Original Article

Exploring the impact of electrocardiographic parameters on the risk of common arrhythmias: a two-sample Mendelian randomization study

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Background: Observational studies have shown that heart rate (HR), heart rate variability (HRV), P-wave terminal force, P-wave duration, T-wave amplitude and PR interval are associated with risk factors for atrial fibrillation (AF) or bradycardia. Arrhythmias are associated with many causes of hospitalization. However, observational studies are susceptible to confounding factors that have not yet been identified. The objective of this study was to clarify the causal relationships by Mendelian randomization analysis.

Methods: We conducted a two-sample and multivariate Mendelian randomization (MVMR) analysis using genome-wide association study (GWAS) data from a European population to assess the total and direct causal effects of HR, three HRV traits, P-wave terminal force, P-wave duration, T-wave top amplitude in five-lead modes, and the PR interval on the risk of AF (N=191,205), bradycardia (N=463,010), and supraventricular tachycardia (SVT) (N=463,010).

Results: The results of the univariate MR analysis revealed the following significant causal effects: the higher the genetically predicted PR interval, the lower the risk of AF; the higher the HR and T-wave top amplitude (aVR leads and V3 + V4 + aVL leads), the lower the risk of bradycardia; and the higher HR and the lower PR interval, the higher the risk of SVT. The multivariate MR results indicated that the HRV_ standard deviation of the normal-to-normal (SDNN) interval had an independent causal effect on the risk of AF [odds ratio (OR): 0.515; 95% confidence interval (CI): 0.278–0.954; P=0.03], and the T-wave top amplitude in the aVR leads (OR: 0.998; 95% CI: 0.996–0.999; P<0.001) and the HRV_SDNN (OR: 0.988; 95% CI: 0.976–1.000; P=0.045) had independent causal effects on the risk of bradycardia.

Conclusions: The HRV_SDNN had an independent causal effect on AF, while the HRV_SDNN and T-wave top amplitude in the aVR leads had independent causal effects on bradycardia, which suggests that some of the electrocardiographic parameters have preventive effects on the incidence of AF and bradycardia.

Keywords: Mendelian randomization (MR); atrial fibrillation (AF); supraventricular tachycardia (SVT); bradycardia

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Introduction

Arrhythmia is a common reason for hospitalization. As life expectancy increases, the prevalence of arrhythmias continues to increase, and arrhythmias have become a leading cause of death (1,2). Atrial fibrillation (AF) and atrial flutter are not only the most common types of arrhythmia but are also risk factors for a variety of cardiovascular diseases (e.g., ischemic stroke and heart failure) (3). AF is associated with increasing morbidity and mortality, and thus represents a growing public health concern and economic burden (4-7). Bradycardia is a common arrhythmia or an abnormal heart rate (HR), for which a large number of pacemakers are implanted each year (8,9). Bradycardia is a concomitant symptom of certain diseases (e.g., severe acute respiratory syndrome coronavirus 2 infection, epilepsy, and multiple myeloma) (10-13) and may even lead to increased mortality in these patients (11,14). Another common type of arrhythmia is supraventricular tachycardia (SVT), which can be divided into several different subtypes according to the different mechanisms and can lead to obvious discomfort, pain, and a higher hospital admission rate (15). SVT is the most common form of tachycardia in infants (16), and the prevalence of SVT is also higher in older populations (it is more than five times more common in people aged ≥65 years than younger people) and in children. The true prevalence of SVT in children is unknown, but it is estimated that 1 in 250–1,000 children has SVT (17,18).

The electrocardiogram (ECG) describes the electrical activity of the heart at a macroscopic level and is the objective basis for the diagnosis of cardiac arrhythmias (19). Prediction for arrhythmias is crucial for early intervention and hence management of arrhythmias. A study has placed high hopes on ECG for arrhythmia prediction (20). Previous observational studies suggest that HR, heart rate variability (HRV), P-wave terminal force, P-wave duration, and the PR interval are all associated with an increased risk of AF (21-30). In addition, increased T-wave amplitude is associated with the risk of AF (31), and an increased PR interval is associated with the risk of atrial flutter (32). T-wave amplitude is associated with the risk of coronary heart disease and myocardial infarction, which may lead to bradycardia (33). However, observational studies are susceptible to confounding factors, such as age, gender, race (23-26), physical activity, and health status, which are risk factors for some HR abnormalities and have a large impact on ECG parameters. If a causal relationship between ECG parameters and arrhythmias can be clarified, it could help clinicians to refine the mechanism of arrhythmogenesis and predict or screen for the occurrence of arrhythmias using a cost-effective, early-identification method.

Mendelian randomization (MR) relies on the natural random assignment of genetic variants in the population during meiosis. These genetic variants are generally unaffected by confounding factors and reverse causality, thus enabling a more reliable assessment of the exposure-outcome causal associations (34). The present study adopted a two-sample MR method to assess the causal effect of different ECG parameters on several common arrhythmias using the latest genome-wide association study (GWAS) summary data. A multivariate Mendelian randomization (MVMR) analysis was also conducted to

Highlight box

Key findings

- Heart rate variability-standard deviation of the normal-to-normal (HRV_SDNN) and atrial fibrillation (AF) have an independent causal effect, the T-wave top amplitude in the aVR leads had independent causal effects on the risk of bradycardia.

What is known and what is new?

- Observational studies have been conducted to correlate changes in electrocardiogram (ECG) parameters with the occurrence of arrhythmias, but the results are inconsistent and susceptible to confounding factors.
- Newly reported evidence for an independent causal effect of HRV_SDNN and AF, as well as T-wave top amplitude in aVR leads and HRV_SDNN had independent causal effects on the risk of bradycardia.

What is the implication, and what should change now?

- This manuscript provides evidence of the causal relationship between some ECG parameters and arrhythmias, which will give a cost-effective early identification method for predicting or screening the occurrence of arrhythmias.
- A clear causal relationship would correlate the mechanism of arrhythmogenesis with the electrophysiological activity represented by ECG-related parameters, which would advance the understanding of arrhythmias.

assess the independent causal relationship between the ECG parameters and arrhythmias. We present this article in accordance with the STROBE-MR reporting checklist (35) (available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-814/rc).

Methods

Study design

To explore the genetic association between the eight ECG-related parameters (exposure) and the risk of AF, bradycardia, and SVT, a two-sample MR analysis was conducted using the GWAS data. In the MR analysis, genetic variants were treated as instrumental variables (IVs) for exposure based on the three following assumptions: (I) relevance: genetic IVs are significantly associated with the eight ECG parameters; (II) independence: genetic IVs are not related to potential confounding factors that may affect the eight ECG parameters and AF, bradycardia, and SVT; (III) exclusion restriction: genetic IVs do not directly affect AF, bradycardia, and SVT, and only affect them through the 8 ECG-related parameters. The whole process is shown in *Figure 1*. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Data source

Publicly available GWAS databases, including the GRASP = HuGeAMP database, were searched to obtain eligible data sets of the eight ECG parameters and AF, bradycardia, and SVT.

Summary-level data on the ECG-related parameters

The summary-level ECG data were derived from several large-scale GWAS meta-analyses covering the eight ECG-related parameters based on European populations. The eight ECG parameters were the resting HR [max N=85,787 (European)] (36), three HRV traits {i.e., the root mean square of successive differences (RMSSD) [max N=26,785 (European)], the peak-valley respiratory sinus arrhythmia or high frequency power (pvRSA/HF) [max N=24,342 (European)], and the standard deviation of the normal-to-normal interval (SDNN) [max N=28,112 (European)]} (37), PR interval [N=271,570 (European)] (38), P-wave duration [N=37,678 (European) + 6,778 (African)], P-wave terminal force [N=33,955 (European) + 6,778 (African)] (39), and T-wave top amplitude [max N=37,977 (European)] in five-lead modes (septal: ECG lead V1 + V2; lateral: ECG lead I + aVL + V5 + V6; inferior: ECG lead II + III + aVF; anterior: ECG lead V3 + V4 + aVL; avr: ECG lead aVR) (40). For further details, see *Table 1*.

Summary-level data on HR abnormalities

Data related to AF and atrial flutter were obtained from the FinnGen consortium (R8, accessed on May 9, 2023, https://www.finngen.fi/en). The data set comprised 34,748 cases and 156,457 controls. The bradycardia and SVT data sets, comprising 1,254 cases and 461,756 controls for bradycardia and 1,306 cases and 461,704 controls for SVT, respectively, were both derived from the IEU GWAS database (https://gwas.mrcieu.ac.uk/) (41-43). All the GWAS summary data on outcomes were based on European populations (*Table 1*).

Statistics

Extraction of IVs

Single-nucleotide polymorphisms (SNPs) in exposure and outcome were searched in the GWAS database according to the above assumptions. SNPs that satisfied the GWAS significance ($P<5\times10^{-8}$) or threshold of $P<1\times10^{-5}$ (44) (when the number of SNPs used for the MR analysis was too few) in the exposure-GWAS were extracted as IVs to ensure that the genetic variants were significantly correlated with exposure. Only independent SNPs not in linkage disequilibrium (LD, $r^2<0.01$ within a 5,000-kb window) were retained. The remaining SNPs were then used to extract relevant information from the outcome-GWAS before coordinating the summary statistics so that the effect of the SNPs on the outcome and exposure was relative to the same allele, and the palindromic SNPs were excluded from the MR analysis [Supplementary file (Appendix 1) and Table S1].

Univariate MR analysis

The MR analysis was conducted following the above three assumptions. The inverse-variance weighted (IVW) (45) method was used as the primary method. To ensure that the three core assumptions were not breached, MR-Egger regression (46,47), weighted median (48,49), and a causal analysis using summary effect estimates (CAUSE) (50) were performed as the sensitivity analyses. We further used several visual plots, such as leave-one-out plots and funnel plots, to detect possible significant outliers. MR-Egger

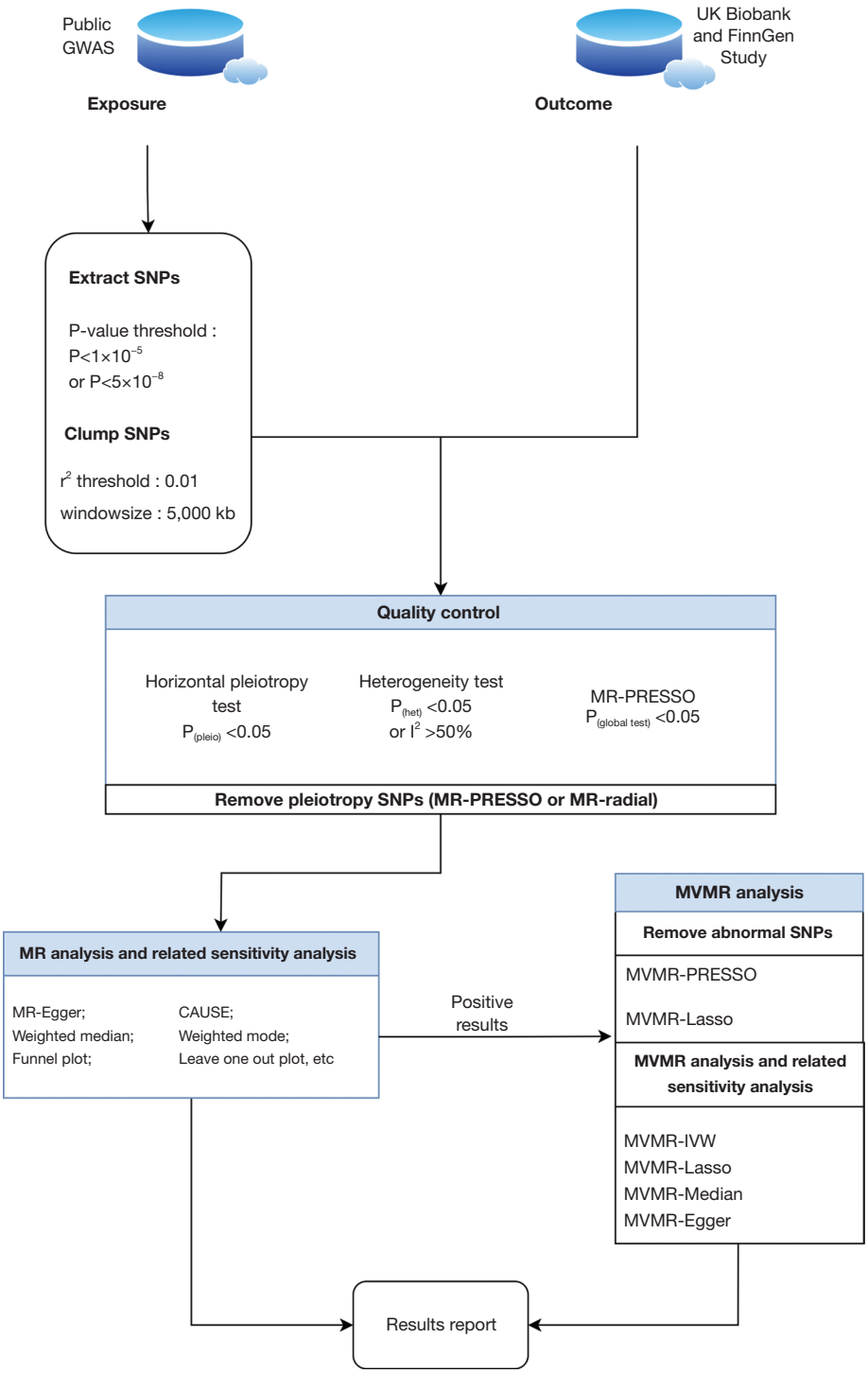

Figure 1 Flow diagram of the Mendelian randomization design. GWAS, genome-wide association study; SNP, single-nucleotide polymorphism; MR, Mendelian randomization; MVMR, multivariate mendelian randomization; het, heterogeneity; pleio, horizontal pleiotropy; CAUSE, causal analysis using summary effect estimates; MR-PRESSO, Mendelian Randomization Pleiotropy RESidual Sum and Outlier; MR-Radial, IVW radial regression and egger radial regression; IVW, inverse-variance weighted.

Table 1 Description of the GWAS data sources used in the Mendelian randomization study

Exposure/outcome	Sample size	Ancestry	PMID/GWAS ID
HR	85,787	European	23583979
HRV_RMSSD	26,785	European	28613276
HRV_pvRSAHF	24,342	European	28613276
HRV_SDNN	28,112	European	28613276
PR interval	271,570	European	32439900
P-wave duration	37,678+6,778	European + African	28794112
P-wave terminal force	33,955+6,778	European + African	28794112
T-wave top amplitude	37,977	European	26962151
Atrial fibrillation and atrial flutter	34,748 cases and 156,457 controls	European	N/A
Bradycardia	1,254 cases and 461,756 controls	European	ukb-b-11664
Supraventricular tachycardia	1,306 cases and 461,704 controls	European	ukb-b-11748

GWAS, genome-wide association study; HR, heart rate (resting); HRV, heart rate variability; RMSSD, root mean square of successive differences; pvRSAHF, peak-valley respiratory sinus arrhythmia or high frequency power; SDNN, standard deviation of the normal-to-normal interval; N/A, not applicable.

follows the INstrument Strength Independent of Direct Effect (InSIDE) assumption (46,47), the weighted median approach assumes that valid IVs provide more than half of the weight (48), and the weighted mode assumes that multiple genetic variants are valid (49).

Heterogeneity was assessed by the Cochran Q heterogeneity test and I^2 statistics. A P value <0.05 or I^2 value >50% implied significant heterogeneity, and outlier SNPs were detected and excluded by the Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) test. If the MR-PRESSO test failed to detect outliers, then IVW radial regression and Egger radial regression were used to further detect and exclude outlier SNPs (51,52). Then, $25\%<I^2<50\%$ implied moderate heterogeneity, and the random-effects model was selected for analysis.

The presence of horizontal pleiotropy would violate the MR core assumptions, and the intercept test (P>0.05 indicates no pleiotropy) of MR-Egger regression was used to assess horizontal pleiotropy. An MR-PRESSO analysis was conducted to detect (the global test) and correct (the outlier test) the abnormal SNPs that might lead to horizontal pleiotropy and to evaluate significant differences in the causal estimate before and after outlier removal (distortion test) (51) [(available online: https://cdn.amegroups.cn/static/public/jtd-24-814-1.xlsx) for details of all the tool variables used for the MR analysis]. A CAUSE analysis was performed to account for correlated and uncorrelated horizontal pleiotropy, and a threshold of $P<1\times10^{-3}$ was used to ensure sufficient SNPs to assess the deleterious parameters. When estimating the correlations between the summary statistics due to sample overlap or population structure, all the variants were used if the total number of SNPs in the summary data set was less than 1,000,000 to avoid poor estimates of the confounding parameters, and the remaining steps were performed according to the default parameters in the CAUSE package (50).

MVMR analysis

Phenotypes with positive results in the univariate MR (P<0.05) analysis were used as exposures. An MVMR analysis was performed after the detection and removal of outlier SNPs by the MR-PRESSO analysis. The MVMR-IVW method was used as the primary multivariate method, and the associated sensitivity tests included the MVMR-Egger, MVMR-Lasso, and MVMR-median tests. MVMR-PRESSO and MVMR-Egger intercept tests were used to identify potential horizontal pleiotropy.

When performing the MVMR analysis with bradycardia as the endpoint, considering that T-wave top amplitude_avr and T-wave top amplitude_anterior are different leads of the same waveband that both essentially respond to ventricular repolarization, the exposures were divided into two groups for the MVMR analysis (HR, HRV_RMSSD,

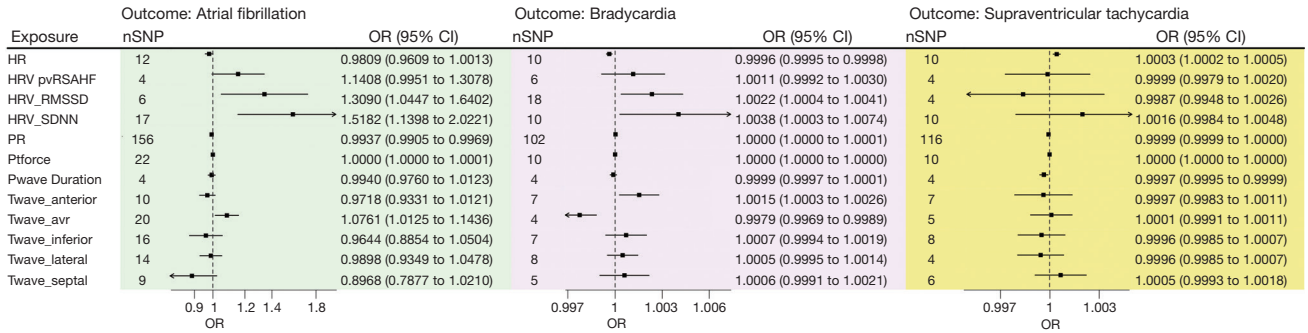

Figure 2 Forest plot of the univariate Mendelian randomization results. The causal effects of the ECG-related parameters on arrhythmia risk were expressed as the OR per unit. Error bars represent the 95% CIs of the estimates. nSNP, the number of single-nucleotide polymorphisms; HR, heart rate (resting); Twave, T-wave top amplitude; septal, ECG lead V1 + V2; lateral, ECG lead I + aVL + V5 + V6; inferior, ECG lead II + III + aVF; anterior, ECG lead V3 + V4 + aVL; avr, ECG lead aVR; Ptforce, P-wave terminal force; Pwave duration, P-wave duration; HRV, heart rate variability; RMSSD, root mean square of successive differences; pvRSAHF, peak-valley respiratory sinus arrhythmia or high frequency power; SDNN, standard deviation of the normal-to-normal interval; PR, PR interval; OR, odds ratio; CI, confidence interval.

HRV_SDNN, and T-wave top amplitude_avr or T-wave top amplitude_anterior). If the Cochran Q of the MVMR-IVW, the MVMR-Egger intercept test, and the MVMR-PRESSO global test all indicated the existence of horizontal pleiotropy or heterogeneity and the MVMR-PRESSO test still failed to detect significant outliers by increasing the number of simulation calculations, we supplemented the MVMR-Lasso method to identify and eliminate abnormal SNPs (53,54). MR-Lasso analysis is an analytical method that identifies some genetic variants as valid IVs and then evaluates causality by fitting the regularized regression model with a standard IVW method with only valid genetic variants (55).

The *F*-statistic for each SNP was calculated using the following formula: *F*-statistic = $beta^2/se^2$ (56). Considering the multiple tests, the significance threshold was set at P<0.00139 (α=0.05/36, 12 exposures and three outcomes) based on Bonferroni correction. Referring to previous articles (44), we considered the results to be strongly significant when P<0.00139 and the evidence to be suggestive when 0.00139<P<0.05. Bonferroni correction was not used in the MVMR analysis due to its mutually adjusted nature (57).

Software

All the analyses were performed with R software (version 4.1.1), and the MR analyses were performed using the "TwoSampleMR", "MendelianRandomization", "MRPRESSO", "RadialMR", and "CAUSE" packages.

Results

Univariate MR

The *F*-statistics for all the included SNPs were greater than 10, which avoided the influence of weak IVs in our study to a certain extent (available online: https://cdn.amegroups.cn/static/public/jtd-24-814-1.xlsx).

ECG-related parameters and AF

Evidence from the primary IVW MR method suggested that the genetically predicted PR interval was negatively associated with the development of AF [odds ratio (OR): 0.994; 95% confidence interval (CI): 0.991–0.997; $P=9.8\times10^{-5}$]. The genetically determined HRV_SDNN, HRV_RMSSD, and T-wave top amplitude_avr were positively associated with the development of AF (HRV_SDNN, OR: 1.518; 95% CI: 1.140–2.022; P=0.004; HRV_RMSSD, OR: 1.309; 95% CI: 1.067–1.605; P=0.01; T-wave top amplitude_avr, OR: 1.076, 95% CI: 1.012–1.144; P=0.02). The other genetically determined ECG-related parameters were not causally associated with the development of AF [*Figure 2* and Supplementary file (Appendix 1), Table S2].

ECG-related parameters and bradycardia

Evidence from the major IVW MR method suggested that the genetically determined T-wave top amplitude_avr, and HR were negatively associated with the occurrence of bradycardia (T-wave top amplitude_avr, OR: 0.998; 95% CI: 0.997–0.999; P<0.001; HR, OR: 1.000; 95% CI: 0.999–1.000; P<0.001). The evidence suggested that the HRV_RMSSD, HRV_SDNN, and T-wave top amplitude_anterior were positively associated with the occurrence of bradycardia (HRV_RMSSD, OR: 1.002; 95% CI: 1.000–1.004; P=0.02; HRV_SDNN, OR: 1.004; 95% CI 1.000–1.007; P=0.03; T-wave top amplitude_anterior, OR: 1.001; 95% CI: 1.000–1.003; P=0.02), whereas the other genetically determined ECG-related parameters were not causally associated with the occurrence of bradycardia [*Figure 2* and Supplementary file (Appendix 1), Table S2].

ECG-related parameters and SVT

Evidence from the primary IVW MR approach suggested a positive correlation between the genetically determined HR and the occurrence of SVT (OR, 1.000; 95% CI: 1.000–1.001; P<0.001). The evidence suggested that the PR interval and P-wave duration were negatively associated with the occurrence of SVT (PR interval, OR: 1.000; 95% CI: 1.000–1.000; P=0.001; P-wave duration, OR: 1.000; 95% CI: 1.000–1.000; P=0.007), whereas the other genetically determined ECG-related parameters were not causally related to the occurrence of bradycardia [*Figure 2* and Supplementary file (Appendix 1), Table S2].

Sensitivity analyses

The results of some sensitivity analyses suggested possible heterogeneity [Supplementary file (Appendix 1), Table S2]; however, there was no evidence of horizontal pleiotropy and heterogeneity (Egger-intercept test and heterogeneity test, P>0.05) [Supplementary file (Appendix 1), Tables S3,S4]. In addition, the MR-PRESSO method and the leave-one-out plot did not detect abnormal SNPs, and the funnel plot was roughly symmetrical [Supplementary file (Appendix 2)].

The higher values of Eta and Q suggested that the results were more influenced by the multiplicity of the correlation levels (50,58). The CAUSE analysis results showed that the causal model did not hold in estimating the causal associations described above (all P>0.05) [Supplementary file (Appendix 1), Table S5].

MVMR

All exposures with positive results (P<0.05) in the univariate MR were selected as exposures for the MVMR analysis.

ECG-related parameters and AF

After adjusting the T-wave top amplitude_avr, PR interval, and HRV_RMSSD, we found that the HRV_SDNN had an independent causal effect on the occurrence of AF (HRV_SDNN, OR: 0.515; 95% CI: 0.278–0.954; P=0.03). In addition, no outliers were detected by the MVMR-PRESSO test, and no heterogeneity and pleiotropy were detected by the MR-Egger intercept test and heterogeneity test (both P>0.05) [*Figure 2* and Supplementary file (Appendix 1), Table S6].

ECG-related parameters and bradycardia

After adjusting the HR and HRV_RMSSD and eliminating the outliers (rs11578508 and rs6127471), we found that the T-wave top amplitude_avr and HRV_SDNN had independent causal effects on the occurrence of bradycardia (T-wave top amplitude_avr, OR: 0.998; 95% CI: 0.996–0.999; P<0.001; HRV_SDNN, OR: 0.988; 95% CI: 0.976–1.000; P=0.045), while the HR and HRV_RMSSD had no independent causal effects on the occurrence of bradycardia. After adjusting for the T-wave top amplitude_anterior and HR and eliminating the outliers (rs7633988 and rs6127471), we found that the HRV_SDNN and HRV_RMSSD were independently causally related to the occurrence of bradycardia (HRV_RMSSD, OR: 1.008; 95% CI: 1.000–1.016; P=0.04; HRV_SDNN, OR: 0.988; 95% CI: 0.977–1.000; P=0.04). All the other sensitivity analysis results were generally consistent with those found using the MVMR-IVW method. In addition, no outliers were detected by the MVMR-PRESSO test, and no heterogeneity or pleiotropy was detected by the MR-Egger intercept test and heterogeneity test (both P>0.05) [*Figure 3* and Supplementary file (Appendix 1), Table S6].

ECG-related parameters and SVT

Of the 116 SNPs significantly associated with the PR interval in the univariate analysis, 39 SNPs were associated with the HR only, 12 SNPs were associated with the P-wave duration only, and 19 SNPs were associated with both the HR and P-wave duration. Considering the overlap of some

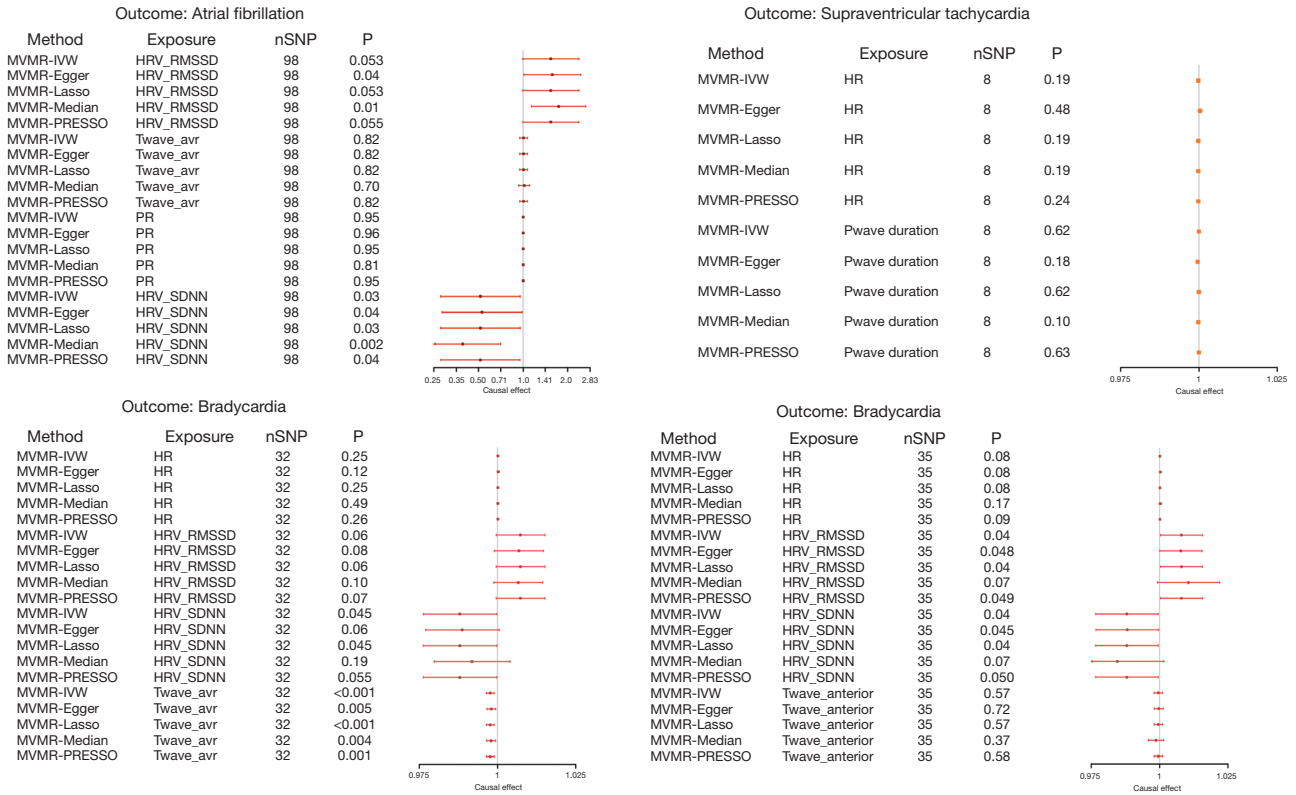

Figure 3 Forest plot of the multivariable Mendelian randomization results. The causal effects of the ECG-related parameters on arrhythmia risk were expressed as the OR per unit. Error bars represent the 95% CIs of the estimates. MVMR, multivariate mendelian randomization; IVW, inverse-variance weighted; HR, heart rate (resting); HRV, heart rate variability; RMSSD, root mean square of successive differences; PR, PR interval; SDNN, standard deviation of the normal-to-normal interval; Twave, T-wave amplitude; avr, ECG lead aVR; anterior, ECG lead V3 + V4 + aVL; Pwave duration, P-wave duration; nSNP, the number of single-nucleotide polymorphisms; OR, odds ratio; CI, confidence interval.

SNPs caused by the inclusion of the P-wave duration in the PR interval, an MVMR analysis was performed with the HR and P-wave duration as exposures (59).

The results suggested that there was no independent causal relationship between the HR or P-wave duration and the occurrence of SVT. The results of the sensitivity analyses were similar to those found using the IVW method. No heterogeneity or horizontal pleiotropy were detected by the heterogeneity and MR-Egger intercept tests, and no outliers were detected by the MR-PRESSO analysis (all P>0.05) [*Figure 3* and Supplementary file (Appendix 1), Table S6].

Discussion

Using a univariate MR analysis, we not only drew conclusions consistent with those drawn by previous studies on the causal relationship between the HRV_SDNN, PR interval, and HRV_RMSSD and the occurrence of AF, as well as the resting HR, PR interval, and P-wave duration and the occurrence of SVT (26,60,61), we also identified novel causal relationships between the T-wave top amplitude (aVR leads) and the occurrence of AF, as well as between the resting HR, HRV_SDNN, HRV_RMSSD, and T-wave top amplitude (aVR leads and V3 + V4 + aVL leads) and the occurrence of bradycardia.

Statistics

Unlike previous studies (26,60,61), the present study employed a more rigorous MR-Radial approach to minimize potential horizontal pleiotropy. However, this may have resulted in the loss of a portion of potentially valid SNPs. Furthermore, taking into account the interactions

between the different ECG parameters, MVMR analyses were conducted to explore the independent effects of ECG parameters on heart rate abnormalities. Notably, the MVMR analysis suggested that there was a causal relationship between the HRV_SDNN and AF, as well as the T-wave top amplitude in the aVR leads and HRV_SDNN and the occurrence of bradycardia remained.

The results of the CAUSE analysis were found to be inconsistent with the aforementioned results obtained via IVW and other sensitivity methods. However, given the relatively relaxed threshold (1×10^{-3}) for extracting IVs and the strictness of removing variants with ambiguous alleles (G/C or A/T), we postulated that the CAUSE analysis might remove SNPs that are strongly correlated with exposure and used in other MR analyses, thereby resulting in the loss of some information, particularly when the number of SNPs used in other MR analyses is low. CAUSE was employed to circumvent false alarms caused by correlated horizontal pleiotropy. The results of the MR-Egger intercept test, MR-PRESSO, and other methods for detecting uncorrelated horizontal pleiotropy did not reach statistical significance. Additionally, the effect size of CAUSE for detecting correlated horizontal pleiotropy was relatively small. Therefore, the disparate results observed between CAUSE and IVW do not indicate that the positive IVW result is a false alarm due to correlated horizontal pleiotropy.

ECG-related parameters and AF

Nevertheless, in contrast to previous study (60), no causal relationships were identified between alterations in HR and the occurrence of AF. This may be because we did not stratify the resting HR in the present study. We might not have found a causal relationship between the total resting HR and the occurrence of AF due to the U-shaped association between the resting HR and the AF risk ratio that has been reported in previous study (60).

The effect of the HRV_SDNN on the occurrence of AF changed, and the significance of the causal relationships between the T-wave top amplitude (aVR leads), HRV_RMSSD, and PR interval and the occurrence of AF, as well as those between the resting HR and P-wave duration and the occurrence of SVT, was not observed in the multivariate analysis results. This result was expected given the strong correlation between the resting HR, HR variability index, and ECG parameters; therefore, the effect of the HRV_SDNN on the occurrence of AF changed after adjusting for other parameters, while the independent causal effect of some parameters on AF or SVT was no longer significant.

ECG-related parameters and bradycardia

As more electrical activity is transmitted by parasympathetic nerves than by sympathetic nerves, the resting HR in humans is mainly determined by the parasympathetic sector (62). Further, the changes in RMSSD also represent parasympathetic activity (63). However, the multivariate analysis results suggested that RMSSD and the resting HR had no independent effect on the occurrence of bradycardia. Even so, the positive results of the univariate MR analysis of RMSSD and the resting HR, and the lower P value of RMSSD in the two multivariate analyses suggested that the acetylcholine (ACh) released from the vagus nerve may play an important role in the occurrence of bradycardia.

The ACh released from the vagus nerve can stimulate Gαi/o-coupled muscarinic M2 receptors and affect membrane excitability via the Gβγ-mediated direct activation of G protein-coupled inwardly rectifying potassium (GIRK) channels (62). The decrease in HRV_RMSSD implies a decrease in the phase change of cardiac vagal activity, which suggests a diminished activation of the GIRK channels. In addition, rs180238 and rs4262 (partial SNPs used for the MVMR analysis) were found to be significantly associated with a guanine nucleotide binding protein (G protein, gamma 11) (*GNG11*) in the expression quantitative trait loci of the HRV SNPs (37), which encodes the Gα βγ heterotrimer of the gamma 11 subunit and is expressed at high levels in the heart (64,65). Reduced gamma-11 availability may reduce Gβγ component-induced GIRK activation. Therefore, the exponential decrease in the HRV_RMSSD may imply an attenuation in the activation affecting GIRK channels. Meanwhile, studies have shown that excessive vagal stimulation is dependent on increased GIRK currents in mice and directly leads to atrioventricular block (which often occurs with bradycardia) (66,67).

The SDNN represents sympathetic and parasympathetic activity, but it is not clear whether changes in HRV are related to sympathetic or vagal nerves (63). Research has shown that knocking out the gene for a key factor (RGS4) in the cardiac vagal pathway may result in unchanged resting HR but bradycardia in mice after the administration of M2 receptor-receptor agonists (68). The independent causal relationship between the SDNN and bradycardia after adjustment for RMSSD and HR may imply that sympathetic nerves play an important role in genetically

mediated bradycardia. Sympathetic nerves in the fight-or-flight response can increase the HR by secreting catecholamines that stimulate β-adrenergic receptors in cardiomyocytes (69), but norepinephrine may have less of an effect on the resting HR and does not reduce the release of ACh in the human heart (70). However, in addition to norepinephrine, sympathetic nerves also release different levels of co-transmitters (e.g., adenosine triphosphate, neuropeptide-Y, and galanin) depending on the level of stimulation. Among these, galanin has been shown to reduce vagally mediated bradycardia (71). Thus, lower sympathetic activity may imply a reduction in co-transmitters, leading to the weakened inhibition of bradycardia.

In the univariate analysis, the T-wave top amplitude (aVR lead) was causally associated with the occurrence of AF and bradycardia. A decrease in the T-wave top amplitude may indicate an abnormal ventricular repolarization process, possibly related to myocardial pathology and autonomic disorders. Bradycardia and T-wave depression caused by alterations in the autonomic nervous system have been observed in athletes (72-74). The aVR lead is the only lead in the body ECG that does not face the "typical" associated wall of the left ventricle (75). A decrease in the T-wave top amplitude detected by the aVR lead may indicate a lesion of the right ventricular myocardium or a disruption of the sympathetic-parasympathetic balance on the right side of the heart, which may play a role in the pathogenesis of AF (76). The different results between the T-wave top amplitude_avr and T-wave top amplitude_anterior in the multivariate analysis of bradycardia suggest that the change in the top amplitude of the T-wave under the aVR leads is more informative.

Limitations

This study had several limitations. First, due to the overlap of SNPs in the GWAS, we did not analyze whether there was an independent causal relationship between the PR interval and the occurrence of SVT, which would require a larger sample of GWAS data for a correlation analysis. Second, referring to previous MR studies and clinical trials (26,60,61,77-81), this study also unified AF with atrial flutter as an outcome. Although atrial flutter is less common and may occur over time in the same individual, caution is needed in interpreting these results (82,83). Third, a U-shaped relationship between some ECG-related parameters and HR abnormalities has been observed in some observational studies; thus, further stratification studies on ECG-related parameters need to be conducted. Fourth, P-wave duration and P-wave terminal force were included in the GWAS for ethnic groups other than Europeans, which could affect the robustness of the MR results. As the relevant GWAS research is mainly conducted among participants of European descent, further research is needed to assess the universality of our results among other ethnic groups. Fifth, despite the relatively low power values observed in our study, this does not affect our significant results [Supplementary file (Appendix 1), Table S7]. Sixth, as with the other two samples of Mendelian randomization, our study is limited to an analysis of causality and does not provide specific cutoff values. Further analysis would require larger studies and individual-level data. Sixth, the sample for the PR interval GWAS was partly from the UK Biobank, and thus might overlap with the SVT and bradycardia samples. Although the exact number of overlapping samples could not be determined, we calculated the sample overlap rate (60,543/463,010=0.13) according to the maximum overlap and used a stronger IV (*F*-statistic: 29.9–1,224.3) to avoid large bias or type I error rates (84). Nevertheless, the results of both sets of MR analyses should be interpreted with caution.

Conclusions

The present study provides new evidence of independent causal relationships between the HRV_SDNN and occurrence of AF, as well as the aVR lead T-wave top amplitude, and HRV_SDNN and the occurrence of bradycardia. Given the limitations of this study, the results should be interpreted with caution in the clinical context; however, the results of this study may still provide some reference for the prediction and prevention of arrhythmias in the future.

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Footnote

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References

1. Lindberg T, Bohman DM, Elmståhl S, et al. Prevalence of unknown and untreated arrhythmias in an older outpatient population screened by wireless long-term recording ECG. Clin Interv Aging 2016;11:1083-90.
2. Kornej J, Börschel CS, Benjamin EJ, et al. Epidemiology of Atrial Fibrillation in the 21st Century: Novel Methods and New Insights. Circ Res 2020;127:4-20.
3. John RM, Michaud GF, Stevenson WG. Atrial fibrillation hospitalization, mortality, and therapy. Eur Heart J 2018;39:3958-60.
4. DeLago AJ, Essa M, Ghajar A, et al. Incidence and Mortality Trends of Atrial Fibrillation/Atrial Flutter in the United States 1990 to 2017. Am J Cardiol 2021;148:78-83.
5. Ruddox V, Sandven I, Munkhaugen J, et al. Atrial fibrillation and the risk for myocardial infarction, all-cause mortality and heart failure: A systematic review and meta-analysis. Eur J Prev Cardiol 2017;24:1555-66.
6. Lippi G, Sanchis-Gomar F, Cervellin G. Global epidemiology of atrial fibrillation: An increasing epidemic and public health challenge. Int J Stroke 2021;16:217-21.
7. Karamitanha F, Ahmadi F, Fallahabadi H. Difference Between Various Countries in Mortality and Incidence Rate of the Atrial Fibrillation Based on Human Development Index in Worldwide: Data From Global Burden of Disease 2010-2019. Curr Probl Cardiol 2023;48:101438.
8. Kany S, Khurshid S. Keeping to the rhythm of cardiovascular health. Eur J Prev Cardiol 2024;31:655-7.
9. Cheng YJ, Deng H, Liao YJ, et al. Role of ideal cardiovascular health metrics in reducing risk of incident arrhythmias. Eur J Prev Cardiol 2024;31:658-66.
10. Li Y, Tang M, Zhong L, et al. Incidence of Arrhythmias and Their Prognostic Value in Patients With Multiple Myeloma. Front Cardiovasc Med 2021;8:753918.
11. Kumar S, Arcuri C, Chaudhuri S, et al. A novel study on SARS-COV-2 virus associated bradycardia as a predictor of mortality-retrospective multicenter analysis. Clin Cardiol 2021;44:857-62.
12. Teo YH, Han R, Leong S, et al. Prevalence, types and treatment of bradycardia in obstructive sleep apnea - A systematic review and meta-analysis. Sleep Med 2022;89:104-13.
13. Serdyuk S, Davtyan K, Burd S, et al. Cardiac arrhythmias and sudden unexpected death in epilepsy: Results of long-term monitoring. Heart Rhythm 2021;18:221-8.
14. Bokade C, Gulhane R, Bagul A, et al. Acute febrile encephalopathy in children and predictors of mortality. J Clin Diagn Res 2014;8:PC09-11.
15. Kotadia ID, Williams SE, O'Neill M. Supraventricular tachycardia: An overview of diagnosis and management. Clin Med (Lond) 2020;20:43-7.
16. Richardson C, Silver ES. Management of Supraventricular Tachycardia in Infants. Paediatr Drugs 2017;19:539-51.
17. Brugada J, Katritsis DG, Arbelo E, et al. 2019 ESC Guidelines for the management of patients with supraventricular tachycardiaThe Task Force for the management of patients with supraventricular tachycardia of the European Society of Cardiology (ESC). Eur Heart J 2020;41:655-720.
18. Salerno JC, Seslar SP. Supraventricular tachycardia. Arch

Pediatr Adolesc Med 2009;163:268-74.
19. Schmitt N, Grunnet M, Olesen SP. Cardiac potassium channel subtypes: new roles in repolarization and arrhythmia. Physiol Rev 2014;94:609-53.
20. Chen LY, Ribeiro ALP, Platonov PG, et al. P Wave Parameters and Indices: A Critical Appraisal of Clinical Utility, Challenges, and Future Research-A Consensus Document Endorsed by the International Society of Electrocardiology and the International Society for Holter and Noninvasive Electrocardiology. Circ Arrhythm Electrophysiol 2022;15:e010435.
21. Nielsen JB, Kühl JT, Pietersen A, et al. P-wave duration and the risk of atrial fibrillation: Results from the Copenhagen ECG Study. Heart Rhythm 2015;12:1887-95.
22. Huang Z, Zheng Z, Wu B, et al. Predictive value of P wave terminal force in lead V1 for atrial fibrillation: A meta-analysis. Ann Noninvasive Electrocardiol 2020;25:e12739.
23. Smith JW, O'Neal WT, Shoemaker MB, et al. PR-Interval Components and Atrial Fibrillation Risk (from the Atherosclerosis Risk in Communities Study). Am J Cardiol 2017;119:466-72.
24. Knuiman M, Briffa T, Divitini M, et al. A cohort study examination of established and emerging risk factors for atrial fibrillation: the Busselton Health Study. Eur J Epidemiol 2014;29:181-90.
25. Agarwal SK, Norby FL, Whitsel EA, et al. Cardiac Autonomic Dysfunction and Incidence of Atrial Fibrillation: Results From 20 Years Follow-Up. J Am Coll Cardiol 2017;69:291-9.
26. Geurts S, Tilly MJ, Arshi B, et al. Heart rate variability and atrial fibrillation in the general population: a longitudinal and Mendelian randomization study. Clin Res Cardiol 2023;112:747-58.
27. Habibi M, Chahal H, Greenland P, et al. Resting Heart Rate, Short-Term Heart Rate Variability and Incident Atrial Fibrillation (from the Multi-Ethnic Study of Atherosclerosis (MESA)). Am J Cardiol 2019;124:1684-9.
28. Giannopoulos G, Tachmatzidis D, Moysidis DV, et al. P-wave Indices as Predictors of Atrial Fibrillation: The Lion from a Claw. Curr Probl Cardiol 2024;49:102051.
29. Jagannatha GNP, Antara IMPS, Kosasih AM, et al. P-wave peak time and P-wave dispersion in surface electrocardiography as initial predictors of new-onset atrial fibrillation in early-onset hypertension. Hypertens Res 2024;47:137-48.
30. Wei Y, Zhou G, Wu X, et al. Latest incidence and electrocardiographic predictors of atrial fibrillation: a prospective study from China. Chin Med J (Engl) 2023;136:313-21.
31. Lehtonen AO, Langén VL, Porthan K, et al. Electrocardiographic predictors of atrial fibrillation in nonhypertensive and hypertensive individuals. J Hypertens 2018;36:1874-81.
32. Rahman F, Wang N, Yin X, et al. Atrial flutter: Clinical risk factors and adverse outcomes in the Framingham Heart Study. Heart Rhythm 2016;13:233-40.
33. Dekker JM, Schouten EG, Klootwijk P, et al. ST segment and T wave characteristics as indicators of coronary heart disease risk: the Zutphen Study. J Am Coll Cardiol 1995;25:1321-6.
34. Emdin CA, Khera AV, Kathiresan S. Mendelian Randomization. JAMA 2017;318:1925-6.
35. Skrivankova VW, Richmond RC, Woolf BAR, et al. Strengthening the Reporting of Observational Studies in Epidemiology Using Mendelian Randomization: The STROBE-MR Statement. JAMA 2021;326:1614-21.
36. den Hoed M, Eijgelsheim M, Esko T, et al. Identification of heart rate-associated loci and their effects on cardiac conduction and rhythm disorders. Nat Genet 2013;45:621-31.
37. Nolte IM, Munoz ML, Tragante V, et al. Genetic loci associated with heart rate variability and their effects on cardiac disease risk. Nat Commun 2017;8:15805.
38. Ntalla I, Weng LC, Cartwright JH, et al. Multi-ancestry GWAS of the electrocardiographic PR interval identifies 202 loci underlying cardiac conduction. Nat Commun 2020;11:2542.
39. Christophersen IE, Magnani JW, Yin X, et al. Fifteen Genetic Loci Associated With the Electrocardiographic P Wave. Circ Cardiovasc Genet 2017;10:e001667.
40. Verweij N, Mateo Leach I, Isaacs A, et al. Twenty-eight genetic loci associated with ST-T-wave amplitudes of the electrocardiogram. Hum Mol Genet 2016;25:2093-103.
41. Hemani G, Zheng J, Elsworth B, et al. The MR-Base platform supports systematic causal inference across the human phenome. Elife 2018;7:e34408.
42. Elsworth B, Lyon M, Alexander T, et al. The MRC IEU OpenGWAS data infrastructure. bioRxiv 2020:2020.08.10.244293.
43. Lyon MS, Andrews SJ, Elsworth B, et al. The variant call format provides efficient and robust storage of GWAS summary statistics. Genome Biol 2021;22:32.
44. Perry BI, Upthegrove R, Kappelmann N, et al. Associations of immunological proteins/traits with schizophrenia, major depression and bipolar disorder: A bi-directional two-sample mendelian randomization study.

Brain Behav Immun 2021;97:176-85.
45. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. Genet Epidemiol 2013;37:658-65.
46. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int J Epidemiol 2015;44:512-25.
47. Bowden J, Del Greco M F, Minelli C, et al. Assessing the suitability of summary data for two-sample Mendelian randomization analyses using MR-Egger regression: the role of the I2 statistic. Int J Epidemiol 2016;45:1961-74.
48. Bowden J, Davey Smith G, Haycock PC, et al. Consistent Estimation in Mendelian Randomization with Some Invalid Instruments Using a Weighted Median Estimator. Genet Epidemiol 2016;40:304-14.
49. Hartwig FP, Davey Smith G, Bowden J. Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. Int J Epidemiol 2017;46:1985-98.
50. Morrison J, Knoblauch N, Marcus JH, et al. Mendelian randomization accounting for correlated and uncorrelated pleiotropic effects using genome-wide summary statistics. Nat Genet 2020;52:740-7.
51. Verbanck M, Chen CY, Neale B, et al. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. Nat Genet 2018;50:693-8.
52. Bowden J, Spiller W, Del Greco M F, et al. Improving the visualization, interpretation and analysis of two-sample summary data Mendelian randomization via the Radial plot and Radial regression. Int J Epidemiol 2018;47:1264-78.
53. Grant AJ, Burgess S. Pleiotropy robust methods for multivariable Mendelian randomization. Stat Med 2021;40:5813-30.
54. Liu J, Chou EL, Lau KK, et al. A Mendelian randomization-based exploration of red blood cell distribution width and mean corpuscular volume with risk of hemorrhagic strokes. HGG Adv 2022;3:100135.
55. Rees JMB, Wood AM, Dudbridge F, et al. Robust methods in Mendelian randomization via penalization of heterogeneous causal estimates. PLoS One 2019;14:e0222362.
56. Pierce BL, Ahsan H, Vanderweele TJ. Power and instrument strength requirements for Mendelian randomization studies using multiple genetic variants. Int J Epidemiol 2011;40:740-52.
57. Yuan S, Tang B, Zheng J, et al. Circulating Lipoprotein Lipids, Apolipoproteins and Ischemic Stroke. Ann Neurol 2020;88:1229-36.
58. Chen L, Fan Z, Sun X, et al. Mendelian Randomization Rules Out Causation Between Inflammatory Bowel Disease and Non-Alcoholic Fatty Liver Disease. Front Pharmacol 2022;13:891410.
59. Hirtz R, Hars C, Naaresh R, et al. Causal Effect of Age at Menarche on the Risk for Depression: Results From a Two-Sample Multivariable Mendelian Randomization Study. Front Genet 2022;13:918584.
60. Siland JE, Geelhoed B, Roselli C, et al. Resting heart rate and incident atrial fibrillation: A stratified Mendelian randomization in the AFGen consortium. PLoS One 2022;17:e0268768.
61. Gajendragadkar PR, Von Ende A, Ibrahim M, et al. Assessment of the causal relevance of ECG parameters for risk of atrial fibrillation: A mendelian randomisation study. PLoS Med 2021;18:e1003572.
62. Stewart A, Huang J, Fisher RA. RGS Proteins in Heart: Brakes on the Vagus. Front Physiol 2012;3:95.
63. Vanderlei LC, Pastre CM, Hoshi RA, et al. Basic notions of heart rate variability and its clinical applicability. Rev Bras Cir Cardiovasc 2009;24:205-17.
64. Hossain MN, Sakemura R, Fujii M, et al. G-protein gamma subunit GNG11 strongly regulates cellular senescence. Biochem Biophys Res Commun 2006;351:645-50.
65. Balcueva EA, Wang Q, Hughes H, et al. Human G protein gamma(11) and gamma(14) subtypes define a new functional subclass. Exp Cell Res 2000;257:310-9.
66. Hardouin SN, Richmond KN, Zimmerman A, et al. Altered cardiovascular responses in mice lacking the M(1) muscarinic acetylcholine receptor. J Pharmacol Exp Ther 2002;301:129-37.
67. Drici MD, Diochot S, Terrenoire C, et al. The bee venom peptide tertiapin underlines the role of I(KACh) in acetylcholine-induced atrioventricular blocks. Br J Pharmacol 2000;131:569-77.
68. Cifelli C, Rose RA, Zhang H, et al. RGS4 regulates parasympathetic signaling and heart rate control in the sinoatrial node. Circ Res 2008;103:527-35.
69. Catterall WA. Regulation of Cardiac Calcium Channels in the Fight-or-Flight Response. Curr Mol Pharmacol 2015;8:12-21.
70. Schwertfeger E, Klein T, Vonend O, et al. Neuropeptide Y inhibits acetylcholine release in human heart atrium by activation of Y2-receptors. Naunyn Schmiedebergs Arch

Pharmacol 2004;369:455-61.
71. Herring N, Cranley J, Lokale MN, et al. The cardiac sympathetic co-transmitter galanin reduces acetylcholine release and vagal bradycardia: implications for neural control of cardiac excitability. J Mol Cell Cardiol 2012;52:667-76.
72. Guasch E, Mont L. Diagnosis, pathophysiology, and management of exercise-induced arrhythmias. Nat Rev Cardiol 2017;14:88-101.
73. Tischer SG, Graff C, Ellervik C, et al. Influence of type of sport on cardiac repolarization assessed by electrocardiographic T-wave morphology combination score. J Electrocardiol 2018;51:296-302.
74. Iellamo F, Pigozzi F, Spataro A, et al. T-wave and heart rate variability changes to assess training in world-class athletes. Med Sci Sports Exerc 2004;36:1342-6.
75. Riera AR, Ferreira C, Ferreira Filho C, et al. Clinical value of lead aVR. Ann Noninvasive Electrocardiol 2011;16:295-302.
76. Khan AA, Lip GYH, Shantsila A. Heart rate variability in atrial fibrillation: The balance between sympathetic and parasympathetic nervous system. Eur J Clin Invest 2019;49:e13174.
77. Lloyd-Jones DM, Wang TJ, Leip EP, et al. Lifetime risk for development of atrial fibrillation: the Framingham Heart Study. Circulation 2004;110:1042-6.
78. Huxley RR, Lopez FL, Folsom AR, et al. Absolute and attributable risks of atrial fibrillation in relation to optimal and borderline risk factors: the Atherosclerosis Risk in Communities (ARIC) study. Circulation 2011;123:1501-8.
79. Krijthe BP, Kunst A, Benjamin EJ, et al. Projections on the number of individuals with atrial fibrillation in the European Union, from 2000 to 2060. Eur Heart J 2013;34:2746-51.
80. Granger CB, Alexander JH, McMurray JJ, et al. Apixaban versus warfarin in patients with atrial fibrillation. N Engl J Med 2011;365:981-92.
81. Hohnloser SH, Crijns HJ, van Eickels M, et al. Effect of dronedarone on cardiovascular events in atrial fibrillation. N Engl J Med 2009;360:668-78.
82. Granada J, Uribe W, Chyou PH, et al. Incidence and predictors of atrial flutter in the general population. J Am Coll Cardiol 2000;36:2242-6.
83. Waldo AL, Feld GK. Inter-relationships of atrial fibrillation and atrial flutter mechanisms and clinical implications. J Am Coll Cardiol 2008;51:779-86.
84. Burgess S, Davies NM, Thompson SG. Bias due to participant overlap in two-sample Mendelian randomization. Genet Epidemiol 2016;40:597-608.

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Appendix 1

Removal of abnormal instrumental variables

Atrial fibrillation

When resting heart rate (HR) was used as the exposure, no duplicated single-nucleotide polymorphisms (SNPs), after the outliers (rs1015451, rs11153730, rs17287293, rs4489968) were detected and removed by Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO), a total of 12 SNPs were extracted as instrumental variables.

When heart rate variability_root mean square of successive differences (HRV_RMSSD) was used as the exposure, no duplicated SNPs, after the outlier (rs10842383) was detected and removed by MR-PRESSO, a total of 6 SNPs were extracted as instrumental variables.

When heart rate variability_peak-valley respiratory sinus arrhythmia or high frequency power (HRV_pvRSA/HF) was used as the exposure, no duplicated SNPs, after the outlier (rs10842383) was detected and removed by MR-PRESSO, a total of 4 SNPs were extracted as instrumental variables.

When heart rate variability_ standard deviation of the normal-to-normal interval (HRV_SDNN) was used as the exposure, the duplicated SNP (rs1384598) was removed, and after the outliers (rs10842383, rs11922153, rs2349556, rs2680344) were detected and removed by MR-PRESSO, a total of 17 SNPs were extracted as instrumental variables.

When the PR interval was used as the exposure, the following duplicated SNPs were removed (rs10786206, rs12190287, rs2395137, rs2540286, rs2912774, rs6084574, rs6496452, rs7660883).

Detection and removal of outliers were conducted by MR-PRESSO (rs10226357, rs1031262, rs10883908, rs11047497, rs11067104, rs11191116, rs12869776, rs12889267, rs144789148, rs1692144, rs17287293, rs17446418, rs1769758, rs1997571, rs2042995, rs214502, rs2403550, rs2629745, rs28709422, rs287606, rs2970854, rs3176326, rs36034102, rs3922843, rs4245338, rs4390169, rs4655001, rs55734480, rs56049025, rs59212267, rs60632610, rs62521284, rs6682872, rs6900627, rs72690486, rs763076, rs7645178, rs883079).

The outliers were removed by IVW radial regression (rs1037100, rs10516755, rs10784418, rs11153730, rs1124477, rs116757907, rs11689011, rs1173743, rs117845584, rs1188285, rs12228985, rs12443745, rs12513783, rs12751675, rs12961264, rs13032472, rs1337024, rs17496249, rs1986524, rs2072966, rs2137897, rs2163107, rs2243857, rs231276, rs2947080, rs29795, rs35680304, rs35816944, rs3747570, rs3794809, rs3825751, rs41306688, rs4871397, rs55679363, rs56352403, rs565720, rs57214150, rs61824887, rs61928421, rs62008078, rs62472627, rs6435953, rs6599230, rs6599240, rs6791171, rs6824178, rs7552783, rs762032, rs78518764, rs7997798, rs881299), and a total of 156 SNPs were extracted as instrumental variables.

When using P-wave terminal force as the exposure, the duplicated SNP (rs251492) was removed, and after the outliers (rs12144965, rs445754) were detected and removed by MR-PRESSO and the outliers (rs10890369, rs11242779, rs4435363, rs7939513) were detected and removed by IVW radial regression, a total of 22 SNPs were extracted as instrumental variables.

When using P-wave duration as the exposure, the duplicated SNPs (rs41312411, rs6773331, rs6790396) were removed, and after the outliers (rs11894252, rs1467026, rs3807989, rs452036, rs7312625) were detected and removed by MR-PRESSO, a total of 4 SNPs were extracted as instrumental variables.

When T-wave top amplitude_anterior [electrocardiogram (ECG) lead V3 + V4 + aVL] was used as the exposure, the duplicated SNP (rs6790396) was removed, and after the outliers (rs7633988, rs7638909) were detected and removed by MR-PRESSO and the outliers (rs11134683, rs16928366) were detected and removed by IVW radial regression, a total of 10 SNPs were retained as instrumental variables.

When T-wave top amplitude_avr (ECG lead aVR) was used as the exposure, the duplicated SNPs (rs12374310, rs4233994) were removed, and after the outliers (rs4076737, rs7638909, rs7846485) were detected and removed by MR-PRESSO and the outliers (rs11134683, rs16928366) were detected and removed by IVW radial regression, a total of 20 SNPs were extracted as instrumental variables.

When T-wave top amplitude_inferior (ECG lead II + III + aVF) was used as the exposure, the duplicated SNP (rs6790396) was removed, and after the outliers (rs7638909, rs9851710) were detected and removed by MR-PRESSO, a total of 16 SNPs were extracted as instrumental variables.

When T-wave top amplitude_lateral (ECG lead I + aVL + V5 + V6) was used as the exposure, no duplicated SNPs, after

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the outliers (rs6783110, rs7633988) were detected and removed by MR-PRESSO and the outliers (rs17128209, rs7191330, rs728926) were detected and removed by IVW radial regression, a total of 14 SNPs were extracted as instrumental variables.

When T-wave top amplitude_septal (ECG lead V1 + V2) was used as the exposure, the duplicated SNP (rs12623169) was removed, and after the outliers (rs10111852, rs10850409) were detected and removed by MR-PRESSO, a total of 9 SNPs were extracted as instrumental variables.

Bradycardia

When heart rate was used as the exposure, a total of 10 SNPs were extracted as instrumental variables, no duplicated SNPs, and no outliers were detected by MR-PRESSO.

When HRV_RMSSD was used as the exposure, a total of 18 SNPs were extracted as instrumental variables, no duplicated SNPs, and no outliers were detected by MR-PRESSO.

When HRV_pvRSA/HF was used as the exposure, a total of 6 SNPs were extracted as instrumental variables, no duplicated SNPs, and no outliers were detected by MR-PRESSO.

When HRV_SDNN was used as the exposure, after removing the duplicated SNP: rs1384598, a total of 10 SNPs were extracted as instrumental variables, and no outliers were detected by MR-PRESSO.

When the PR interval was used as the exposure, the duplicated SNPs (rs10786206, rs2540286, rs6084574 and rs6496452) were removed.

Detection and removal of outliers (rs12190287 and rs3746471) were conducted by MR-PRESSO.

The outliers (rs13032472, rs34187723, rs3795063, rs62008078, rs66507060 and rs696) were removed by IVW radial regression and Egger radial regression, and a total of 102 SNPs were extracted as instrumental variables.

When using P-wave terminal force as the exposure, a total of 10 SNPs were extracted as instrumental variables after removing the duplicated SNP (rs251492), and no outliers were detected by MR-PRESSO.

When using P-wave duration as the exposure, the duplicated SNP (rs6790396) was removed, and no outliers were detected by MR-PRESSO, but the heterogeneity test suggested the existence of outliers, so the outliers were removed by IVW radial regression and Egger radial regression (rs1467026 and rs4276421), and a total of 4 SNPs were retained as instrumental variables.

When T-wave top amplitude_anterior was used as the exposure, the duplicated SNP (rs6790396) was removed, no outliers were detected by MR-PRESSO, but the heterogeneity test suggested the existence of outliers, so the outlier was removed by IVW radial regression and Egger radial regression (rs686930), a total of 7 SNPs were extracted as instrumental variables.

When T-wave top amplitude_avr was used as the exposure, there were no duplicated SNPs and no outliers were detected by MR-PRESSO, but the heterogeneity test suggested the existence of outliers, so the outlier was removed by IVW radial regression and Egger radial regression (rs10842350), a total of 4 SNPs were extracted as instrumental variables.

When T-wave top amplitude_inferior was used as the exposure, the duplicated SNP (rs6790396) was removed, and no outliers were detected by MR-PRESSO, a total of 7 SNPs were extracted as instrumental variables.

When T-wave top amplitude_lateral was used as the exposure, there were no duplicated SNPs, and after the outliers (rs10842350, rs6783110, rs7191330 and rs7633988) were detected and removed by MR-PRESSO a total of 8 SNPs were extracted as instrumental variables.

When T-wave top amplitude_septal was used as the exposure, the duplicated SNP (rs12623169) was removed. MR-PRESSO did not detect outliers, and a total of 5 SNPs were extracted as instrumental variables.

Supraventricular tachycardia

When heart rate was used as the exposure, a total of 10 SNPs were extracted as instrumental variables, no duplicated SNPs, and no outliers were detected by MR-PRESSO.

When HRV_RMSSD was used as the exposure, a total of 4 SNPs were extracted as instrumental variables, no duplicated SNPs, and no outliers were detected by MR-PRESSO.

When HRV_pvRSA/HF was used as the exposure, a total of 4 SNPs were extracted as instrumental variables, no duplicated SNPs, and no outliers were detected by MR-PRESSO.

When HRV_SDNN was used as the exposure, after removing the duplicated SNP (rs1384598), a total of 10 SNPs were

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extracted as instrumental variables, and no outliers were detected by MR-PRESSO.

When the PR interval was used as the exposure, the following duplicated SNPs (rs10786206, rs2540286, rs6084574, rs6496452) were removed. A total of 116 SNPs were extracted as instrumental variables, and no outliers were detected by MR-PRESSO.

When using P-wave terminal force as the exposure, a total of 10 SNPs were extracted as instrumental variables after removing the duplicated SNP (rs251492). and no outliers were detected by MR-PRESSO.

When using P-wave duration as the exposure, after removing the duplicated SNP (rs6790396) and detecting and removing the outlier (rs452036) by MR-PRESSO, the heterogeneity test still suggested that there were potential outliers affecting the results, and after removing the outlier (rs11894252) by IVW radial regression and Egger radial regression, a total of 4 SNPs were extracted as instrumental variables.

When T-wave top amplitude_anterior was used as the exposure, the duplicated SNP (rs6790396) was removed. After detecting and removing the outlier (rs6807275) by MR-PRESSO, a total of 7 SNPs were retained as instrumental variables.

When T-wave top amplitude_avr was used as the exposure, there were no duplicated SNPs and no outliers were detected by MR-PRESSO, and a total of 5 SNPs were extracted as instrumental variables.

When T-wave top amplitude_inferior was used as the exposure, the duplicated SNP (rs6790396) was removed. MR-PRESSO did not detect outliers, and a total of 8 SNPs were extracted as instrumental variables.

When T-wave top amplitude_lateral was used as the exposure, there were no duplicated SNPs and no outliers were detected by MR-PRESSO, and a total of 4 SNPs were extracted as instrumental variables.

When T-wave top amplitude_septal was used as the exposure, the duplicated SNP (rs12623169) was removed. MR-PRESSO did not detect outliers, and a total of 6 SNPs were extracted as instrumental variables.

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Table S1 Removal of abnormal instrument variables

Outcome	Exposure	Threshold	Duplicated SNPs	MR-PRESSO	MR-Radial	nSNP
AF	HR	5×10^{-8}	√	4	√	12
	HRV_RMSSD	5×10^{-8}	√	1	√	6
	HRV_pvRSA/HF	5×10^{-8}	√	1	√	4
	HRV_SDNN	1×10^{-5}	1	4	√	17
	PR	5×10^{-8}	8	38	51	156
	Ptforce	1×10^{-5}	1	2	4	22
	Pwave Duration	5×10^{-8}	3	5	√	4
	Twave_anterior	1×10^{-5}	1	2	2	10
	Twave_avr	1×10^{-5}	2	3	4	20
	Twave_inferior	1×10^{-5}	1	2	√	16
	Twave_lateral	1×10^{-5}	√	2	3	14
	Twave_septal	1×10^{-5}	1	2	√	9
Bradycardia	HR	5×10^{-8}	√	√	√	10
	HRV_RMSSD	1×10^{-5}	√	√	√	18
	HRV_pvRSA/HF	1×10^{-5}	√	√	√	6
	HRV_SDNN	1×10^{-5}	1	√	√	10
	PR	5×10^{-8}	4	2	6	102
	Ptforce	1×10^{-5}	1	√	√	10
	Pwave Duration	5×10^{-8}	1	√	2	4
	Twave_anterior	1×10^{-5}	1	√	1	7
	Twave_avr	5×10^{-8}	√	√	1	4
	Twave_inferior	1×10^{-5}	1	√	√	7
	Twave_lateral	1×10^{-5}	4	√	√	8
	Twave_septal	1×10^{-5}	1	√	√	5
Supraventricular tachycardia	HR	5×10^{-8}	√	√	√	10
	HRV_RMSSD	5×10^{-8}	√	√	√	4
	HRV_pvRSA/HF	5×10^{-8}	√	√	√	4
	HRV_SDNN	1×10^{-5}	1	√	√	10
	PR	5×10^{-8}	4	√	√	116
	Ptforce	1×10^{-5}	1	√	√	10
	Pwave Duration	5×10^{-8}	1	1	1	4
	Twave_anterior	1×10^{-5}	√	1	√	7
	Twave_avr	5×10^{-8}	√	√	√	5
	Twave_inferior	1×10^{-5}	1	√	√	8
	Twave_lateral	5×10^{-8}	√	√	√	4
	Twave_septal	1×10^{-5}	1	√	√	6

SNP, single-nucleotide polymorphism; MR-PRESSO, number of SNPs removed by MR-PRESSO; MR-Radial, number of SNPs removed by IVW radial regression and Egger radial regression; nSNP, number of SNPs used for MR analysis; √, no abnormal SNPs were removed; AF, atrial fibrillation; HR, resting heart rate; Twave, T-wave top amplitude; septal: ECG lead V1 + V2; lateral: ECG lead I + aVL + V5 + V6; inferior, ECG lead II + III + aVF; anterior: ECG lead V3 + V4 + aVL; avr, ECG lead aVR; Ptforce, P-wave terminal force; Pwave duration, P-wave duration; PR, PR interval; HRV, heart rate variability; RMSSD, root mean square of successive differences; pvRSA/HF, peak-valley respiratory sinus arrhythmia or high frequency power; SDNN, standard deviation of the normal-to-normal interval.

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Table S2 Results of univariate Mendelian randomization

Outcome	Exposure	Method	nSNP	P value	OR	or_lci95	or_uci95
AF	HR	Inverse variance weighted	12	0.07	0.981	0.961	1.001
		MR Egger	12	0.68	1.018	0.938	1.104
		Weighted median	12	0.71	0.996	0.974	1.018
		Weighted mode	12	0.89	0.998	0.971	1.026
		Inverse variance weighted (multiplicative random effects)	12	0.07	0.981	0.961	1.001
	HRV_RMSSD	Inverse variance weighted	6	0.02	1.309	1.045	1.640
		MR Egger	6	0.78	0.919	0.525	1.610
		Weighted median	6	0.33	1.154	0.868	1.535
		Weighted mode	6	0.55	1.123	0.786	1.603
		Inverse variance weighted (multiplicative random effects)	6	0.01	1.309	1.067	1.605
	HRV_pvRSA/HF	Inverse variance weighted	4	0.06	1.141	0.995	1.308
		MR Egger	4	0.95	0.990	0.770	1.273
		Weighted median	4	0.33	1.082	0.925	1.267
		Weighted mode	4	0.498	1.068	0.903	1.264
		Inverse variance weighted (multiplicative random effects)	4	0.06	1.141	0.995	1.308
	HRV_SDNN	Inverse variance weighted	17	0.004	1.518	1.140	2.022
		MR Egger	17	0.26	2.092	0.604	7.247
		Weighted median	17	0.12	1.338	0.930	1.926
		Weighted mode	17	0.23	1.406	0.820	2.411
		Inverse variance weighted (multiplicative random effects)	17	0.004	1.518	1.140	2.022
	PR	Inverse variance weighted	156	<0.001	0.994	0.991	0.997
		MR Egger	156	0.19	0.995	0.987	1.003
		Weighted median	156	0.003	0.993	0.988	0.998
		Weighted mode	156	0.07	0.993	0.985	1.001
		Inverse variance weighted (multiplicative random effects)	156	<0.001	0.994	0.991	0.997
	Ptforce	Inverse variance weighted	22	0.46	1.000	1.000	1.000
		MR Egger	22	0.80	1.000	1.000	1.000
		Weighted median	22	0.81	1.000	1.000	1.000
		Weighted mode	22	0.81	1.000	1.000	1.000
		Inverse variance weighted (multiplicative random effects)	22	0.43	1.000	1.000	1.000
	Pwave duration	Inverse variance weighted	4	0.52	0.994	0.976	1.012
		MR Egger	4	0.68	0.985	0.924	1.050
		Weighted median	4	0.54	0.994	0.973	1.014
		Weighted mode	4	0.50	0.989	0.962	1.017
		Inverse variance weighted (multiplicative random effects)	4	0.10	0.994	0.987	1.001
	Twave_anterior	Inverse variance weighted	10	0.17	0.972	0.933	1.012
		MR Egger	10	0.14	0.962	0.918	1.008
		Weighted median	10	0.15	0.965	0.920	1.013
		Weighted mode	10	0.16	0.965	0.921	1.010
		Inverse variance weighted (multiplicative random effects)	10	0.13	0.972	0.937	1.008
	Twave_avr	Inverse variance weighted	20	0.02	1.076	1.012	1.144
		MR Egger	20	0.30	1.099	0.926	1.304
		Weighted median	20	0.07	1.083	0.993	1.181
		Weighted mode	20	0.31	1.069	0.945	1.209
		Inverse variance weighted (multiplicative random effects)	20	0.005	1.076	1.022	1.133
	Twave_inferior	Inverse variance weighted	16	0.41	0.964	0.885	1.050
		MR Egger	16	0.28	0.830	0.600	1.148
		Weighted median	16	0.61	0.969	0.861	1.092
		Weighted mode	16	0.76	0.967	0.789	1.186
		Inverse variance weighted (multiplicative random effects)	16	0.41	0.964	0.885	1.050
	Twave_lateral	Inverse variance weighted	14	0.72	0.990	0.935	1.048
		MR Egger	14	0.99	1.000	0.909	1.101
		Weighted median	14	0.48	0.971	0.897	1.052
		Weighted mode	14	0.45	0.952	0.843	1.075
		Inverse variance weighted (multiplicative random effects)	14	0.69	0.990	0.941	1.041
	Twave_septal	Inverse variance weighted	9	0.10	0.897	0.788	1.021
		MR Egger	9	0.66	1.100	0.730	1.658
		Weighted median	9	0.50	0.953	0.829	1.095
		Weighted mode	9	0.71	0.969	0.828	1.134
		Inverse variance weighted (multiplicative random effects)	9	0.10	0.897	0.788	1.021

Table S2 (*continued*)

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Table S2 (*continued*)

Outcome	Exposure	Method	nSNP	P value	OR	or_lci95	or_uci95
Bradycardia	HR	Inverse variance weighted	10	<0.001	1.000	0.999	1.000
		MR Egger	10	0.93	1.000	0.999	1.001
		Weighted median	10	0.001	1.000	0.999	1.000
		Weighted mode	10	0.02	0.999	0.999	1.000
		Inverse variance weighted (multiplicative random effects)	10	<0.001	1.000	0.999	1.000
	HRV_RMSSD	Inverse variance weighted	18	0.02	1.002	1.000	1.004
		MR Egger	18	0.59	1.003	0.992	1.014
		Weighted median	18	0.21	1.002	0.999	1.004
		Weighted mode	18	0.85	1.000	0.995	1.004
		Inverse variance weighted (multiplicative random effects)	18	0.01	1.002	1.001	1.004
	HRV_pvRSA/HF	Inverse variance weighted	6	0.26	1.001	0.999	1.003
		MR Egger	6	0.76	1.002	0.992	1.011
		Weighted median	6	0.92	1.000	0.998	1.002
		Weighted mode	6	0.74	0.999	0.996	1.003
		Inverse variance weighted (multiplicative random effects)	6	0.26	1.001	0.999	1.003
	HRV_SDNN	Inverse variance weighted	10	0.03	1.004	1.000	1.007
		MR Egger	10	0.14	1.016	0.997	1.035
		Weighted median	10	0.058	1.004	1.000	1.009
		Weighted mode	10	0.09	1.006	1.000	1.013
		Inverse variance weighted (multiplicative random effects)	10	0.03	1.004	1.000	1.007
	PR	Inverse variance weighted	102	0.12	1.000	1.000	1.000
		MR Egger	102	0.53	1.000	1.000	1.000
		Weighted median	102	0.89	1.000	1.000	1.000
		Weighted mode	102	0.62	1.000	1.000	1.000
		Inverse variance weighted (multiplicative random effects)	102	0.12	1.000	1.000	1.000
	Ptforce	Inverse variance weighted	10	0.98	1.000	1.000	1.000
		MR Egger	10	0.81	1.000	1.000	1.000
		Weighted median	10	0.83	1.000	1.000	1.000
		Weighted mode	10	0.72	1.000	1.000	1.000
		Inverse variance weighted (multiplicative random effects)	10	0.98	1.000	1.000	1.000
	Pwave Duration	Inverse variance weighted	4	0.30	1.000	1.000	1.000
		MR Egger	4	0.70	0.999	0.997	1.002
		Weighted median	4	0.19	1.000	1.000	1.000
		Weighted mode	4	0.36	1.000	1.000	1.000
		Inverse variance weighted (multiplicative random effects)	4	0.15	1.000	1.000	1.000
	Twave_anterior	Inverse variance weighted	7	0.02	1.001	1.000	1.003
		MR Egger	7	0.29	1.002	0.998	1.006
		Weighted median	7	0.01	1.002	1.000	1.003
		Weighted mode	7	0.06	1.002	1.000	1.004
		Inverse variance weighted (multiplicative random effects)	7	0.02	1.001	1.000	1.003
	Twave_avr	Inverse variance weighted	4	<0.001	0.998	0.997	0.999
		MR Egger	4	0.61	0.999	0.996	1.002
		Weighted median	4	<0.001	0.998	0.997	0.999
		Weighted mode	4	0.09	0.998	0.997	1.000
		Inverse variance weighted (multiplicative random effects)	4	<0.001	0.998	0.997	0.998
	Twave_inferior	Inverse variance weighted	7	0.29	1.001	0.999	1.002
		MR Egger	7	0.04	1.004	1.001	1.007
		Weighted median	7	0.11	1.001	1.000	1.003
		Weighted mode	7	0.11	1.002	1.000	1.003
		Inverse variance weighted (multiplicative random effects)	7	0.29	1.001	0.999	1.002
	Twave_lateral	Inverse variance weighted	8	0.33	1.000	1.000	1.001
		MR Egger	8	0.68	1.000	0.998	1.003
		Weighted median	8	0.35	1.001	0.999	1.002
		Weighted mode	8	0.42	1.001	0.999	1.002
		Inverse variance weighted (multiplicative random effects)	8	0.33	1.000	1.000	1.001
	Twave_septal	Inverse variance weighted	5	0.45	1.001	0.999	1.002
		MR Egger	5	0.16	1.009	0.999	1.018
		Weighted median	5	0.25	1.001	0.999	1.003
		Weighted mode	5	0.32	1.001	0.999	1.004
		Inverse variance weighted (multiplicative random effects)	5	0.42	1.001	0.999	1.002

Table S2 (*continued*)

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Table S2 (*continued*)

Outcome	Exposure	Method	nSNP	P value	OR	or_lci95	or_uci95
Supraventricular tachycardia	HR	Inverse variance weighted	10	<0.001	1.000	1.000	1.001
		MR Egger	10	0.73	1.000	0.999	1.001
		Weighted median	10	0.006	1.000	1.000	1.001
		Weighted mode	10	0.10	1.000	1.000	1.001
		Inverse variance weighted (multiplicative random effects)	10	<0.001	1.000	1.000	1.001
	HRV_RMSSD	Inverse variance weighted	4	0.52	0.999	0.995	1.003
		MR Egger	4	0.37	0.984	0.957	1.011
		Weighted median	4	0.13	0.997	0.993	1.001
		Weighted mode	4	0.24	0.997	0.993	1.001
		Inverse variance weighted (multiplicative random effects)	4	0.52	0.999	0.995	1.003
	HRV_pvRSA/HF	Inverse variance weighted	4	0.93	1.000	0.998	1.002
		MR Egger	4	0.64	1.004	0.991	1.017
		Weighted median	4	0.27	0.999	0.997	1.001
		Weighted mode	4	0.47	0.999	0.995	1.002
		Inverse variance weighted (multiplicative random effects)	4	0.93	1.000	0.998	1.002
	HRV_SDNN	Inverse variance weighted	10	0.33	1.002	0.998	1.005
		MR Egger	10	0.82	0.998	0.979	1.017
		Weighted median	10	0.89	1.000	0.995	1.004
		Weighted mode	10	0.60	0.998	0.991	1.005
		Inverse variance weighted (multiplicative random effects)	10	0.33	1.002	0.998	1.005
	PR	Inverse variance weighted	116	0.001	1.000	1.000	1.000
		MR Egger	116	0.02	1.000	1.000	1.000
		Weighted median	116	0.03	1.000	1.000	1.000
		Weighted mode	116	0.03	1.000	1.000	1.000
		Inverse variance weighted (multiplicative random effects)	116	0.001	1.000	1.000	1.000
	Ptforce	Inverse variance weighted	10	0.94	1.000	1.000	1.000
		MR Egger	10	0.36	1.000	1.000	1.000
		Weighted median	10	0.51	1.000	1.000	1.000
		Weighted mode	10	0.52	1.000	1.000	1.000
		Inverse variance weighted (multiplicative random effects)	10	0.92	1.000	1.000	1.000
	Pwave Duration	Inverse variance weighted	4	0.007	1.000	1.000	1.000
		MR Egger	4	0.72	1.001	0.996	1.006
		Weighted median	4	0.02	1.000	0.999	1.000
		Weighted mode	4	0.16	1.000	0.999	1.000
		Inverse variance weighted (multiplicative random effects)	4	<0.001	1.000	1.000	1.000
	Twave_anterior	Inverse variance weighted	7	0.68	1.000	0.998	1.001
		MR Egger	7	0.68	1.001	0.996	1.006
		Weighted median	7	0.75	1.000	0.998	1.001
		Weighted mode	7	0.76	1.000	0.998	1.001
		Inverse variance weighted (multiplicative random effects)	7	0.68	1.000	0.998	1.001
	Twave_avr	Inverse variance weighted	5	0.87	1.000	0.999	1.001
		MR Egger	5	0.77	1.001	0.997	1.004
		Weighted median	5	0.88	1.000	0.999	1.001
		Weighted mode	5	0.89	1.000	0.999	1.001
		Inverse variance weighted (multiplicative random effects)	5	0.87	1.000	0.999	1.001
	Twave_inferior	Inverse variance weighted	8	0.50	1.000	0.998	1.001
		MR Egger	8	0.14	1.003	1.000	1.006
		Weighted median	8	0.75	1.000	0.998	1.001
		Weighted mode	8	0.85	1.000	0.998	1.001
		Inverse variance weighted (multiplicative random effects)	8	0.50	1.000	0.998	1.001
	Twave_lateral	Inverse variance weighted	4	0.45	1.000	0.998	1.001
		MR Egger	4	0.93	1.000	0.995	1.006
		Weighted median	4	0.61	1.000	0.998	1.001
		Weighted mode	4	0.91	1.000	0.998	1.001
		Inverse variance weighted (multiplicative random effects)	4	0.33	1.000	0.999	1.000
	Twave_septal	Inverse variance weighted	6	0.40	1.001	0.999	1.002
		MR Egger	6	0.22	0.998	0.994	1.001
		Weighted median	6	0.83	1.000	0.998	1.001
		Weighted mode	6	0.43	0.999	0.998	1.001
		Inverse variance weighted (multiplicative random effects)	6	0.40	1.001	0.999	1.002

nSNP, number of SNPs used as instrumental variables; SNP, single-nucleotide polymorphism; OR, odds ratio; or_lci95, lower limit of the odds ratio; or_uci95, upper limit of the odds ratio; AF, atrial fibrillation; HR, resting heart rate; Twave, T-wave top amplitude; septal, ECG lead V1 + V2; lateral, ECG lead I + aVL + V5 + V6; inferior: ECG lead II + III + aVF; anterior: ECG lead V3 + V4 + aVL; avr, ECG lead aVR; Ptforce: P-wave terminal force; Pwave duration, P-wave duration; PR, PR interval; HRV, heart rate variability; RMSSD, root mean square of successive differences; pvRSA/HF, peak-valley respiratory sinus arrhythmia or high frequency power; SDNN, standard deviation of the normal-to-normal interval.

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Table S3 Results of heterogeneity test

Outcome	Exposure	Method	Q	Q_df	Q_pval	I^2
Atrial fibrillation	HR	Inverse variance weighted	17.595	11	0.091	37.482%
	HR	Inverse variance weighted (multiplicative random effects)	17.595	11	0.091	37.482%
	HR	MR Egger	16.237	10	0.093	38.413%
	HRV_RMSSD	MR Egger	2.266	4	0.687	0
	HRV_RMSSD	Inverse variance weighted	4.090	5	0.537	0
	HRV_RMSSD	Inverse variance weighted (multiplicative random effects)	4.090	5	0.537	0
	HRV_pvRSA/HF	MR Egger	1.620	2	0.445	0
	HRV_pvRSA/HF	Inverse variance weighted	3.293	3	0.349	8.893%
	HRV_pvRSA/HF	Inverse variance weighted (multiplicative random effects)	3.293	3	0.349	8.893%
	HRV_SDNN	Inverse variance weighted	23.366	16	0.104	31.526%
	HRV_SDNN	Inverse variance weighted (multiplicative random effects)	23.366	16	0.104	31.526%
	HRV_SDNN	MR Egger	22.952	15	0.085	34.647%
	PR	Inverse variance weighted	142.605	155	0.754	0
	PR	Inverse variance weighted (multiplicative random effects)	142.605	155	0.754	0
	PR	MR Egger	142.487	154	0.737	0
	Ptforce	Inverse variance weighted	18.606	21	0.610	0
	Ptforce	Inverse variance weighted (multiplicative random effects)	18.606	21	0.610	0
	Ptforce	MR Egger	18.407	20	0.561	0
	Pwave Duration	Inverse variance weighted	0.454	3	0.929	0
	Pwave Duration	Inverse variance weighted (multiplicative random effects)	0.454	3	0.929	0
	Pwave Duration	MR Egger	0.365	2	0.833	0
	Pwave Duration	Inverse variance weighted	7.337	9	0.602	0
	Pwave Duration	Inverse variance weighted (multiplicative random effects)	7.337	9	0.602	0
	Pwave Duration	MR Egger	6.573	8	0.583	0
	Twave_anterior	Inverse variance weighted	13.471	19	0.814	0
	Twave_anterior	Inverse variance weighted (multiplicative random effects)	13.471	19	0.814	0
	Twave_anterior	MR Egger	13.405	18	0.767	0
	Twave_avr	Inverse variance weighted	19.693	15	0.184	23.830%
	Twave_avr	Inverse variance weighted (multiplicative random effects)	19.693	15	0.184	23.830%
	Twave_avr	MR Egger	18.524	14	0.184	24.421%
	Twave_inferior	Inverse variance weighted	10.084	13	0.687	0
	Twave_inferior	Inverse variance weighted (multiplicative random effects)	10.084	13	0.687	0
	Twave_inferior	MR Egger	10.011	12	0.615	0
	Twave_lateral	MR Egger	12.383	7	0.089	43.473%
	Twave_lateral	Inverse variance weighted	14.258	8	0.075	43.893%
	Twave_lateral	Inverse variance weighted (multiplicative random effects)	14.258	8	0.075	43.893%
	Twave_septal	Inverse variance weighted	17.595	11	0.091	37.482%
	Twave_septal	Inverse variance weighted (multiplicative random effects)	17.595	11	0.091	37.482%
	Twave_septal	MR Egger	16.237	10	0.093	38.413%
Bradycardia	HR	Inverse variance weighted	9.612	9	0.383	6.367%
		MR Egger	8.506	8	0.386	5.953%
		Inverse variance weighted (multiplicative random effects)	9.612	9	0.383	6.367%
	HRV_RMSSD	Inverse variance weighted	13.940	17	0.671	0
		MR Egger	13.921	16	0.605	0
		Inverse variance weighted (multiplicative random effects)	13.940	17	0.671	0
	HRV_pvRSA/HF	Inverse variance weighted	7.298	5	0.199	31.492%
		MR Egger	7.275	4	0.122	45.019%
		Inverse variance weighted (multiplicative random effects)	7.298	5	0.199	31.492%
	HRV_SDNN	Inverse variance weighted	11.281	9	0.257	20.217%
		MR Egger	9.450	8	0.306	15.347%
		Inverse variance weighted (multiplicative random effects)	11.281	9	0.257	20.217%
	PR	Inverse variance weighted	107.607	101	0.308	6.140%
		MR Egger	107.586	100	0.284	7.051%
		Inverse variance weighted (multiplicative random effects)	107.607	101	0.308	6.140%
	Ptforce	Inverse variance weighted	7.320	9	0.604	0
		MR Egger	7.252	8	0.510	0
		Inverse variance weighted (multiplicative random effects)	7.320	9	0.604	0

Table S3 (*continued*)

https://dx.doi.org/10.21037/jtd-24-814

Table S3 (*continued*)

Outcome	Exposure	Method	Q	Q_df	Q_pval	I^2
	Pwave Duration	Inverse variance weighted	1.576	3	0.665	0
		MR Egger	1.434	2	0.488	0
		Inverse variance weighted (multiplicative random effects)	1.576	3	0.665	0
		MR Egger	1.299	2	0.522	0
		Inverse variance weighted (multiplicative random effects)	1.353	3	0.717	0
	Twave_anterior	Inverse variance weighted	7.400	6	0.285	18.917%
		MR Egger	7.121	5	0.212	29.784%
		Inverse variance weighted (multiplicative random effects)	7.400	6	0.285	18.917%
	Twave_avr	Inverse variance weighted	0.884	3	0.829	0
		MR Egger	0.195	2	0.907	0
		Inverse variance weighted (multiplicative random effects)	0.884	3	0.829	0
	Twave_inferior	Inverse variance weighted	9.769	6	0.135	38.583%
		MR Egger	3.940	5	0.558	0
		Inverse variance weighted (multiplicative random effects)	9.769	6	0.135	38.583%
	Twave_lateral	Inverse variance weighted	7.979	7	0.334	12.271%
		MR Egger	7.979	6	0.240	24.804%
		Inverse variance weighted (multiplicative random effects)	7.979	7	0.334	12.271%
	Twave_septal	Inverse variance weighted	3.403	4	0.493	0
		MR Egger	0.390	3	0.942	0
		Inverse variance weighted (multiplicative random effects)	3.403	4	0.493	0
Supraventricular tachycardia	HR	Inverse variance weighted	7.357	9	0.600	0
		MR Egger	5.700	8	0.681	0
		Inverse variance weighted (multiplicative random effects)	7.357	9	0.600	0
	HRV_RMSSD	Inverse variance weighted	4.580	3	0.205	34.503%
		MR Egger	2.906	2	0.234	31.176%
		Inverse variance weighted (multiplicative random effects)	4.580	3	0.205	34.503%
	HRV_pvRSA/HF	Inverse variance weighted	3.924	3	0.270	23.552%
		MR Egger	3.369	2	0.186	40.639%
		Inverse variance weighted (multiplicative random effects)	3.924	3	0.270	23.552%
	HRV_SDNN	Inverse variance weighted	9.032	9	0.434	0.359%
		MR Egger	8.845	8	0.356	9.551%
		Inverse variance weighted (multiplicative random effects)	9.032	9	0.434	0.359%
	PR	Inverse variance weighted	113.915	115	0.511	0
		MR Egger	113.152	114	0.505	0
		Inverse variance weighted (multiplicative random effects)	113.915	115	0.511	0
	Ptforce	Inverse variance weighted	5.792	9	0.761	0
		MR Egger	4.813	8	0.777	0
		Inverse variance weighted (multiplicative random effects)	5.792	9	0.761	0
	Pwave Duration	Inverse variance weighted	0.276	3	0.965	0
		MR Egger	0.009	2	0.995	0
		Inverse variance weighted (multiplicative random effects)	0.276	3	0.965	0
	Twave_anterior	Inverse variance weighted	10.546	6	0.103	43.105%
		MR Egger	9.844	5	0.080	49.208%
		Inverse variance weighted (multiplicative random effects)	10.546	6	0.103	43.105%
	Twave_avr	Inverse variance weighted	4.522	4	0.340	11.535%
		MR Egger	4.393	3	0.222	31.711%
		Inverse variance weighted (multiplicative random effects)	4.522	4	0.340	11.535%
	Twave_inferior	Inverse variance weighted	10.088	7	0.184	30.609%
		MR Egger	5.881	6	0.437	0
		Inverse variance weighted (multiplicative random effects)	10.088	7	0.184	30.609%
	Twave_lateral	Inverse variance weighted	1.843	3	0.606	0
		MR Egger	1.770	2	0.413	0
		Inverse variance weighted (multiplicative random effects)	1.843	3	0.606	0
	Twave_septal	Inverse variance weighted	5.734	5	0.333	12.807%
		MR Egger	2.131	4	0.712	0
		Inverse variance weighted (multiplicative random effects)	5.734	5	0.333	12.807%

AF, atrial fibrillation; HR, resting heart rate; Twave, T-wave top amplitude; septal, ECG lead V1 + V2; lateral, ECG lead I+ aVL+ V5+ V6; inferior: ECG lead II + III + aVF; anterior: ECG lead V3 + V4 + aVL; avr: ECG lead aVR; Ptforce, P-wave terminal force; Pwave duration, P-wave duration; PR, PR interval; HRV, heart rate variability; RMSSD, root mean square of successive differences; pvRSA/HF, peak-valley respiratory sinus arrhythmia or high frequency power; SDNN, standard deviation of the normal-to-normal interval.

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Table S4 Results of MR-Egger intercept test

Outcome	Exposure	egger_intercept	se	P value
AF	HR	–0.016	0.017	0.382
	HRV_RMSSD	0.017	0.012	0.248
	HRV_pvRSA/HF	0.015	0.012	0.325
	HRV_SDNN	–0.008	0.016	0.610
	PR	–0.001	0.003	0.732
	Ptforce	0.002	0.004	0.661
	Pwave Duration	0.006	0.021	0.794
	Twave_anterior	0.004	0.004	0.408
	Twave_avr	–0.002	0.008	0.800
	Twave_inferior	0.013	0.013	0.363
	Twave_lateral	–0.002	0.006	0.791
	Twave_septal	–0.017	0.017	0.338
Bradycardia	HR	0.000	0.000	0.338
	HRV_RMSSD	0.000	0.000	0.892
	HRV_pvRSA/HF	0.000	0.000	0.915
	HRV_SDNN	0.000	0.000	0.248
	PR	0.000	0.000	0.889
	Ptforce	0.000	0.000	0.801
	Pwave Duration	0.000	0.001	0.743
	Twave_anterior	0.000	0.000	0.677
	Twave_avr	0.000	0.000	0.494
	Twave_inferior	0.000	0.000	0.061
	Twave_lateral	0.000	0.000	0.998
	Twave_septal	–0.001	0.000	0.181
Supraventricular tachycardia	HR	0.000	0.000	0.234
	HRV_RMSSD	0.001	0.000	0.395
	HRV_pvRSA/HF	0.000	0.000	0.624
	HRV_SDNN	0.000	0.000	0.691
	PR	0.000	0.000	0.384
	Ptforce	0.000	0.000	0.351
	Pwave Duration	–0.001	0.001	0.657
	Twave_anterior	0.000	0.000	0.577
	Twave_avr	0.000	0.000	0.786
	Twave_inferior	0.000	0.000	0.086
	Twave_lateral	0.000	0.000	0.811
	Twave_septal	0.000	0.000	0.130

AF, atrial fibrillation; HR, resting heart rate; Twave, T-wave top amplitude; septal, ECG lead V1 + V2; lateral: ECG lead I + aVL + V5 + V6; inferior, ECG lead II + III + aVF; anterior, ECG lead V3 + V4 + aVL; avr, ECG lead aVR; Ptforce, P-wave terminal force; Pwave duration, P-wave duration; PR, PR interval; HRV, heart rate variability; RMSSD, root mean square of successive differences; pvRSA/HF, peak-valley respiratory sinus arrhythmia or high frequency power; SDNN, standard deviation of the normal-to-normal interval.

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Table S5 Results of CAUSE

Outcome	Exposure	Model	Gamma	Eta	Q	P
AF	HR	Sharing		–0.04 (-0.14, 0.13)	0.08 (0, 0.32)	0.29
		Causal	–0.02 (–0.03, 0)	0.02 (-0.12, 0.19)	0.05 (0, 0.27)	0.27
	HRV_RMSSD	Sharing		0.24 (–1.7, 1.79)	0.05 (0, 0.26)	0.93
		Causal	0.12 (–0.08, 0.32)	–0.15 (–1.85, 1.59)	0.05 (0, 0.27)	0.63
	HRV_pvRSA/HF	Sharing		0.37 (-0.89, 1.68)	0.05 (0, 0.25)	0.62
		Causal	0.09 (–0.03, 0.21)	0.12 (–1.06, 1.6)	0.04 (0, 0.24)	0.43
	HRV_SDNN	Sharing		0.98 (-2.25, 3)	0.07 (0, 0.31)	0.32
		Causal	0.32 (–0.06, 0.69)	–0.13 (-3.11, 2.68)	0.05 (0, 0.27)	0.31
	PR	Sharing		0.01 (–0.08, 0.1)	0.03 (0, 0.2)	0.90
		Causal	0 (0, 0.01)	0.01 (–0.08, 0.08)	0.05 (0, 0.28)	0.96
	Ptforce	Sharing		0 (0, 0)	0.02 (0, 0.17)	0.95
		Causal	0 (0, 0)	0 (0, 0)	0.04 (0, 0.26)	0.96
	Pwave Duration	Sharing		–0.02 (-0.19, 0.1)	0.02 (0, 0.15)	0.87
		Causal	0 (–0.01, 0.01)	–0.03 (–0.07, 0.06)	0.09 (0, 0.31)	0.56
	Twave_anterior	Sharing		-0.1 (–1.07, 0.97)	0.06 (0, 0.26)	0.81
		Causal	–0.04 (-0.24, 0.15)	–0.02 (–1.04, 1.03)	0.06 (0, 0.29)	0.98
	Twave_avr	Sharing		0.07 (–1.07, 1.22)	0.07 (0, 0.31)	0.30
		Causal	0.2 (-0.39, 0.79)	0.02 (–1.15, 1.18)	0.07 (0, 0.33)	0.77
	Twave_inferior	Sharing		–0.02 (–1.03, 0.96)	0.05 (0, 0.26)	1.00
		Causal	0 (-0.19, 0.18)	–0.02 (–1.03, 0.95)	0.05 (0, 0.28)	1.00
	Twave_lateral	Sharing		–0.03 (–1.51, 1.4)	0.07 (0, 0.31)	0.43
		Causal	–0.07 (-0.84, 0.7)	–0.02 (–1.52, 1.45)	0.07 (0, 0.33)	1.00
	Twave_septal	Sharing		0.14 (-0.73, 0.97)	0.06 (0, 0.29)	0.69
		Causal	0.1 (-0.1, 0.3)	0.03 (-0.8, 0.91)	0.06 (0, 0.29)	0.70
Bradycardia	HR	Sharing		0 (0, 0)	0.27 (0.06, 0.52)	0.01
		Causal	0 (0, 0)	0 (0, 0)	0.05 (0, 0.26)	0.02
	HRV_RMSSD	Sharing		0 (–0.01, 0.01)	0.1 (0, 0.35)	0.22
		Causal	0 (0, 0)	0 (–0.01, 0.01)	0.05 (0, 0.28)	0.20
	HRV_pvRSA/HF	Sharing		0 (–0.01, 0.01)	0.06 (0, 0.26)	0.89
		Causal	0 (0, 0)	0 (–0.01, 0.01)	0.07 (0, 0.31)	1.00
	HRV_SDNN	Sharing		0.01 (–0.02, 0.02)	0.07 (0, 0.32)	0.36
		Causal	0 (0, 0.01)	0 (–0.02, 0.02)	0.05 (0, 0.28)	0.33
	PR	Sharing		0 (0, 0)	0.05 (0, 0.26)	0.55
		Causal	0 (0, 0)	0 (0, 0)	0.05 (0, 0.3)	0.57
	Ptforce	Sharing		0 (0, 0)	0.08 (0, 0.31)	0.30
		Causal	NA	0 (0, 0)	0.05 (0, 0.28)	0.28
	Pwave Duration	Sharing		0 (0, 0)	0.06 (0, 0.24)	0.46
		Causal	0 (0, 0)	0 (0, 0)	0.07 (0, 0.32)	0.55
	Twave_anterior	Sharing		0 (–0.01, 0.01)	0.06 (0, 0.31)	0.47
		Causal	0 (0, 0)	0 (–0.01, 0.01)	0.06 (0, 0.29)	0.43
	Twave_avr	Sharing		0 (0, 0.01)	0.05 (0, 0.25)	1.00
		Causal	0 (0, 0)	0 (0, 0.01)	0.06 (0, 0.3)	1.00
	Twave_inferior	Sharing		0 (0, 0.01)	0.06 (0, 0.3)	0.52
		Causal	0 (0, 0)	0 (–0.01, 0)	0.06 (0, 0.29)	0.43
	Twave_lateral	Sharing		0 (–0.01, 0.01)	0.06 (0, 0.27)	0.95
		Causal	0 (0, 0)	0 (–0.01, 0.01)	0.07 (0, 0.32)	0.96
	Twave_septal	Sharing		0 (–0.01, 0.01)	0.06 (0, 0.27)	0.81
		Causal	0 (0, 0)	0 (–0.01, 0.01)	0.07 (0, 0.3)	0.99
Supraventricular tachycardia	HR	Sharing		0 (0, 0)	0.05 (0, 0.26)	0.87
		Causal	0 (0, 0)	0 (0, 0)	0.06 (0, 0.3)	0.79
	HRV_RMSSD	Sharing		0 (–0.02, 0.01)	0.05 (0, 0.27)	0.56
		Causal	0 (0, 0)	0 (–0.02, 0.01)	0.05 (0, 0.26)	0.52
	HRV_pvRSA/HF	Sharing		0 (–0.01, 0.01)	0.04 (0, 0.24)	0.98
		Causal	0 (0, 0)	0 (–0.01, 0.01)	0.05 (0, 0.28)	1.00
	HRV_SDNN	Sharing		0 (–0.02, 0.02)	0.05 (0, 0.26)	0.99
		Causal	0 (0, 0)	0 (–0.02, 0.02)	0.05 (0, 0.28)	0.98
	PR	Sharing		0 (0, 0)	0.16 (0.01, 0.43)	0.07
		Causal	0 (0, 0)	0 (0, 0)	0.03 (0, 0.23)	0.06
	Ptforce	Sharing		0 (0, 0)	0.09 (0, 0.34)	0.24
		Causal	0 (0, 0)	0 (0, 0)	0.04 (0, 0.26)	0.20
	Pwave Duration	Sharing		0 (0, 0)	0.04 (0, 0.22)	0.97
		Causal	0 (0, 0)	0 (0, 0)	0.05 (0, 0.28)	0.98
	Twave_anterior	Sharing		0 (–0.01, 0.01)	0.06 (0, 0.29)	0.80
		Causal	0 (0, 0)	0 (–0.01, 0.01)	0.06 (0, 0.3)	0.69
	Twave_avr	Sharing		0 (0, 0.01)	0.07 (0, 0.32)	0.38
		Causal	0 (0, 0)	0 (–0.01, 0)	0.05 (0, 0.27)	0.30
	Twave_inferior	Sharing		0 (–0.01, 0.01)	0.07 (0, 0.3)	0.37
		Causal	0 (0, 0)	0 (–0.01, 0.01)	0.07 (0, 0.3)	0.55
	Twave_lateral	Sharing		0 (–0.01, 0.01)	0.05 (0, 0.27)	0.88
		Causal	0 (0, 0)	0 (–0.01, 0.01)	0.06 (0, 0.28)	0.60
	Twave_septal	Sharing		0 (0, 0.01)	0.07 (0, 0.33)	0.36
		Causal	0 (0, 0)	0 (–0.01, 0.01)	0.06 (0, 0.29)	0.26

AF, atrial fibrillation; HR, resting heart rate; Twave, T-wave top amplitude; septal, ECG lead V1 + V2; lateral, ECG lead I + aVL + V5 + V6; inferior: ECG lead II + III + aVF; anterior: ECG lead V3 + V4 + aVL; avr, ECG lead aVR; Ptforce, P-wave terminal force; Pwave duration, P-wave duration; PR, PR interval; HRV, heart rate variability; RMSSD, root mean square of successive differences; pvRSA/HF, peak-valley respiratory sinus arrhythmia or high frequency power; SDNN, standard deviation of the normal-to-normal interval; Sharing, means two traits have shared genetics; Causal, means the exposure can causally affect the outcome; Gamma, the effect size of exposure on outcome; Eta: the effect size of correlated pleiotropy; Q, the proportion of variants exhibiting correlated pleiotropy; P, the probability of accepting the model.

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Table S6 Results of multivariable Mendelian randomization

Outcome	Methods	Exposure	nSNP	OR	or_lci95	or_uci95	pval
AF	MVMR-IVW	HRV_RMSSD	98	1.538	0.995	2.379	0.053
	MVMR-Egger	HRV_RMSSD	98	1.576	1.014	2.450	0.04
	MVMR-Lasso	HRV_RMSSD	98	1.538	0.995	2.379	0.053
	MVMR-Median	HRV_RMSSD	98	1.737	1.140	2.646	0.01
	MVMR-PRESSO	HRV_RMSSD	98	1.538	0.997	2.374	0.055
	MVMR-IVW	Twave_avr	98	1.007	0.948	1.069	0.82
	MVMR-Egger	Twave_avr	98	1.007	0.948	1.069	0.82
	MVMR-Lasso	Twave_avr	98	1.007	0.948	1.069	0.82
	MVMR-Median	Twave_avr	98	1.017	0.935	1.106	0.70
	MVMR-PRESSO	Twave_avr	98	1.007	0.949	1.069	0.82
	MVMR-IVW	PR	98	1.000	0.995	1.005	0.95
	MVMR-Egger	PR	98	1.000	0.995	1.005	0.96
	MVMR-Lasso	PR	98	1.000	0.995	1.005	0.95
	MVMR-Median	PR	98	1.001	0.994	1.008	0.81
	MVMR-PRESSO	PR	98	1.000	0.995	1.005	0.95
	MVMR-IVW	HRV_SDNN	98	0.515	0.278	0.954	0.03
	MVMR-Egger	HRV_SDNN	98	0.529	0.284	0.983	0.04
	MVMR-Lasso	HRV_SDNN	98	0.515	0.278	0.954	0.03
	MVMR-Median	HRV_SDNN	98	0.392	0.217	0.705	0.002
	MVMR-PRESSO	HRV_SDNN	98	0.515	0.279	0.951	0.04
Bradycardia	MVMR-IVW	HR	32	1.000	1.000	1.000	0.25
	MVMR-Egger	HR	32	1.000	1.000	1.001	0.13
	MVMR-Lasso	HR	32	1.000	1.000	1.000	0.25
	MVMR-Median	HR	32	1.000	1.000	1.000	0.49
	MVMR-PRESSO	HR	32	1.000	1.000	1.000	0.26
	MVMR-IVW	HRV_RMSSD	32	1.007	1.000	1.015	0.06
	MVMR-Egger	HRV_RMSSD	32	1.007	0.999	1.015	0.09
	MVMR-Lasso	HRV_RMSSD	32	1.007	1.000	1.015	0.06
	MVMR-Median	HRV_RMSSD	32	1.007	0.999	1.014	0.10
	MVMR-PRESSO	HRV_RMSSD	32	1.007	1.000	1.015	0.07
	MVMR-IVW	HRV_SDNN	32	0.988	0.976	1.000	0.045
	MVMR-Egger	HRV_SDNN	32	0.989	0.977	1.001	0.06
	MVMR-Lasso	HRV_SDNN	32	0.988	0.976	1.000	0.045
	MVMR-Median	HRV_SDNN	32	0.992	0.98	1.004	0.19
	MVMR-PRESSO	HRV_SDNN	32	0.988	0.976	1.000	0.055
	MVMR-IVW	Twave_avr	32	0.998	0.996	0.999	<0.001
	MVMR-Egger	Twave_avr	32	0.998	0.997	0.999	0.005
	MVMR-Lasso	Twave_avr	32	0.998	0.996	0.999	<0.001
	MVMR-Median	Twave_avr	32	0.998	0.997	0.999	0.004
	MVMR-PRESSO	Twave_avr	32	0.998	0.996	0.999	0.001
Bradycardia	MVMR-IVW	HR	35	1.000	1.000	1.000	0.08
	MVMR-Egger	HR	35	1.000	1.000	1.001	0.08
	MVMR-Lasso	HR	35	1.000	1.000	1.000	0.08
	MVMR-Median	HR	35	1.000	1.000	1.001	0.17
	MVMR-PRESSO	HR	35	1.000	1.000	1.000	0.09
	MVMR-IVW	HRV-RMSSD	35	1.008	1.000	1.016	0.04
	MVMR-Egger	HRV-RMSSD	35	1.008	1.000	1.016	0.048
	MVMR-Lasso	HRV-RMSSD	35	1.008	1.000	1.016	0.04
	MVMR-Median	HRV-RMSSD	35	1.011	0.999	1.022	0.07
	MVMR-PRESSO	HRV-RMSSD	35	1.008	1.000	1.016	0.049
	MVMR-IVW	HRV-SDNN	35	0.988	0.977	1.000	0.04
	MVMR-Egger	HRV-SDNN	35	0.988	0.977	1.000	0.04
	MVMR-Lasso	HRV-SDNN	35	0.988	0.977	1.000	0.04
	MVMR-Median	HRV-SDNN	35	0.985	0.968	1.002	0.07
	MVMR-PRESSO	HRV-SDNN	35	0.988	0.977	1.000	0.050
	MVMR-IVW	Twave_anterior	35	1.000	0.998	1.001	0.57
	MVMR-Egger	Twave_anterior	35	1.000	0.998	1.001	0.73
	MVMR-Lasso	Twave_anterior	35	1.000	0.998	1.001	0.57
	MVMR-Median	Twave_anterior	35	0.999	0.996	1.002	0.37
	MVMR-PRESSO	Twave_anterior	35	1.000	0.998	1.001	0.58
Supraventricular tachycardia	MVMR-IVW	HR	8	1.000	0.999	1.000	0.19
	MVMR-Egger	HR	8	1.000	0.999	1.001	0.48
	MVMR-Lasso	HR	8	1.000	0.999	1.000	0.19
	MVMR-Median	HR	8	1.000	0.999	1.000	0.19
	MVMR-PRESSO	HR	8	1.000	0.999	1.000	0.24
	MVMR-IVW	Pwave duration	8	1.000	1.000	1.000	0.62
	MVMR-Egger	Pwave duration	8	1.000	0.999	1.000	0.18
	MVMR-Lasso	Pwave duration	8	1.000	1.000	1.000	0.62
	MVMR-Median	Pwave duration	8	1.000	1.000	1.000	0.10
	MVMR-PRESSO	Pwave duration	8	1.000	1.000	1.000	0.63

nSNP, number of SNPs used as instrumental variables; pval, P value; or_lci95, lower limit of the odds ratio; or_uci95, upper limit of the odds ratio; MVMR, Multivariate Mendel randomization; IVW, inverse-variance weighted; OR, odds ratio; SNP, single-nucleotide polymorphism; AF, atrial fibrillation; HR, resting heart rate; HRV, heart rate variability; RMSSD, root mean square of successive differences; pvRSA/HF, peak-valley respiratory sinus arrhythmia or high frequency power; SDNN, standard deviation of the normal-to-normal interval; Twave, T-wave top amplitude; anterior, ECG lead V3 + V4 + aVL; avr, ECG lead aVR; Pwave duration, P-wave duration.

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Table S7 Power for Mendelian randomization

Outcome	Exposures	Power
AF	HR	0.05
	HRV_RMSSD	0.23
	HRV_pvRSAHF	0.07
	HRV_SDNN	0.69
	PR interval	0.05
	P-wave duration	NA
	P-wave terminal force	NA
	Twave_anterior	0.05
	Twave_avr	0.07
	Twave_inferior	0.05
	Twave_lateral	0.05
	Twave_septal	0.07
Bradycardia	HR	0.05
	HRV_RMSSD	0.05
	HRV_pvRSAHF	0.05
	HRV_SDNN	0.05
	PR interval	0.05
	P-wave duration	NA
	P-wave terminal force	NA
	Twave_anterior	0.05
	Twave_avr	0.05
	Twave_inferior	0.05
	Twave_lateral	0.05
	Twave_septal	0.05
Supraventricular tachycardia	HR	0.05
	HRV_RMSSD	0.05
	HRV_pvRSAHF	0.05
	HRV_SDNN	0.05
	PR interval	0.05
	P-wave duration	NA
	P-wave terminal force	NA
	Twave_anterior	0.05
	Twave_avr	0.05
	Twave_inferior	0.05
	Twave_lateral	0.05
	Twave_septal	0.05

Online tools were used to calculate the power of Mendelian randomization (https://shiny.cnsgenomics.com/mRnd/). The R^2 used in the calculation is determined by the formula: $R^2=2\times (1\text{-MAF}) \times \text{MAF} \times \beta^2/(se^2 \times N)$. Given that the GWAS data for P-wave duration and P-wave terminal force did not provide effect allele frequencies, we did not calculate power for P-wave duration and P-wave terminal force. AF, atrial fibrillation; HR, resting heart rate; Twave, T-wave top amplitude; septal: ECG lead V1 + V2; lateral: ECG lead I+ aVL+ V5+ V6; inferior: ECG lead II + III + aVF; anterior: ECG lead V3 + V4 + aVL; avr, ECG lead aVR; HRV, heart rate variability; RMSSD, root mean square of successive differences; pvRSA/HF, peak-valley respiratory sinus arrhythmia or high frequency power; SDNN, standard deviation of the normal-to-normal interval.

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Appendix 2

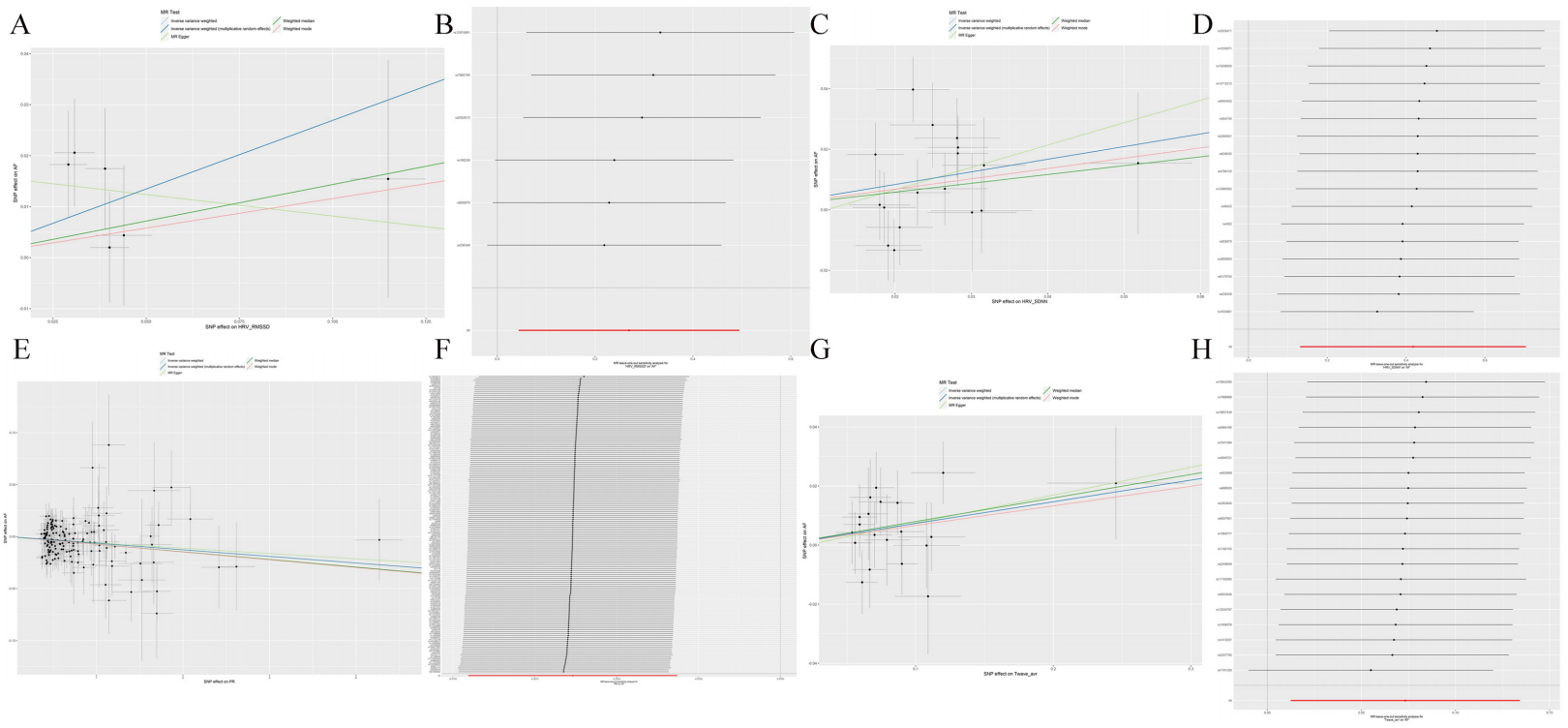

Figure S1 Visualization of Atrial fibrillation outcomes. (A) Scatter plot of HRV-RMSSD and AF; (B) Leave one out plot of HRV-RMSSD and AF; (C) Scatter plot of HRV-SDNN and AF; (D) Leave one out plot of HRV-SDNN and AF; (E) Scatter plot of PR and AF; (F) Leave one out plot of PR and AF; (G) Scatter plot of Twave_avr and AF; (H) Leave one out plot of Twave_avr and AF. Twave, T-wave top amplitude; avr, ECG lead aVR; PR, PR interval; HRV, heart rate variability; RMSSD, root mean square of successive differences; SDNN, standard deviation of the normal-to-normal interval; AF, atrial fibrillation.

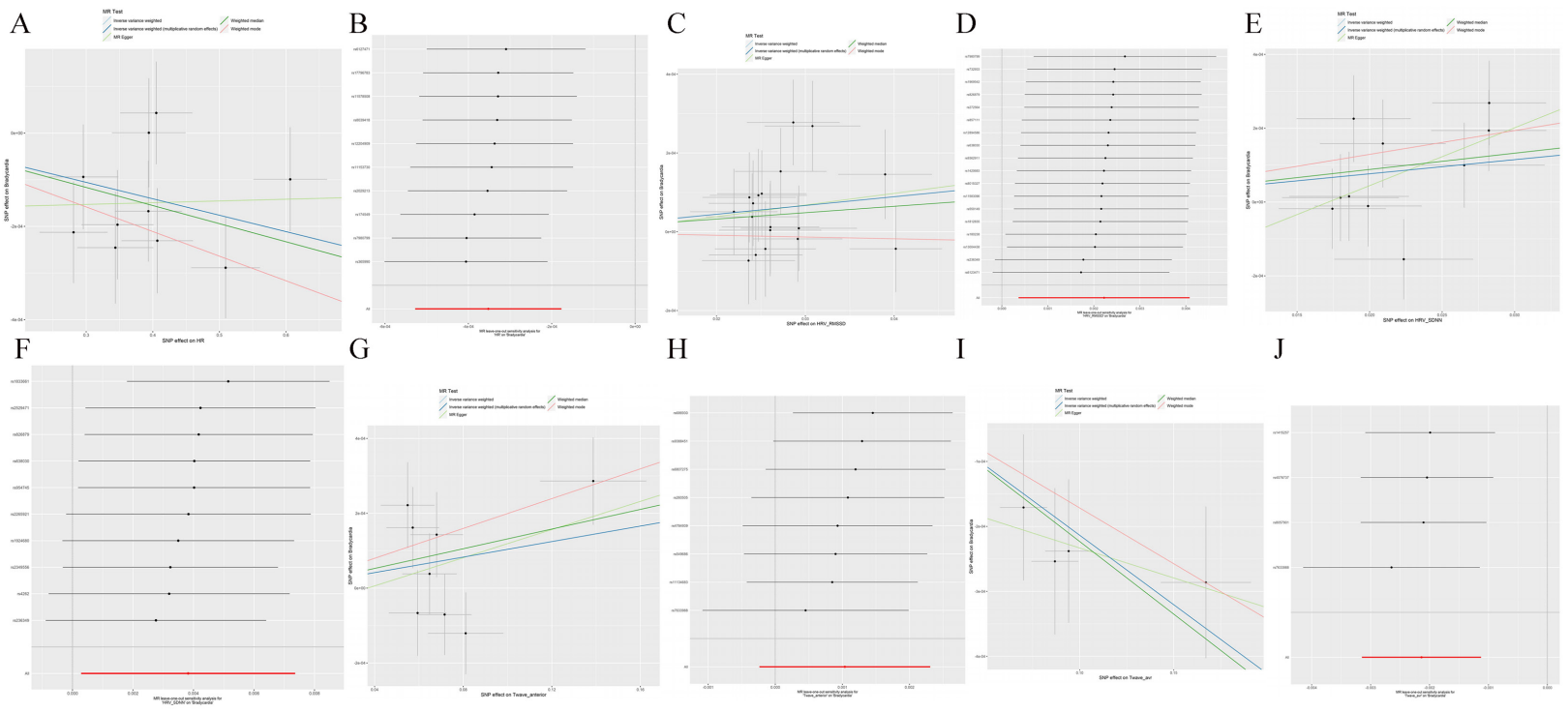

Figure S2 Visualization of bradycardia outcomes. (A) Scatter plot of HR and Bradycardia; (B) Leave one out plot of HR and Bradycardia; (C) Scatter plot of HRV-RMSSD and Bradycardia; (D) Leave one out plot of HRV-RMSSD and Bradycardia; (E) Scatter plot of HRV-SDNN and Bradycardia; (F) Leave one out plot of HRV-SDNN and Bradycardia; (G) Scatter plot of Twave_anterior and Bradycardia; (H) Leave one out plot of Twave_anterior and Bradycardia; (I) Scatter plot of Twave_avr and Bradycardia; J, Leave one out plot of Twave_avr and Bradycardia. HR, heart rate (resting); Twave, T-wave top amplitude; anterior, ECG lead V3 + V4 + aVL; avr, ECG lead aVR; HRV, heart rate variability; RMSSD, root mean square of successive differences; SDNN, standard deviation of the normal-to-normal interval.

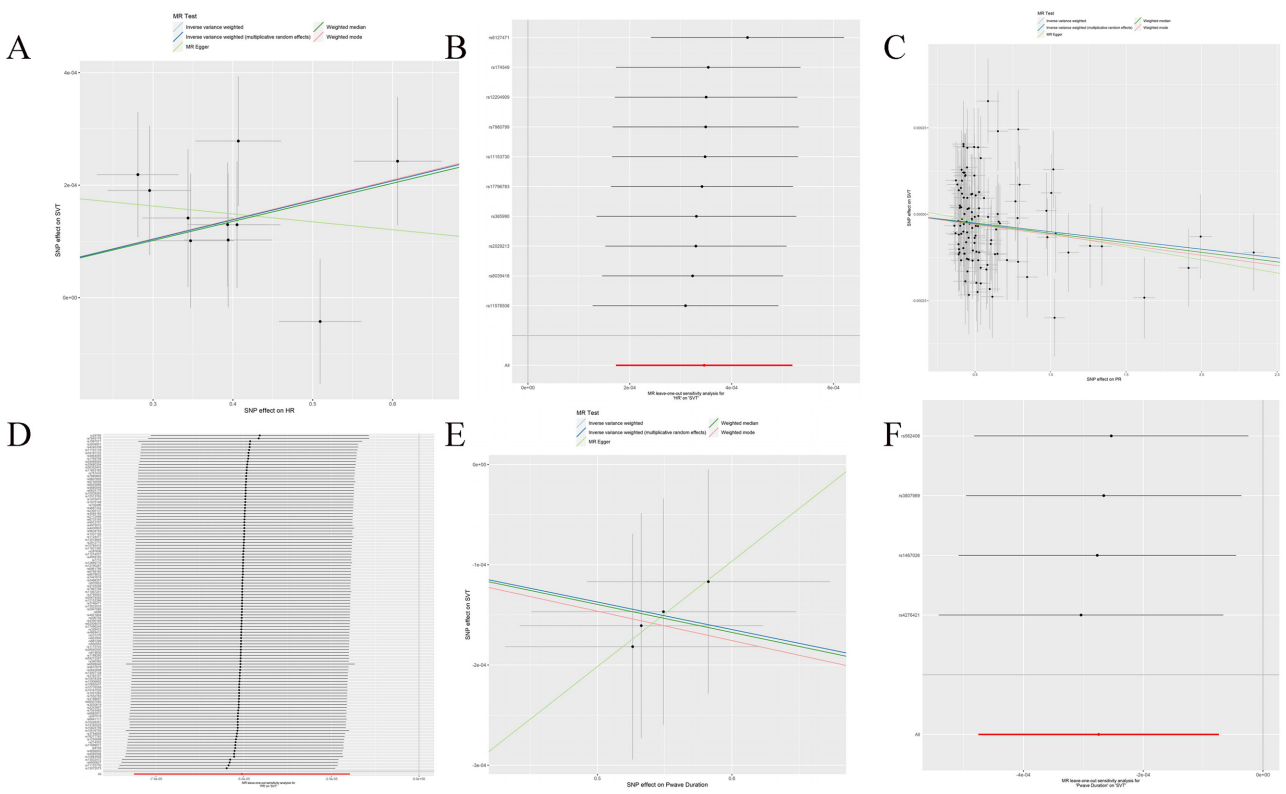

Figure S3 Visualization of supraventricular tachycardia outcomes. (A) Scatter plot of HR and SVT; (B) Leave one out plot of HR and SVT; (C) Scatter plot of PR and SVT; (D) Leave one out plot of PR and SVT; (E) Scatter plot of P-wave duration and SVT; (F) Leave one out plot of P-wave duration and SVT. HR, heart rate (resting); PR, PR interval; Pwave duration, P-wave duration; SVT, Supraventricular tachycardia.

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