Figures

Figure 1. Study Flowchart showing the study design, in silico annotations and function analyses.
A) Schematic overview of the study design for the discovery and replication of genetic loci associated with resting heart rate (RHR). The black bordered boxes show the methodology, the red bordered boxes show the most important results. B) Analyses performed to evaluate RHR variants and to gain further insights in the underlying biology. C) Schematic presentation of the MR analyses of RHR on mortality and cardiovascular diseases. Effect sizes were taken from the IC-RHR data to test the associations with mortality and cardiovascular diseases in the UK Biobank. Effect sizes were taken from the UK Biobank to test the association with coronary artery disease and myocardial infarction in the CARDIoGRAMplusC4D cohort, atrial fibrillation in the AFGen cohort and any, ischemic, cardio-embolic, large artery and small vessel stroke within the MEGASTROKE consortium. BMI = body mass index, GWAS = genome-wide association study, HRC = Haplotype Reference Panel, IC-RHR = International Consortium for Resting Heart Rate, MB = megabase, N = sample size, Neff = Effective sample size, PC = principal components, RHR = resting heart rate, SNPs = single nucleotide polymorphisms, QC = quality control, 1000G = 1000 Genomes.

Figure 2. Overview of the findings in the genome-wide association study and *in silico* search of candidate causal genes.
A) Manhattan plot showing the -$\log_{10}$(*P*-value) for the association of all genotyped or imputed genetics variants with resting heart rate (RHR). Red indicates novel and internally replicated RHR associated loci and black indicates novel but unreplicated RHR associated loci. Dark grey indicates RHR associated genetic variants within 1 MB of previously identified RHR associated loci, which were internally replicated in the current study. Light grey indicates RHR associated genetic variants within 1 MB of previously identified RHR associated loci, which were not internally replicated in the current study. B) Venn diagram of the 352 identified loci. Of the 352 loci, 332 were internally replicated. C) Quantile-quantile (QQ) plot of the final meta-analysis. The black dots represent the observed statistic for the genotyped genetic variants against the corresponding expected statistic. The linkage disequilibrium score regression intercept after the final meta-analysis was 1.051, suggesting little evidence of genomic inflation due to non-polygenic signal. D) Venn diagram of the prioritization of the 670 unique candidate causal genes as identified by one or multiple strategies. Venn plot shows overlap of genes tagged by one or multiple strategies, including 1) by proximity, the nearest gene or any gene within 10 kb; 2) genes containing coding variants in LD with RHR associated variants at R^2>0.8; 3) eQTL genes in LD (R^2 >0.8) with RHR associated variants; and 4) DEPICT gene mapping using variants that achieved $P<1 \times 10^{-8}$. DEPICT = Data-driven Expression Prioritized Integration for Complex Traits, eQTL = expression quantitative trait loci.

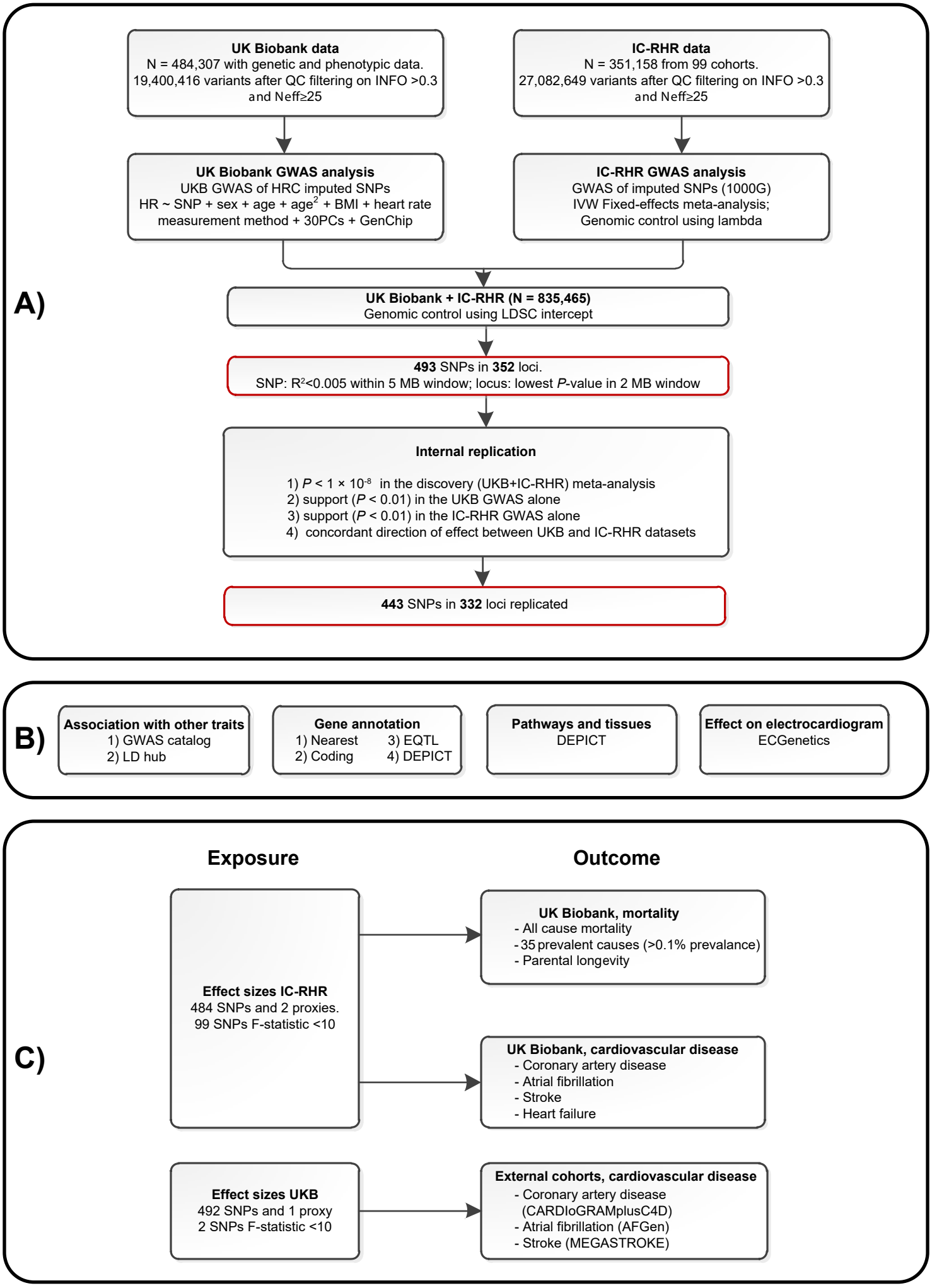
Figure 3. Conditional analyses of tissue enrichment by DEPICT emphasizes cardiac tissue for RHR biology.
A) Shows the results of the depict tissue enrichment analysis. The Y-axis shows the tissues clustered by first MeSH term, ordered on Z value per cluster. The X-axis shows the Z-value. An FDR <0.05, corresponding to a *P*-value < 9.75×10^{-3} and Z-value of 2.585 was considered to be statistically significant. Significant tissues are plotted in red and annotated, other tissues are plotted in grey. Conditional analyses were performed by correcting for the tissue with the highest Z value to investigate whether significant tissues were independently associated with RHR. Not a single tissue remained significant at a FDR < 0.05 after three consecutive corrections (for heart, heart valve and arteries). Panel B), C) and D) show Z values of all tissues after consecutive correction for respectively heart and heart valves, heart and arteries and heart valve and arteries and jointly provide information on which the other tissues co-dependent.

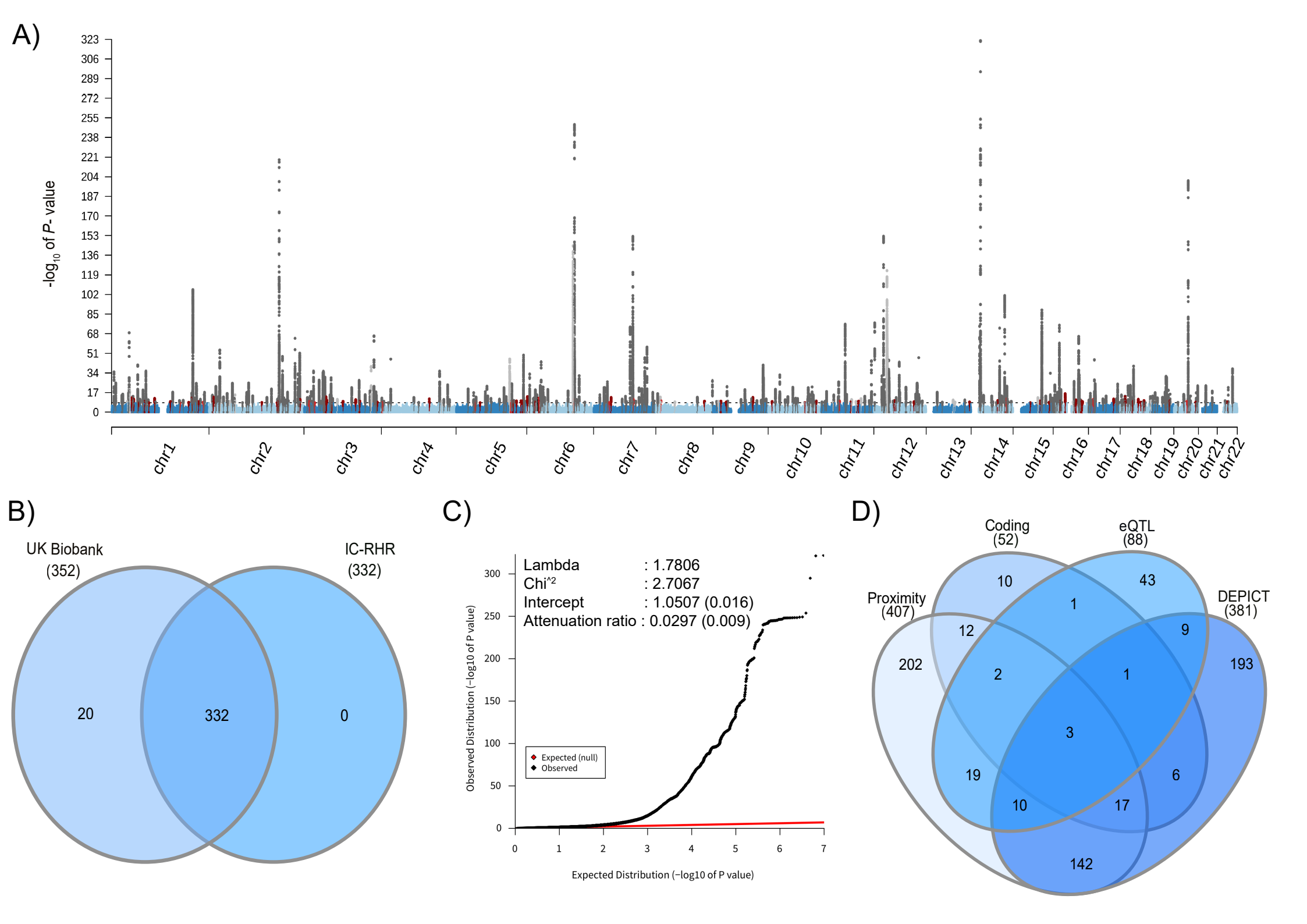
Figure 4. Mendelian randomization shows absence of linear and non-linear associations between genetically predicted RHR and all-cause mortality.
Linear and non-linear Mendelian randomization analyses were performed to test the association between genetically predicted RHR and all-cause mortality. Panel A) shows a forestplot of the linear

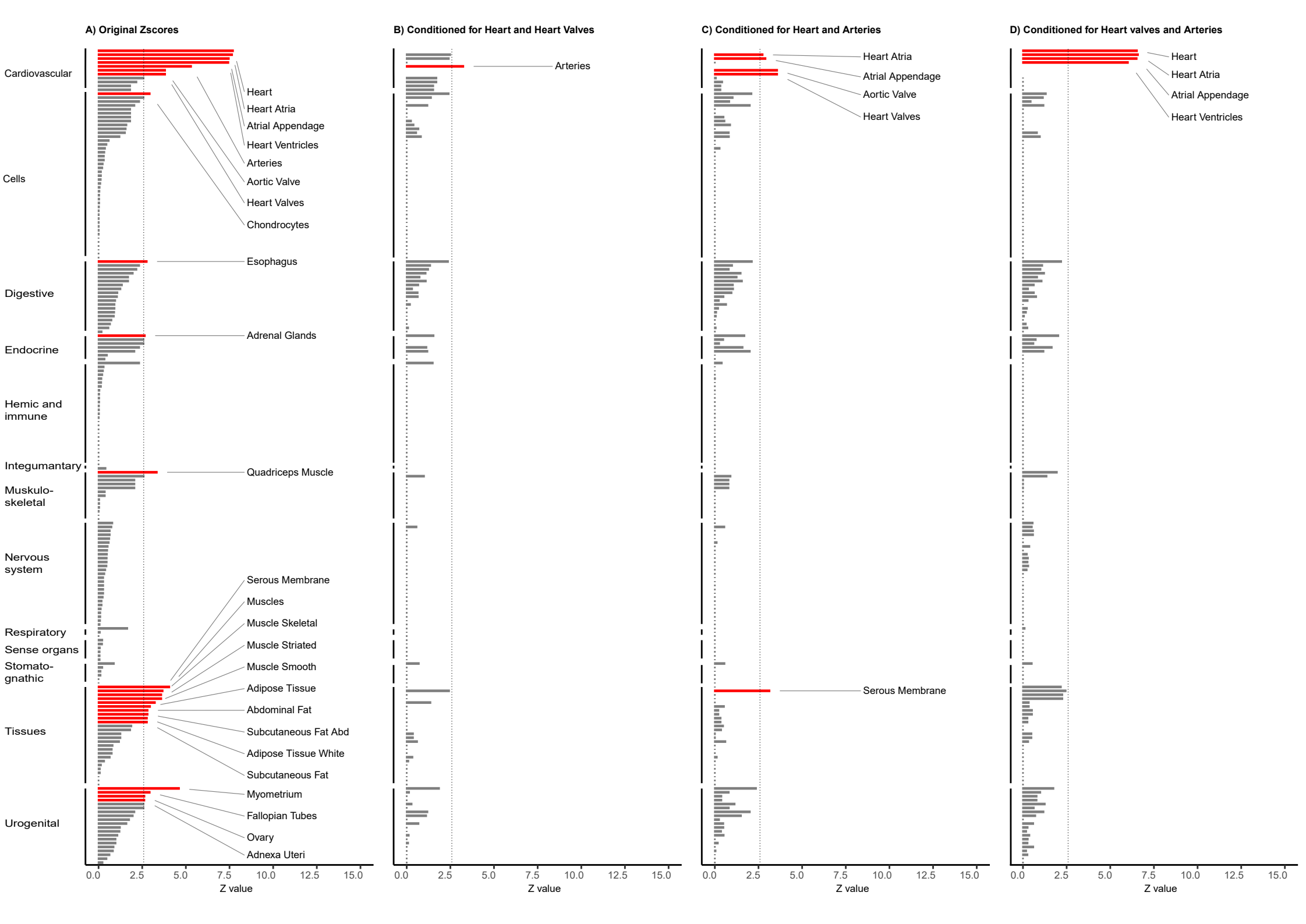
MR analyses between genetically predicted RHR and all-cause mortality. Hazard ratios and 95% confidence intervals are shown. Panel B) shows the dose-response curve of the non-linear MR analyses between genetically predicted RHR and all-cause mortality. The comparisons are conducted within strata and therefore the graph provides information on the expected average change in the outcome if a person with a RHR of (say) 70 bpm instead had a RHR value of 90 bpm. The gradient at each point of the curve is the localized average causal effect. Shaded areas represent 95% confidence intervals. RHR = resting heart rate; HR = hazard ratio; CI = Confidence interval; MR = Mendelian randomization; IVW = inverse variance weighted; FE = Fixed effects; MRE = multiplicative random effects.

Figure 5. Mendelian randomization of genetically predicted RHR on cardiovascular diseases.
Forestplots of the linear Mendelian randomization analyses of resting heart rate (RHR) on cardiovascular diseases. Effect sizes were taken from the IC-RHR data to test the associations with mortality and cardiovascular diseases in the UK Biobank (panel A). Effect sizes were taken from the UK Biobank to test the association with cardiovascular diseases in in the CARDIoGRAMplusC4D, AFGen and MEGASTROKE consortia (panel B). Results of the MR-IVW, outlier-robust MR-Lasso and plurality valid MR-Mix are provided. Odds ratios and 95% confidence intervals are shown. RHR = resting heart rate; MR = Mendelian randomization; IVW = inverse variance weighted multiplicative random effects; OR = odds ratio; CI = Confidence interval.

Figure 6. Multivariable Mendelian randomization reveals pulse pressure and atrial fibrillation as potential mediators of the association of genetically predicted RHR with ischemic and cardio-embolic stroke, respectively.
Forestplots of the results of the two-sample multivariable Mendelian randomization analyses of resting heart rate on any, ischemic and cardio-embolic stroke, when using atrial fibrillation, systolic, diastolic and pulse pressure as secondary exposures. Shown in red are the univariable Mendelian randomization estimates which represent the total estimates of resting heart rate on the outcome. In black are the multivariable Mendelian randomization estimates, which show the direct effect of RHR when corrected for the secondary exposure. These results indicate that atrial fibrillation attenuates the beneficial effect of higher resting heart rate on cardio-embolic stroke, while pulse pressure attenuates the beneficial effect on any and ischemic stroke. MR-Steiger sensitivity analysis indicated that the association between the RHR associated genetic variants and pulse pressure is unlikely mediated through RHR entirely and biological pleiotropic effects are therefore more likely to cause the attenuation of the association between RHR and stroke when correcting for pulse pressure. Odds ratios and 95% confidence intervals are shown. RHR = resting heart rate; MV = multivariable, Nsnp = number of SNPs.

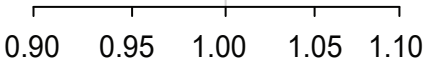

A)
UK Biobank data
N = 484,307 with genetic and phenotypic data.
19,400,416 variants after QC filtering on INFO >0.3 and Neff≥25
IC-RHR data
N = 351,158 from 99 cohorts.
27,082,649 variants after QC filtering on INFO >0.3 and Neff≥25
UK Biobank GWAS analysis
UKB GWAS of HRC imputed SNPs
HR ~ SNP + sex + age + age2 + BMI + heart rate measurement method + 30PCs + GenChip
IC-RHR GWAS analysis
GWAS of imputed SNPs (1000G)
IVW Fixed-effects meta-analysis;
Genomic control using lambda
UK Biobank + IC-RHR (N = 835,465)
Genomic control using LDSC intercept
493 SNPs in 352 loci.
SNP: R2<0.005 within 5 MB window; locus: lowest P-value in 2 MB window
Internal replication
1) P < 1 × 10-8 in the discovery (UKB+IC-RHR) meta-analysis
2) support (P < 0.01) in the UKB GWAS alone
3) support (P < 0.01) in the IC-RHR GWAS alone
4) concordant direction of effect between UKB and IC-RHR datasets
443 SNPs in 332 loci replicated
B)
Association with other traits
1) GWAS catalog
2) LD hub
Gene annotation
1) Nearest
2) Coding
3) EQTL
4) DEPICT
Pathways and tissues
DEPICT
Effect on electrocardiogram
ECGenetics
C)
Exposure
Outcome
Effect sizes IC-RHR
484 SNPs and 2 proxies.
99 SNPs F-statistic <10
UK Biobank, mortality
- All cause mortality
- 35 prevalent causes (>0.1% prevalance)
- Parental longevity
UK Biobank, cardiovascular disease
- Coronary artery disease
- Atrial fibrillation
- Stroke
- Heart failure
Effect sizes UKB
492 SNPs and 1 proxy
2 SNPs F-statistic <10
External cohorts, cardiovascular disease
- Coronary artery disease (CARDIoGRAMplusC4D)
- Atrial fibrillation (AFGen)
- Stroke (MEGASTROKE)


A)
-log10 of P- value
323
306
289
272
255
238
221
204
187
170
153
136
119
102
85
68
51
34
17
0
chr1
chr2
chr3
chr4
chr5
chr6
chr7
chr8
chr9
chr10
chr11
chr12
chr13
chr14
chr15
chr16
chr17
chr18
chr19
chr20
chr21
chr22
B)
UK Biobank
(352)
IC-RHR
(332)
20
332
0
C)
Lambda : 1.7806
Chi^2 : 2.7067
Intercept : 1.0507 (0.016)
Attenuation ratio : 0.0297 (0.009)
Observed Distribution (−log10 of P value)
Expected Distribution (−log10 of P value)
Expected (null)
Observed
0
50
100
150
200
250
300
0
1
2
3
4
5
6
7
D)
Coding
(52)
eQTL
(88)
Proximity
(407)
DEPICT
(381)
10
43
1
12
9
202
2
1
193
3
19
6
10
17
142

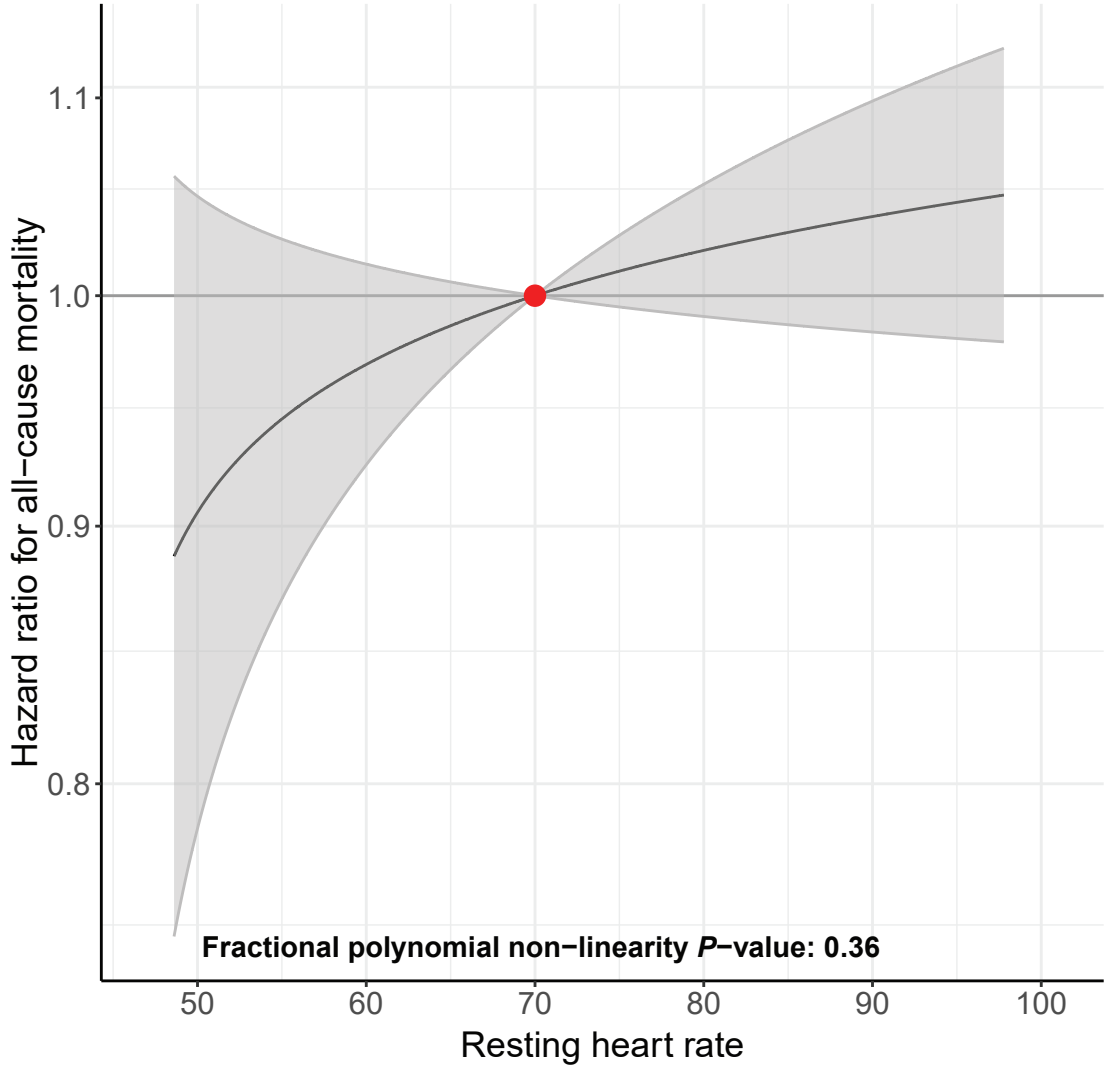

A) Original Zscores
B) Conditioned for Heart and Heart Valves
C) Conditioned for Heart and Arteries
D) Conditioned for Heart valves and Arteries
Cardiovascular
Cells
Digestive
Endocrine
Hemic and immune
Integumentary
Muskulo-skeletal
Nervous system
Respiratory
Sense organs
Stomato-gnathic
Tissues
Urogenital
Heart
Heart Atria
Atrial Appendage
Heart Ventricles
Arteries
Aortic Valve
Heart Valves
Chondrocytes
Esophagus
Adrenal Glands
Quadriceps Muscle
Serous Membrane
Muscles
Muscle Skeletal
Muscle Striated
Muscle Smooth
Adipose Tissue
Abdominal Fat
Subcutaneous Fat Abd
Adipose Tissue White
Subcutaneous Fat
Myometrium
Fallopian Tubes
Ovary
Adnexa Uteri
Arteries
Heart Atria
Atrial Appendage
Aortic Valve
Heart Valves
Serous Membrane
Heart
Heart Atria
Atrial Appendage
Heart Ventricles
0.0
2.5
5.0
7.5
10.0
12.5
15.0
Z value

A) Forestplot of the linear MR between RHR and all−cause mortality

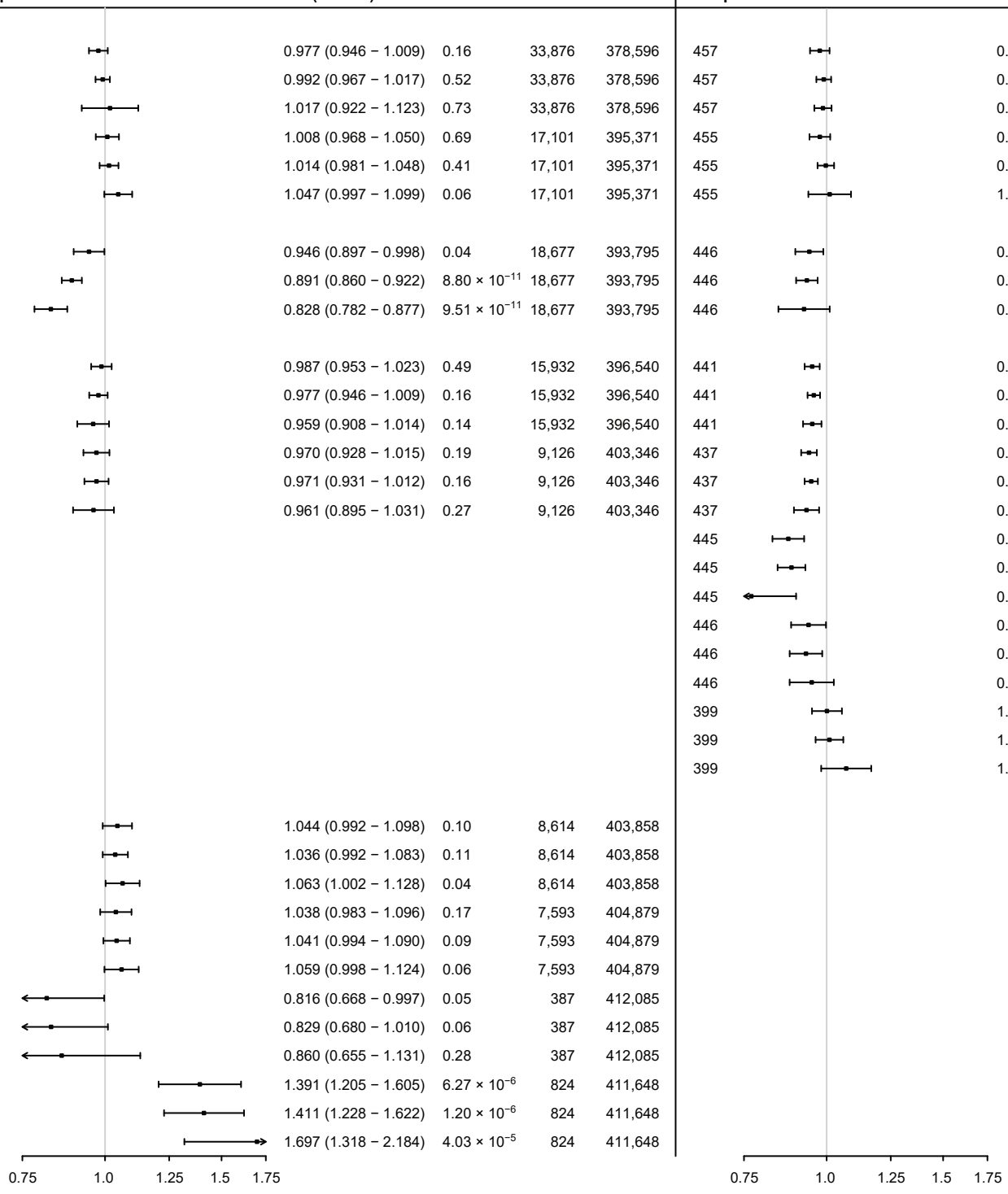
Method	Nsnp	HR (95% CI)	*P*−value
MR IVW−FE	472	1.024 (0.994 − 1.055)	0.12
MR IVW−MRE	472	1.024 (0.993 − 1.057)	0.13
MR Egger	472	1.019 (0.960 − 1.082)	0.54
Weighted median	472	1.042 (0.992 − 1.094)	0.10
MR Lasso	472	1.023 (0.992 − 1.055)	0.15
MR contamination mixture model	472	1.033 (0.987 − 1.081)	0.16



B) Dose response curve of the non−linear MR between RHR and all−cause mortality



		UK Biobank as outcome cohort					CARDIoGRAMplusC4D, AFGen or MEGASTROKE as outcome cohorts				
Outcome	**Method**	**Nsnp**	**OR (95% CI)**	***P*-value**	**Ncases**	**Ncontrols**	**Nsnp**	**OR (95% CI)**	***P*-value**	**Ncases**	**Ncontrols**
Ischemic heart disease											
Coronary artery disease	MR IVW−MRE	470	0.977 (0.946 − 1.009)	0.16	33,876	378,596	457	0.976 (0.944 − 1.010)	0.17	60,801	123,504
	MR Lasso	470	0.992 (0.967 − 1.017)	0.52	33,876	378,596	457	0.990 (0.965 − 1.016)	0.44	60,801	123,504
	MR conmix	470	1.017 (0.922 − 1.123)	0.73	33,876	378,596	457	0.987 (0.958 − 1.017)	0.40	60,801	123,504
Myocardial infarction	MR IVW−MRE	470	1.008 (0.968 − 1.050)	0.69	17,101	395,371	455	0.977 (0.942 − 1.013)	0.20	43,676	128,199
	MR Lasso	470	1.014 (0.981 − 1.048)	0.41	17,101	395,371	455	0.997 (0.969 − 1.026)	0.85	43,676	128,199
	MR conmix	470	1.047 (0.997 − 1.099)	0.06	17,101	395,371	455	1.011 (0.938 − 1.089)	0.78	43,676	128,199
Rythm disorders											
Atrial fibrillation	MR IVW−MRE	471	0.946 (0.897 − 0.998)	0.04	18,677	393,795	446	0.942 (0.897 − 0.989)	0.02	17,931	115,142
	MR Lasso	471	0.891 (0.860 − 0.922)	8.80×10^{-11}	18,677	393,795	446	0.934 (0.899 − 0.969)	3.15×10^{-04}	17,931	115,142
	MR conmix	471	0.828 (0.782 − 0.877)	9.51×10^{-11}	18,677	393,795	446	0.924 (0.845 − 1.011)	0.08	17,931	115,142
Stroke and subtypes											
Any stroke	MR IVW−MRE	472	0.987 (0.953 − 1.023)	0.49	15,932	396,540	441	0.951 (0.926 − 0.976)	1.59×10^{-4}	67,162	454,450
	MR Lasso	472	0.977 (0.946 − 1.009)	0.16	15,932	396,540	441	0.956 (0.936 − 0.977)	5.68×10^{-5}	67,162	454,450
	MR conmix	472	0.959 (0.908 − 1.014)	0.14	15,932	396,540	441	0.951 (0.921 − 0.982)	2.22×10^{-3}	67,162	454,450
Ischemic stroke	MR IVW−MRE	472	0.970 (0.928 − 1.015)	0.19	9,126	403,346	437	0.940 (0.915 − 0.967)	1.08×10^{-5}	60,341	454,450
	MR Lasso	472	0.971 (0.931 − 1.012)	0.16	9,126	403,346	437	0.948 (0.926 − 0.970)	5.28×10^{-6}	60,341	454,450
	MR conmix	472	0.961 (0.895 − 1.031)	0.27	9,126	403,346	437	0.932 (0.892 − 0.974)	1.72×10^{-3}	60,341	454,450
Cardio−embolic stroke	MR IVW−MRE						445	0.875 (0.828 − 0.925)	2.11×10^{-6}	9,006	403,807
	MR Lasso						445	0.885 (0.843 − 0.929)	6.86×10^{-7}	9,006	403,807
	MR conmix						445	0.769 (0.658 − 0.899)	9.79×10^{-4}	9,006	403,807
Large artery stroke	MR IVW−MRE						446	0.939 (0.884 − 0.998)	0.04	6,688	345,629
	MR Lasso						446	0.931 (0.879 − 0.985)	0.01	6,688	345,629
	MR conmix						446	0.949 (0.879 − 1.025)	0.19	6,688	345,629
Small vessel stroke	MR IVW−MRE						399	1.001 (0.950 − 1.055)	0.97	11,710	391,899
	MR Lasso						399	1.010 (0.962 − 1.060)	0.69	11,710	391,899
	MR conmix						399	1.071 (0.981 − 1.168)	0.13	11,710	391,899
Heart failure and subtypes											
Heart failure	MR IVW−MRE	472	1.044 (0.992 − 1.098)	0.10	8,614	403,858					
	MR Lasso	472	1.036 (0.992 − 1.083)	0.11	8,614	403,858					
	MR conmix	472	1.063 (1.002 − 1.128)	0.04	8,614	403,858					
Heart failure, excluding cardiomyopathies	MR IVW−MRE	472	1.038 (0.983 − 1.096)	0.17	7,593	404,879					
	MR Lasso	472	1.041 (0.994 − 1.090)	0.09	7,593	404,879					
	MR conmix	472	1.059 (0.998 − 1.124)	0.06	7,593	404,879					
Hypertrophic cardiomyopathy	MR IVW−MRE	471	0.816 (0.668 − 0.997)	0.05	387	412,085					
	MR Lasso	471	0.829 (0.680 − 1.010)	0.06	387	412,085					
	MR conmix	471	0.860 (0.655 − 1.131)	0.28	387	412,085					
Dilated cardiomyopathy	MR IVW−MRE	468	1.391 (1.205 − 1.605)	6.27×10^{-6}	824	411,648					
	MR Lasso	468	1.411 (1.228 − 1.622)	1.20×10^{-6}	824	411,648					
	MR conmix	468	1.697 (1.318 − 2.184)	4.03×10^{-5}	824	411,648					



A) Multivariable MR of RHR on any stroke (Ncases = 67,162; Ncontrols = 454,450)

	Secondary exposure atrial fibrillation Percentage change = 11.7%		Secondary exposure systolic blood pressure Percentage change = -2.6%		Secondary exposure diastolic blood pressure Percentage change = -0.4%		Secondary exposure pulse pressure Percentage change = 28.1%	
Method	**Nsnp**	***P*-value**	**Nsnp**	***P*-value**	**Nsnp**	***P*-value**	**Nsnp**	***P*-value**
Univariable IVW	390	2.36×10^{-4}	372	7.66×10^{-4}	354	3.94×10^{-4}	372	7.66×10^{-4}
MV IVW	390	1.36×10^{-3}	372	5.24×10^{-4}	354	4.05×10^{-4}	372	0.02
MV MR Egger	390	7.53×10^{-3}	372	6.28×10^{-3}	354	0.02	372	0.03
MV MR Median	390	3.90×10^{-3}	372	4.65×10^{-3}	354	1.88×10^{-3}	372	0.14
MV MR Lasso	390	6.54×10^{-4}	372	2.47×10^{-5}	354	3.13×10^{-5}	372	1.59×10^{-3}

B) Multivariable MR of RHR on any ischemic stroke (Ncases = 60,341; Ncontrols = 454,450)

	Secondary exposure atrial fibrillation Percentage change = 10.5%		Secondary exposure systolic blood pressure Percentage change = -1.6%		Secondary exposure diastolic blood pressure Percentage change = -0.1%		Secondary exposure pulse pressure Percentage change = 28.1%	
Method	**Nsnp**	***P*-value**	**Nsnp**	***P*-value**	**Nsnp**	***P*-value**	**Nsnp**	***P*-value**
Univariable IVW	390	3.42×10^{-5}	373	1.19×10^{-4}	354	1.04×10^{-4}	373	1.19×10^{-4}
MV IVW	390	2.38×10^{-4}	373	7.99×10^{-5}	354	9.68×10^{-5}	373	9.14×10^{-3}
MV MR Egger	390	1.83×10^{-3}	373	1.13×10^{-3}	354	4.12×10^{-3}	373	0.01
MV MR Median	390	3.18×10^{-4}	373	0.02	354	6.94×10^{-3}	373	0.06
MV MR Lasso	390	3.38×10^{-4}	373	3.67×10^{-6}	354	5.50×10^{-6}	373	8.77×10^{-4}

C) Multivariable MR of RHR on cardio-embolic stroke (Ncases = 9,006; Ncontrols = 403,807)

	Secondary exposure atrial fibrillation Percentage change = 18.4%		Secondary exposure systolic blood pressure Percentage change = 0.3%		Secondary exposure diastolic blood pressure Percentage change = 2.1%		Secondary exposure pulse pressure Percentage change = -5.6%	
Method	**Nsnp**	***P*-value**	**Nsnp**	***P*-value**	**Nsnp**	***P*-value**	**Nsnp**	***P*-value**
Univariable IVW	398	6.81×10^{-6}	377	4.85×10^{-5}	356	6.19×10^{-5}	378	4.93×10^{-5}
MV IVW	398	1.78×10^{-4}	377	5.01×10^{-5}	356	1.09×10^{-4}	378	1.64×10^{-5}
MV MR Egger	398	0.01	377	2.81×10^{-3}	356	0.02	378	9.25×10^{-4}
MV MR Median	398	1.95×10^{-3}	377	0.01	356	0.03	378	2.09×10^{-3}
MV MR Lasso	398	3.84×10^{-4}	377	2.08×10^{-5}	356	1.56×10^{-4}	378	2.39×10^{-5}