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CLINICAL RESEARCH

Insight into the relationship between resting heart rate and atrial fibrillation: a Mendelian randomization study

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Aims A low resting heart rate (RHR) implies a more efficient heart function and a lower risk of cardiovascular disease. However, observational studies have reported a U-shaped association between RHR and atrial fibrillation (AF). In contrast, Mendelian randomization (MR) studies have found an inverse causal association between RHR and AF. Hence, the causal nature of the relationship is not clear. The aim is to investigate the causal association and its shape between RHR on AF using linear and non-linear MR (NLMR).

Methods and results Linear and non-linear MR were performed on individual-level data in the Trøndelag Health Study (HUNT) and UK Biobank (UKB). HUNT consists of 69 155 individuals with 7,062 AF cases, while UKB provides data on 431 852 individuals with 20 452 AF cases. The linear MR found an inverse relationship between RHR and AF with an OR = 0.95 [95% confidence interval (CI): 0.93–0.98] and OR = 0.96 (95% CI: 0.95–0.97) per unit decrease in RHR in HUNT and UKB, respectively. The NLMR was supportive of an inverse linear relationship in both HUNT and UKB for RHR values <90 beats per minute (bpm). Several sensitivity analyses were also consistent.

Conclusion In contrast with the current observational knowledge of RHR and AF, an inverse causal association between RHR and AF was demonstrated in both linear and non-linear MR for RHR values up to 90 bpm. Further exploring the underlying mechanisms of the genetic instrument for RHR may shed light on whether pleiotropy is biasing this association.

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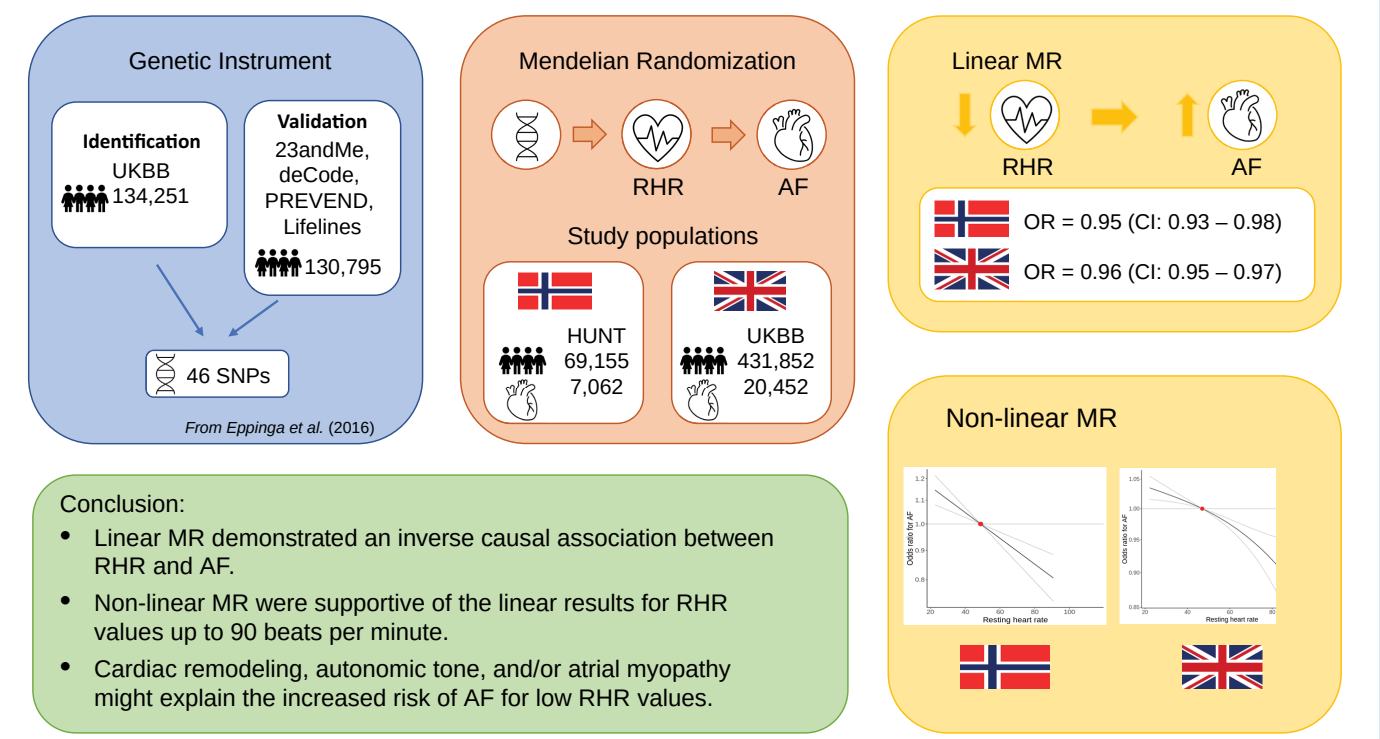
Graphical Abstract



Keywords Resting heart rate • Atrial fibrillation • Mendelian randomization • Non-linear Mendelian randomization • UK Biobank

What's new?

- Linear Mendelian randomization provides evidence of a possible inverse causal association between resting heart rate and atrial fibrillation (AF), and this study confirms this association in a new cohort.
- Non-linear Mendelian randomization suggests a linear, inverse causal association between resting heart rate and AF for resting heart rate values up to 90 b.p.m., in contrast to the U-shaped association demonstrated in observational studies (and our present observational analyses).
- A genetic predisposition for a low resting heart rate seems to increase the risk of AF, potentially due to a causal effect or pleiotropic pathway on cardiac remodelling, atrial myopathy, autonomic tone, and/or heart structure. However, we lack data and evidence of any protection for AF by increasing resting heart rate above normal values.

Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide, hence improving the understanding of the underlying mechanisms of CVD has great potential in preventing morbidity and mortality, as well as lowering healthcare costs for this large patient group. Elevated resting heart rate (RHR) is an independent risk factor for CVD, both for patients with hypertension, coronary artery disease, and heart failure, but also for healthy individuals.[1] Furthermore, high RHR is established as a predictor of overall mortality.[2,3] Atrial fibrillation (AF) is the most common sustained cardiac arrhythmia, affecting more than 33 million people worldwide.[4] Patients with incident AF have a 3.5-fold risk of death compared to subjects without AF.[5]

RHR is defined as the number of heart beats per minute (bpm) at rest and is an objective measure that is easy for clinicians and individuals themselves to measure. For adults, a normal RHR ranges from 60 to 100 bpm. RHR is a complex trait regulated by different biological factors and pathways including the sinus node, autonomous nervous system, central cortex, baroreceptors, and cardiac regulatory mechanics.[6,7] The genetic contribution of RHR is estimated to be ~22% in family studies and up to 65% in twin studies.[8–10] In general, a lower RHR implies a more efficient heart function and a higher cardiovascular fitness. However, in the case of AF, the shape of the association between RHR and risk of AF is not clear. While some studies suggest that only lower rates of RHR are associated with AF,[11] others have found that an elevated RHR is associated with AF.[12] Both a J- and U-shaped association has been reported from observational studies, indicating that individuals with both low and high RHR are at increased risk of AF.[13] One observational study found that a low mean heart rate measured from a 24-h electrocardiogram was an independent predictor of AF.[14] There was no association between a low mean heart rate and increased supraventricular activity.

Mendelian randomization (MR) is an approach for investigating causality between a modifiable exposure and an outcome, by using genetic variants as instruments, illustrated in *Figure 1*.[15] Given certain assumptions, MR can be thought as analogous to a randomized controlled trial (RCT), which is considered the gold standard study design for assessing causality, as genetic variants are independently assorted and randomly allocated at conception, illustrated in *Figure 2*.[16] As the genetic variants are determined at birth, the effect estimate obtained from MR should not be affected by confounders. In addition, reverse causation is unlikely, as the

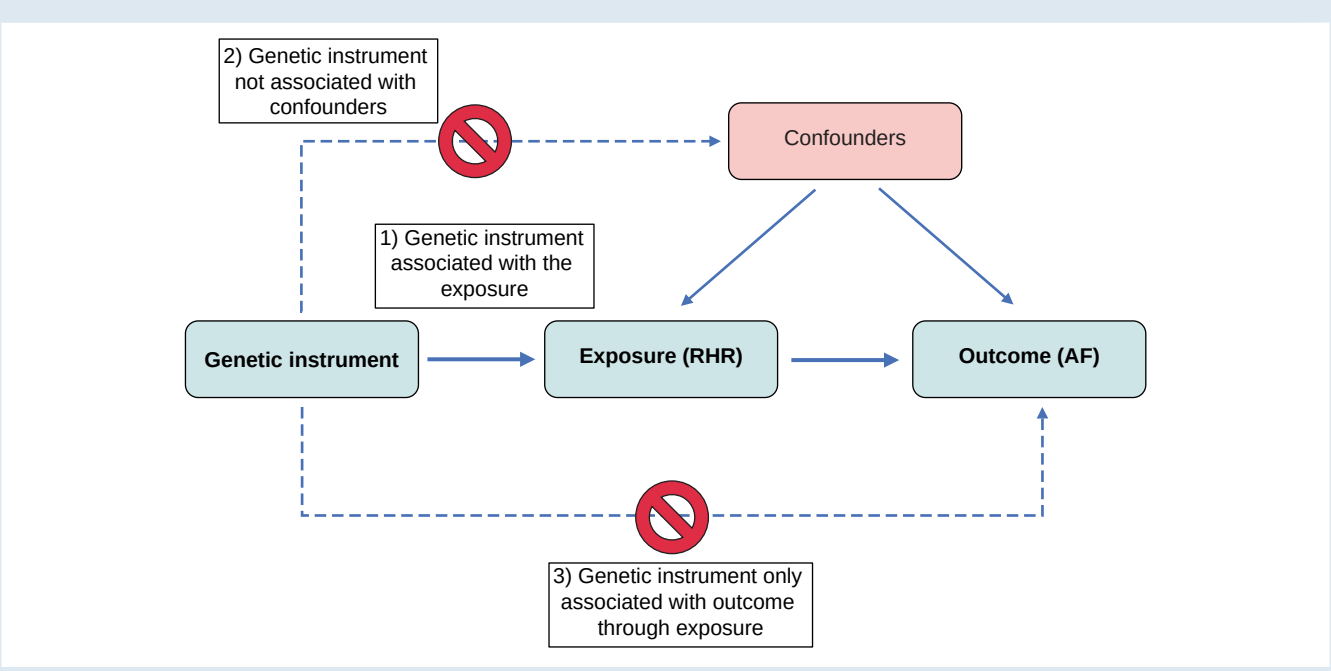


Figure 1 An illustration of the Mendelian randomization approach. The genetic instrument is used as a proxy to estimate the causal effect of the exposure on the outcome. The Mendelian randomization approach is based on three assumptions (i) the genetic instrument is associated with the exposure, (ii) the genetic instrument is not associated with any confounders, and (iii) the genetic instrument influences the outcome only through the exposure. RHR, resting heart rate; AF, atrial fibrillation.

outcome cannot affect which genetic variants one inherits. In short, the causal effect estimate from MR can be obtained using a two-stage least squares method, which involves regressing the exposure on the genetic variants, and then the outcome is regressed on the predicted values of the exposure from the first regression. Consequently, when the assumptions of MR are fulfilled, the MR estimate is a causal estimate of the relationship between an exposure and an outcome. Non-linear MR (NLMR) is a novel method used to investigate the shape of the causal relationship between an exposure and an outcome by the same concepts as linear MR.[17,18] The method divides the population into strata based on the exposure distribution and estimates a causal effect in each stratum. Further, the fractional polynomial methods perform meta-regression on these estimates, resulting in a non-linear causal estimate of the relationship between the exposure and the outcome. In a large RCT, it has been shown that lowering RHR in heart failure patients leads to a reduced risk of cardiovascular death and worsening of heart failure, indicating that RHR is a modifiable and causal risk factor, making it a candidate for MR.[19]

There are some previous studies using MR to investigate the causal relationship of RHR on different CVDs.[20–22] One study that assessed the effect of reduced heart rate increase during exercise and reduced heart rate recovery after exercise found an inverse relationship between RHR and AF and a relationship between RHR and ischaemic stroke.[22] Another study performed a stratified MR, dividing the study population into three strata of RHR, and found an inverse relationship between RHR and AF only in the individuals with low RHR (<65 bpm).[21] To our knowledge, no studies have used NLMR to investigate the shape of the relationship between RHR and AF, hence all previous MR estimates are limited to the linear setting. Also, all previous MR studies looking at RHR and AF have used data from UK Biobank (UKB), hence it will add value to these previous findings to replicate these results in an independent cohort.

Consequently, the current knowledge from observational studies suggests that there is a U-shaped association between RHR and AF. However, previous MR studies have shown an inverse causal effect of RHR on AF, but these studies are limited to linear MR. Hence, in this study, we aimed at investigating the causal effect of RHR on AF, and to shed light on the shape of the association by using both linear and NLMR. In addition, the analyses were performed in two independent cohorts, making the results more reliable.

Methods

Study populations

This study was conducted using data from the Trøndelag Health Study (HUNT) and UKB. HUNT is a prospective health study of ~229 000 participants from four sub waves, HUNT1 (1984–1986), HUNT2 (1995–1997), HUNT3 (2006–2008), and HUNT4 (2017–2019).[23,24] In this study, participants from HUNT2 and HUNT3 with available genotype data and RHR measurements were included, resulting in 69 155 participants. The UKB consists of >500 000 participants where all have been genotyped, and the study and participants have been described in detail elsewhere.[25] By including participants with European ancestry and RHR measures available the study population from UKB consisted of 431 852 participants.

Definition of RHR and AF

All participants in HUNT underwent pulse measures at three subsequent events, with the lowest measure used as RHR. All participants with available pulse measures and genotype data from HUNT2 and HUNT3 were included. Pulse measures from HUNT2 were used if the participant had pulse measures from both HUNT2 and HUNT3, resulting in data from HUNT2 for ~70% of the included participants and data from HUNT3 for ~30% of the included participants. AF status was estimated using the international classification of diseases (ICD)-9 codes 427.31 and 427.32, and ICD-10 codes I48 from hospital registry data, resulting in 7062 cases with AF and 62 093 controls in HUNT. The HUNT2 measurements were collected during 1995–1997, HUNT3 measurements were collected during 2006–2008, and the hospital registry data were collected until 2016. In UKB RHR was assessed by an automated reading during blood pressure (BP) measurement (ID field 95 and 102), and by the pulse rate form obtained from arterial

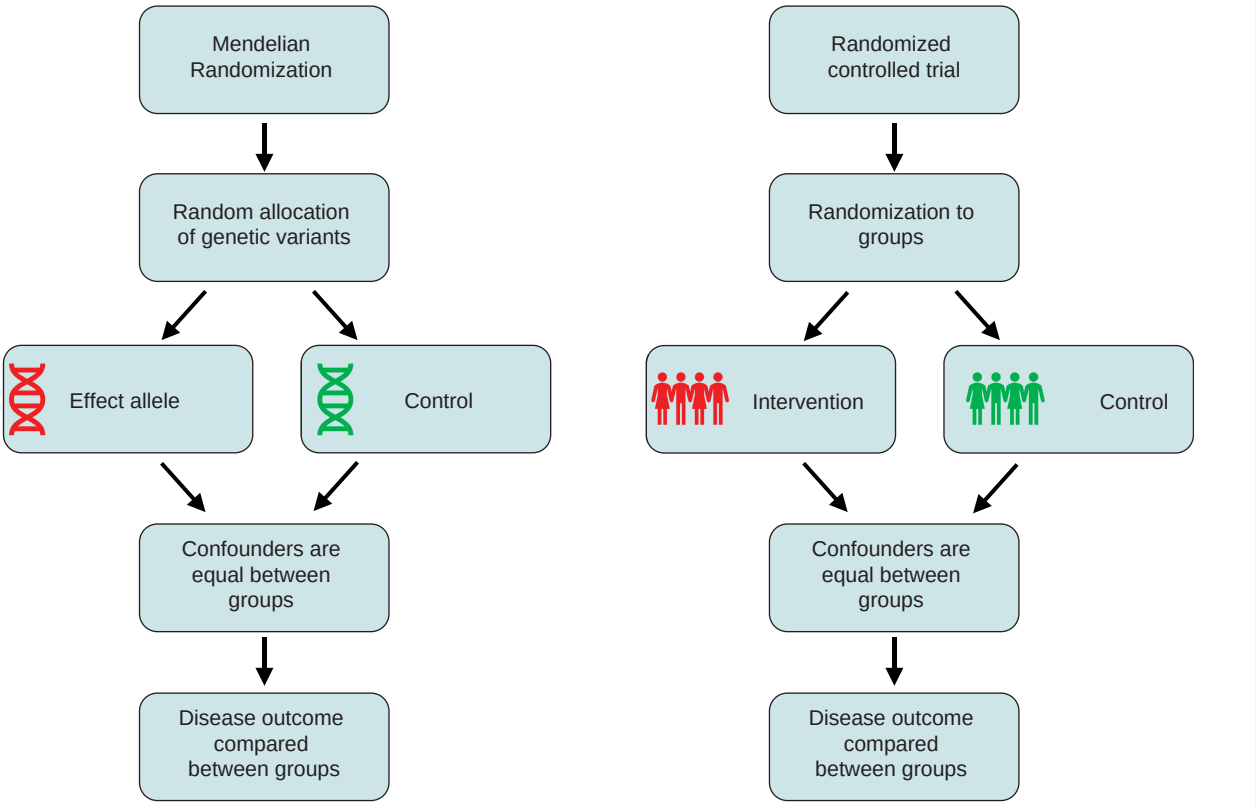


Figure 2 An illustration of the concept of Mendelian randomization and a randomized controlled trial, and how they are comparable to each other. Both methods are used to assess causality, and a randomized controlled trial is considered the gold standard for assessing causality.

stiffness measurement (ID field 4194). If several measurements were available from one participant, the RHR was defined as the lowest measured heart rate using ID-fields 102, 4194, and 95. AF status was calculated using ICD-9 codes 427.31 and 427.32, and ICD-10 codes I48 from hospital registry data (ID field 41 271 and 41 270, respectively), resulting in 20 452 AF cases and 411 400 controls in UKB. The RHR measures from UKB were collected during 2006–2010, and the hospital registry data were collected until 2021.

Genetic instrument

The genetic instrument for RHR comprised the 46 independent significant single-nucleotide polymorphisms (SNPs) that were identified and validated in the genome-wide association study (GWAS) on RHR by Eppinga *et al.*[26] In short, a GWAS was conducted using 134 251 individuals from UKB as the discovery cohort, and 130 795 individuals from a meta-analysis of 23andMe, deCODE, PREVEND, and LifeLines sample collections as the replication cohort. The genetic variant with the strongest signal (lowest *P*-value) in the genomic region of ±1Mb and pairwise linkage disequilibrium $r^2 < 0.1$ was considered as the independent genetic loci. In our study, the 46 SNPs from Eppinga *et al.* were used to estimate a weighted allele score for each participant by multiplying the effect estimate from the validation cohort for each SNP with the number of alleles carried by the participant in HUNT/UKB. 42 and 43 of the 46 SNPs from Eppinga *et al.* were available in UKB and HUNT, respectively (rs145358377, rs1468333, rs138186803, and rs10880689 not present in UKB, and rs145358377, rs564190295, and rs138186803 not present in HUNT).

Statistical analyses

Linear MR

To investigate the causal effect of RHR on AF, linear MR was conducted on individual-level data in HUNT and UKB separately, using the *OneSampleMR* package in R (v4.1.2). The causal, linear MR estimate was obtained using the two-stages predictor substitution method, which in brief involves fitting a first-stage model of the exposure regressed upon the genetic instrument, and then a second-stage model fitted of the outcome regressed upon the predicted values of the exposure. The causal MR estimate is presented as odds ratio (OR), with 95% confidence intervals (CI) per one bpm rise in RHR.

Non-linear MR

A fractional polynomial approach was used in the MR framework to estimate the causal non-linear shape of the relationship between the risk factor (RHR) and the outcome (AF). The NLMR method was applied to individual-level data in both HUNT and UKB separately. Both the HUNT and UKB cohorts were divided into 100 strata based on the exposure distribution adjusted for age, sex, principal components 1–10, and batch. As there are few participants with RHR values >90 bpm, all these participants are merged into one stratum, resulting in no NLMR estimates for RHR values >90 bpm. An estimate from a linear logistic MR was used to estimate a localized average causal effect in each stratum. The localized average causal effect estimates were then meta-analyzed against the mean of the exposure in each stratum in a flexible semiparametric framework, resulting in the best-fitted fractional polynomial.

Sensitivity analyses

In MR, an important assumption is that the genetic instrument is not associated with any confounders. Therefore, the association of the genetic instrument with possible confounders affecting RHR and AF was investigated using a linear model for continuous variables and analysis of variance for categorical variables. Based on prior knowledge, age, sex, body mass index (BMI), systolic blood pressure (SBP), maximal oxygen consumption (VO_{2max})/cardiorespiratory fitness (CRF) (directly measured VO_{2max} available for ∼4500 participants in HUNT, and estimated CRF from a submaximal bicycle test available for ∼59 000 participants in UKB), physical activity (PA), and smoking status were evaluated as possible confounders. To further explore the

relationship between RHR and AF restricted cubic splines of the observed association of RHR and AF were plotted using the function *rcspline.plot* in R. Both the linear and NLMR analyses were conducted on males and females separately in both HUNT and UKB, and these sex-specific analyses are considered as sensitivity analyses. As an additional sensitivity analysis, the linear and NLMR analyses were conducted on a subset of the total population including only participants with SBP < 140 mmHg both in HUNT and UKB.

Results

Study participants

The number of participants used in the different cohorts is presented in Supplementary material online, *Table S1*. In HUNT, the mean RHR was 69.9 bpm [standard deviation (SD): 12.4], while the mean RHR was 67.0 bpm in UKB (SD: 11.1). The prevalence of AF was 10.2% and 4.7% in HUNT and UKB, respectively. The distribution of RHR separated by AF cases and controls in HUNT and UKB are presented in Supplementary material online, *Figure S1*. The restricted cubic spline was used to plot the observed association between RHR and AF in HUNT and UKB separately, *Figure 3*. In UKB there seems to be a U-shaped observed association between RHR and AF, with increased risk of AF for both low and high RHR values. The lowest risk of AF seems to be around 60–80 bpm. In HUNT the observed association between RHR and AF is much the same as in UKB, but the increased risk for RHR values above 80 is more moderate. The plot of the observed association between RHR and AF for each sex is comparable to the plots for the total population (see Supplementary material online, *Figure S2*). The genetic instrument explained 1% of the variation of RHR in UKB, and 0.6% in HUNT, and the F-statistic was 4241 and 414.2 in UKB and HUNT, respectively.

Instrument association with confounders

In UKB, the genetic instrument was associated with SBP (beta = 1.1×10^{-2}, *P*-value = 5.1×10^{-6}) and CRF (beta = 1.2×10^{-3}, *P*-value = 1.6×10^{-4}) (see Supplementary material online, *Table S2*). The genetic instrument explained 5.0×10^{-5} of the variation in SBP and 2.3×10^{-4} of the variation in CRF. The genetic instrument was not associated with age, sex, BMI, PA, or smoking status. In HUNT, the genetic instrument was not substantially associated with any of the tested covariates, including age, sex, BMI, SBP, VO_{2max}, PA, or smoking status (see Supplementary material online, *Table S3*).

Linear MR

Linear MR was performed on individual-level data in HUNT and UKB separately. The linear MR estimate indicated an inverse relationship between RHR and AF with an OR = 0.96 (95% CI: 0.95–0.97) in UKB. The linear MR estimate suggests that one bpm increase in RHR is associated with a 4.0% (95% CI: 2.8%–5.2%) reduced risk of AF in UKB. The linear MR estimate in HUNT was consistent with the estimate from UKB with an OR = 0.95 (95% CI: 0.93–0.98). In HUNT the MR estimate shows that one bpm increase in RHR is associated with a 5.0% (95% CI: 2.5%–7.5%) reduced risk of AF. The sensitivity analyses including the sex-specific analyses and the subpopulation with SBP < 140 mmHg were consistent, and all results are presented in *Table 1*.

Non-linear effects of RHR on AF

To further assess the shape of the relationship between RHR and AF, NLMR was performed in UKB and HUNT. In general, the NLMR was supportive of the findings from the linear MR. In UKB a decreasing risk of AF for increasing RHR values was observed (*Figure 4* and Supplementary material online, *Table S4*). The NLMR suggested a polynomial of degree one as the best fit of the association (*P*-value = 0.009). Also in HUNT, a polynomial of degree one was suggested as the best fit of the association (*P*-value = 5.62×10^{-6}) (*Figure 4* and Supplementary material online, *Table S4*). For all values of RHR, the OR for AF is decreasing with increasing RHR in both UKB and HUNT. However, it is important to note that the NLMR only has estimates up to ~90 bpm, as the method divides the population into 100 strata based on the RHR distribution, and all participants with RHR values >90 bpm are merged into one of the 100 strata.

The sex-specific NLMR are supportive of the linear- and non-linear findings in the total population, and they are presented in Supplementary material online, *Figure S3* and *Table S4*. In general, all sex-specific analyses showed decreasing risk of AF for increasing values of RHR, but with slightly different shapes in the different subcohorts. The sensitivity analysis with the subcohort consisting of participants from the total population with SBP < 140 mmHg was also supportive of the linear- and non-linear findings with decreasing risk of AF for increasing values of RHR in both HUNT and UKB (see Supplementary material online, *Figure S4* and *Table S4*).

Discussion

This study aimed to investigate if there is a causal effect of RHR on AF and to elucidate the shape of this relationship. Previous observational studies have reported a J–/U-shaped association between RHR and risk of AF, in contrast to previous MR studies that have reported an inverse causal effect of RHR on AF. However, the previous MR studies have been restricted to the linear setting. In the linear MR, we found an inversely significant causal estimate between RHR and AF, with a 5% and 4% reduced risk of AF per bpm increase in RHR in HUNT and UKB, respectively. The NLMR was supportive of the linear MR results in both HUNT and UKB, with decreasing risk of AF for increasing values of RHR, but only with estimates up to 90 bpm. More data is required to investigate the shape of the association above 90 bpm. All sensitivity analyses, including analyses on males and females separately and a subcohort of participants with SBP < 140 mmHg, supported the linear, inverse causal effect, both in the linear and in the NLMR.

To further increase the understanding of the results, the observed association between RHR and AF was plotted in both HUNT and UKB. In UKB, the observed association of RHR and AF was U-shaped with increased risk of AF at both ends and with the lowest risk of AF for RHR values between 60 and 80 bpm. This is in concordance with findings from previous observational studies, including a meta-analysis of the observed association of RHR and AF.[13] In HUNT, somewhat the same pattern was observed, but the increased risk of AF for RHR values above ~80 bpm was more moderate, and the observed association between RHR and risk of AF was comparable to a vertically flipped J. In HUNT the observed risk of AF is higher for low RHR values than for high RHR values. Interestingly, the observed association in both HUNT and UKB is different from the MR estimates from the NLMR using the same individual-level data. However, as the NLMR divides the population into 100 strata based on the RHR distribution, the method only provides MR estimates for RHR up to ~90 bpm, in contrast to the observed association where there are associations up to ~140 bpm. There are few participants with RHR values >90 bpm, making the observed decreased risk for this group more uncertain. The significant linear estimate from the linear MR and the confirmed linearity from the NLMR might predict that there is a linear trend also for RHR values >90 bpm, but as there is not enough data available for the NLMR it is difficult to say much about the causality for RHR values >90 bpm. Therefore, our findings may likely only reflect the strong inverse association in the lower range of RHR (35–65 bpm).

A previous MR study assessing RHR and risk of AF, found an inverse causal association between RHR and AF (OR = 0.98, *P*-value = 9.8×10^{-6}),[22] which is consistent with our results, though we demonstrated stronger effects in our cohorts. Another study using MR to assess the potential causal

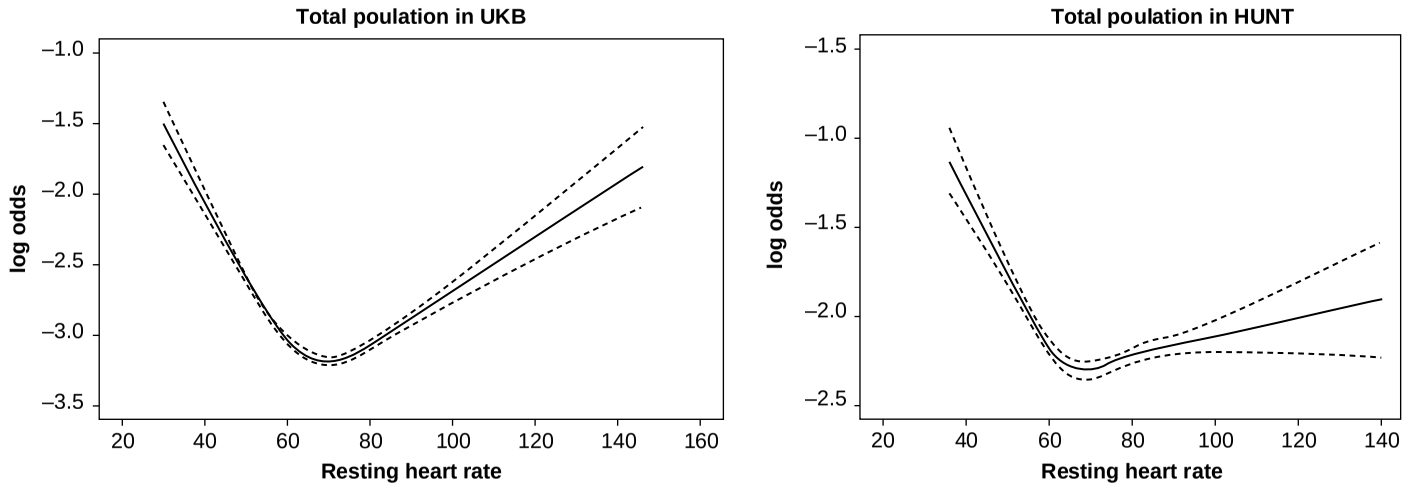


Figure 3 The observed relationship between resting heart rate and risk of atrial fibrillation plotted with the estimated restricted cubic spline using five knots in UK Biobank to the left and the Trøndelag health study to the right. Resting heart rate in beats per minute is on the *x*-axis and the log odds of atrial fibrillation is on the *y*-axis. The solid line is the dose-response curve between resting heart rate and risk of atrial fibrillation, and the dotted line represents the 95% confidence interval.

Table 1 Linear Mendelian randomization estimates of the causal association between resting heart rate and atrial fibrillation

Cohort	Population	*n*	OR	CI lower	CI upper	% lower risk of AF
HUNT	Total	69 155	0.9499	0.9253	0.9753	5.01%
HUNT	Women	36 595	0.9455	0.9087	0.9837	5.45%
HUNT	Men	3 2560	0.9534	0.9200	0.9879	4.66%
HUNT	SBP < 140 mmHg	44 972	0.9512	0.9119	0.9922	4.88%
UKB	Total	431 852	0.9600	0.9477	0.9724	4.00%
UKB	Women	234 385	0.9678	0.9468	0.9893	3.22%
UKB	Men	197 467	0.9561	0.9413	0.9711	4.39%
UKB	SBP < 140 mmHg	226 568	0.9550	0.9346	0.9759	4.50%

n, sample size; OR, odds ratio; CI, confidence interval; AF, atrial fibrillation. SBP, systolic blood pressure.

relationship between RHR and AF stratified the study population in low, medium, and high RHR, and found an inverse causal association between RHR and AF only for the individuals in the low strata for RHR (<65 bmp).[21] This result is in the same direction as our results, although we found the same for RHR values up to 90 bpm, not only for the participants with a low RHR. A clear limitation of that study was the low power with a sample size of only 39 000 participants which again was divided into three groups. Hence, their lack of an inverse significant relationship for the two remaining strata could be due to a lack of statistical power. Their actual finding in the strata with low RHR, despite their low statistical power adds confidence to the fact that low RHR values (<65 bpm) causally lead to an increased risk of AF. A third MR study also found an inverse causal association between RHR and AF, which also supports our findings.[20] All previous MR studies are based on data from UKB alone, hence our novel result in an independent cohort, HUNT, adds value and strength to the previously reported results.

Results from both observational studies and MR studies demonstrate that a low RHR is associated with an increased risk of AF, and in the MR setting a low RHR causally leads to an increased risk of AF. However, a high RHR has been shown to increase the risk of AF in observational studies, but in MR studies a there is an indication of a decreasing risk of AF for increasing RHR values. In the observational setting, the association could be affected by confounders for the observed high RHR values, as high RHR often occurs in individuals with low PA, high BP, high BMI, smoking, and other unhealthy factors. In the linear MR setting, it could be that the association is stronger in the lower RHR levels than that of higher RHR levels resulting in an overall average decreased risk which is not representative of the shape of the association. The NLMR was limited to investigate the association below 90 bpm due to few participants with RHR values above 90 bpm. Additionally, in the MR setting it could be that there is mis-specification of the primary phenotype or correlated pleiotropy. This means that it might be difficult to interpret our findings as causal effects without knowing the underlying mechanisms of the genetic instrument for RHR and our estimates could result from biological antecedents of RHR.

The underlying mechanisms regulating this relationship are still unclear. Larsson *et al.* suggest that the inverse relationship could involve alterations in autonomic tone or subclinical sinus node dysfunction. The autonomic tone, which balances the sympathetic and parasympathetic pathways, is suggested to be central in the pathophysiology of heart failure, hypertension, and arrhythmias, and could be one of the mechanisms behind the observed causal effect of RHR on AF.[27] In the atrial myocardium, both sympathetic and parasympathetic stimulation shortens action potential duration, hence vagal stimulation is arrhythmogenic in the atria and could lead to AF. Another study assessing at sinus node dysfunction, found that AF patients have a smaller number of earliest atrial activation sites and a poorer response to β-stimulations compared with participants free from AF.[28]

PA, CRF, and RHR are related to each other in such manner that increased PA most often leads to an increased CRF, which again may induce a lower RHR. PA is previously reported to have a J-shaped association with AF. Moderate PA was associated with a reduced risk of AF, while low PA, and in particular high PA was associated with an increased risk of AF.[29] In the same study, an increased risk of AF with decreasing RHR values was observed, and PA did not moderate this association. It has been demonstrated that systematic endurance exercise and high VO_{2max} are associated with an increased risk of AF.[30] Furthermore, a high prevalence of AF in endurance athletes, especially in males has been observed.[31] It is suggested that high exercise volumes over many years might induce cardiac adaptations including cardiac remodelling that could lead to an increased risk of AF.[32] Also, regular endurance exercise leads to atrial enlargement and left ventricular hypertrophy, which also predisposes AF.[33,34] An animal study found that chronic endurance exercise increased AF susceptibility in rats.[35] They identified autonomic changes, atrial dilation, and fibrosis as potential mechanistic contributors. Other possible mechanisms involved in the observed increased prevalence of AF in individuals with high exercise volumes may involve increased atrial ectopic activity, greater parasympathetic activation, and blunted sympathetic tone which might increase vulnerability to atrial arrhythmia, and/or exercise-induced cardiac fibrosis.[33] In conclusion, the relationship between genetic factors and AF is multifaceted. While PA, CRF, and RHR are interconnected, the impact of these factors on AF risk is complex.

Limitations and strengths

In UKB, the genetic instrument was associated with SBP and CRF. Hence the second assumption of MR, that the genetic instrument is not associated with any confounders, might be violated. However, the genetic instrument was not associated with any of the confounders under consideration in HUNT. This does not mean that the instrument is not associated with any confounders at all, since it can be associated with unmeasured and/or unknown confounders. However, to the best of our knowledge, the genetic instrument does not substantially violate the second assumption of MR in the HUNT cohort. Since the analyses reported similar results in HUNT and UKB, it seems that the estimates in UKB are not significantly affected by the genetic instrument being associated with some potential confounders. Also, the density plots of RHR by AF status in both HUNT and UKB showed that the AF cases had similar RHR distributions as the controls. Hence, the tested confounders, and other possible confounders including medications like beta-blockers or other comorbidities like hypertension or heart failure,

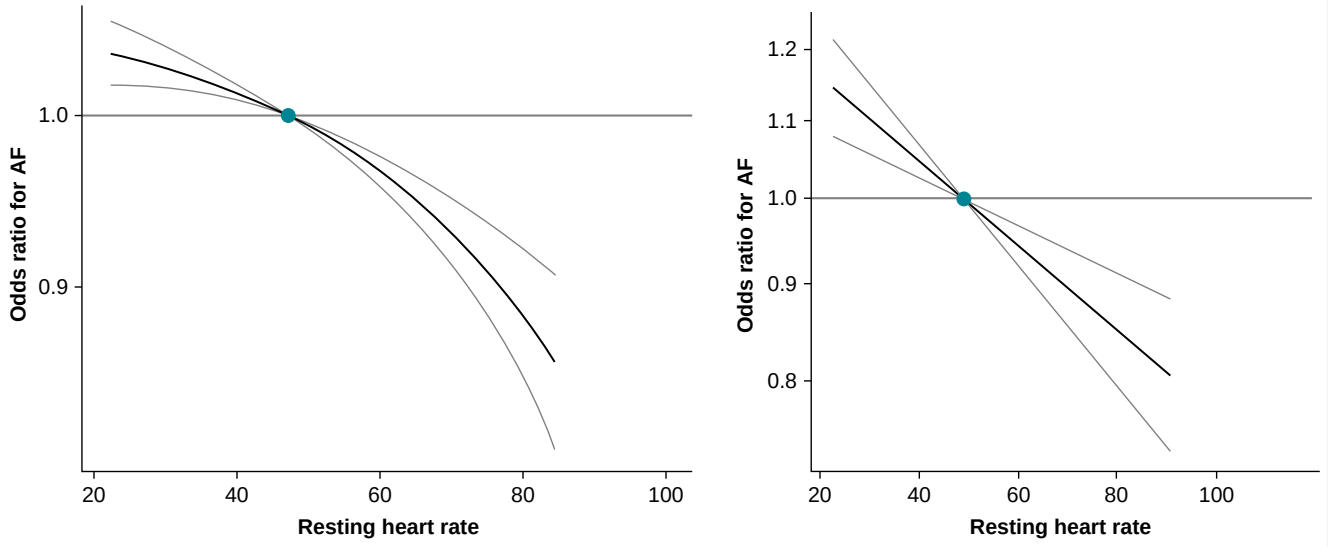


Figure 4 The causal association between resting heart rate and risk of atrial fibrillation from non-linear Mendelian randomization. Here, the resting heart rate distribution is divided into 100 strata, and an estimate from linear Mendelian randomization is made in each stratum. The black line is the dose-response curve between resting heart rate and risk of atrial fibrillation, with the grey lines indicating the 95% confidence intervals, in UK Biobank to the left and The Trøndelag Health Study to the right. AF, atrial fibrillation.

seem to be equally distributed between AF cases and controls, and by that not affecting the causal estimate. Similarly, the sensitivity analyses in the subpopulation with SBP < 140 mmHg were consistent with the linear and non-linear estimates from the main analyses, making it less likely that SBP and hypertension affect the results.

The fact that the genetic instrument was associated with SBP and CRF in UKB could imply that the genetic variants affecting RHR also affects SBP and/or CRF. BP is highly genetic, with heritability estimates of 30–50%.[36] Elevated RHR is associated with elevated BP.[37,38] In a GWAS of more than 1 million people, several of the novel genetic loci identified to be associated with BP were also associated with RHR.[39] Also, CRF is highly genetic with heritability estimates of 50–60%.[40,41] There are few genetic studies on CRF, as it is a time-consuming and costly phenotype to quantify, but one study demonstrated that the CRF slope, defined as an estimate of the rate of the increase in heart rate during exercise, was genetically correlated with RHR ($r = -0.31$, $se = 0.07$).[42] Therefore, the likelihood of pleiotropic effect biasing the causal estimates in this study exists.

In addition to what has already been discussed, RHR is closely related to different mechanisms and other factors like heart size and heart structure. Accordingly, it could be difficult to differentiate whether the genetic instrument affects RHR and/or these interrelated phenotypes. Therefore, pleiotropic effects could affect the causal estimates in the MR analysis. MR results should always be interpreted with caution as the underlying assumptions are difficult to test. However, the combined evidence from this study and previous observational studies and MR studies, suggests that there might be a causal effect of RHR on AF especially for low RHR values (<65 bpm), and potentially up to 90 bpm.

In HUNT, the RHR measures were collected either between 1995–1997 (70% of the participants) or between 2006 and 2008 (30% of the participants), with a follow-up of hospital registry data until 2016, making the follow-up period 21–19 years for 70% of the participants, and 10–8 years for 30% of the participants. In UKB, the RHR measures were collected from 2006–2010, with hospital registry data available until 2021, making the follow-up period 15–11 years. Hence, the follow-up period is longer for the majority in HUNT compared to UKB, which can explain why there are more AF cases in HUNT (10.2%) than in UKB (4.7%). In the previous MR study by Siland *et al.* that used individual-level data in a one sample MR setting, the prevalence of AF was 12%, while the prevalence of AF was 4.2% in the observational meta-analysis by Liu *et al.* Again, there are similar results in both HUNT and UKB, implying that this likely does not affect the results.

A strength of the reported data is that this is the first study using NLMR to investigate the shape of the causal effect of RHR on AF. This is also the largest MR study on RHR and AF using individual-level data. The genetic instrument used is validated in a cohort independent of both HUNT and UKB, avoiding sample overlap. In addition, the identification and validation of the genetic instrument are completely independent of the HUNT population. This is the first MR study looking at RHR and AF using another population than the UKB. Hence, the findings from HUNT, which confirms what has previously been found in UKB, are novel and add strength and validity to the previous findings.

Conclusion

Both linear and NLMR were used to investigate the shape of the potential causal association between RHR and AF. A linear, inverse relationship between RHR and AF was demonstrated, and the NLMR estimates were supportive of the linear MR, showing a decreased risk of AF with increasing RHR values up to 90 bpm. We speculate however that this likely does not indicate that increasing RHR above normal values provides any protection for AF. A genetic predisposition for a low RHR seems to increase the risk of AF, potentially due to a causal effect or pleiotropic pathway on cardiac remodelling, atrial myopathy, autonomic tone, and/or heart structure. Future investigations of the underlying mechanisms of the genetic instrument, RHR, and investigating possible confounding with other phenotypes such as heart size, heart structure, CRF, or SBP are desired.

Authors' contributions

Conceptualization: M.K., A.B., and B.M.B.; Data curation: M.K. and B.M.B.; Formal analysis: M.K.; Software: M.K., H.R., and B.M.B.;

Supervision: A.B., E.M., B.M.B., and P.R.R.; Writing-original draft: M.K.; Writing-review & editing: M.K., H.R., P.R.R., E.M., B.M.B., and A.B.

Ethics declaration

This study was approved by the Regional Committee for Medical Research Ethics (2019/29771), the HUNT, the Norwegian Data Inspectorate, and by the National Directorate of Health. The study is in conformity with Norwegian laws and the Helsinki Declaration, and written informed consent was obtained from all participants in the HUNT study. This research has been conducted using the UKB Resource under application number 40135. The UKB research protocol and study design were approved by the NHS National Research Ethics Service, and all study participants provided written informed consent. Ethical approval was obtained from the Northwest Centre for Research Ethics Committee (MREC, 11/NW/0382). In Scotland, the UKB has approval from the Community Health Index Advisory Group (CHIAG).

Supplementary material

Supplementary material is available at *Europace* online.

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Data availability

The data underlying this article are available in the article and in its online supplementary material.

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