P < 5 \times 10^{-8}$ ) as covariates in a conditional analysis. Potential modifier effects of gender,  $\beta$ -adrenergic receptor-blocking agent (beta-blockers), and calcium-channels blockers drugs on resting heart rate were assessed by an interaction test (Bonferroni adjusted for the number ( $n$ ) of tests ( $P < 0.05/n$ )).

We used genetic variants as instrumental variables to study the relationship of resting heart rate with outcomes (Mendelian Randomization). To this end we defined a larger set of independent loci at the previously specified hypothesis-generating threshold ( $P < 1 \times 10^{-5}$ ) in order to increase power<sup>34,35</sup>. For our main analysis we calculated  $\beta_3$  values (the putative association between resting heart rate (per 5 beats per minute (bpm) and outcome mediated through that variant) from the direct measurements of  $\beta_1$  (the effect size of the association between the variant and resting heart rate) and  $\beta_2$  (the effect size of the association between the variant and outcome), as described previously<sup>36</sup>. The value of  $\beta_3$  can be interpreted as the hazard ratio for outcome per 5 bpm increase in genetically determined resting heart rate. Inverse-variance-weighted random-effects meta-analysis was used to combine individual  $\beta_3$  estimates providing additional power to assess the overall association between genetically determined resting heart rate and mortality. Cochran's Q statistic was used to assess heterogeneity among  $\beta_3$  estimates. We also created a weighted genetic risk score (GRS) by first multiplying for each individual the effect size of the association between the variant and resting heart rate ( $\beta_1$ ) with the number of alleles 0-2 of each genetic variant and then summing all products. An unweighted GRS was created by summing the number of resting heart rate-increasing alleles 0-2 of each associated genetic variant.

To examine the robustness of our findings as well as the possibility of pleiotropic or other confounding and mediation effects we included covariates and the phenotype resting heart rate into the Cox regression models. We excluded all genetic variants that were also individually nominally associated ( $P < 0.05$ ) with covariates, and performed multivariable Mendelian randomization<sup>37</sup> to account for variables not available in UK



Biobank, and used the MR-Egger regression method to test for evidence of pleiotropy<sup>38</sup> (details provided in **Supplementary Note and Supplementary Figures 9-10**). As an alternative strategy to exclude confounding due to prevalent disease or medication use, we estimated the associations of each genetic variant with resting heart rate ( $\beta_1$ ) in the subgroup of 11,405 healthy individuals (defined above) to calculate the hazard ratio for outcome. We estimated the impact on life expectancy using the National Life Tables of the United Kingdom provided by the Office of National Statistics (ONS; [www.ons.gov.uk](http://www.ons.gov.uk)) of 2011-2013 separately for males and females (**Supplementary Note**).

Details of analyses performed to gain insights in the biological pathways and tissues underlying the genome-wide significant loci are provided in the **Supplementary Note**.

## Methods-only References

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