Circulation: Arrhythmia and Electrophysiology

ORIGINAL ARTICLE

Cardiovascular Predictive Value and Genetic Basis of Ventricular Repolarization Dynamics

BACKGROUND: Early prediction of cardiovascular risk in the general population remains an important issue. The T-wave morphology restitution (TMR), an ECG marker quantifying ventricular repolarization dynamics, is strongly associated with cardiovascular mortality in patients with heart failure. Our aim was to evaluate the cardiovascular prognostic value of TMR in a UK middle-aged population and identify any genetic contribution.

METHODS: We analyzed ECG recordings from 55 222 individuals from a UK middle-aged population undergoing an exercise stress test in UK Biobank (UKB). TMR was used to measure ventricular repolarization dynamics, exposed in this cohort by exercise (TMR during exercise, TMR^{ex}) and recovery from exercise (TMR during recovery, TMR^{rec}). The primary end point was cardiovascular events; secondary end points were all-cause mortality, ventricular arrhythmias, and atrial fibrillation with median follow-up of 7 years. Genome-wide association studies for TMR^{ex} and TMR^{rec} were performed, and genetic risk scores were derived and tested for association in independent samples from the full UKB cohort (N=360 631).

RESULTS: A total of 1743 (3.2%) individuals in UKB who underwent the exercise stress test had a cardiovascular event, and TMR^{rec} was significantly associated with cardiovascular events (hazard ratio, 1.11; $P=5\times10^{-7}$), independent of clinical variables and other ECG markers. TMR^{rec} was also associated with all-cause mortality (hazard ratio, 1.10) and ventricular arrhythmias (hazard ratio, 1.16). We identified 12 genetic loci in total for TMR^{ex} and TMR^{rec}, of which 9 are associated with another ECG marker. Individuals in the top 20% of the TMR^{rec} genetic risk score were significantly more likely to have a cardiovascular event in the full UKB cohort (18 997, 5.3%) than individuals in the bottom 20% (hazard ratio, 1.07; $P=6\times10^{-3}$).

CONCLUSIONS: TMR and TMR genetic risk scores are significantly associated with cardiovascular risk in a UK middle-aged population, supporting the hypothesis that increased spatio-temporal heterogeneity of ventricular repolarization is a substrate for cardiovascular risk and the validity of TMR as a cardiovascular risk predictor.

VISUAL OVERVIEW: A visual overview is available for this article.

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Key Words: exercise ■ genetic analyses ■ genetic risk score ■ middle aged ■ risk ■ T-wave morphology

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WHAT IS KNOWN?

- The T-wave morphology restitution (TMR) is a recently proposed ECG marker that quantifies the rate of variation of the T-wave morphology with heart rate.
- TMR is a strong predictor of sudden cardiac death in chronic heart failure patients.

WHAT THE STUDY ADDS?

- TMR at 1-minute recovery from exercise (TMR during recovery) was associated with cardiovascular risk (hazard ratio, 1.11; P=5×10^{-7}), all-cause mortality (hazard ratio, 1.10), and ventricular arrhythmic risk (hazard ratio 1.16) independent of clinical variables, resting corrected QT interval, and resting and recovery heart rate from an analysis of 60 000 individuals from a UK middle-aged population participating in an exercise stress test.
- Genetic loci for TMR during exercise and TMR during recovery were identified, of which 9 had been previously associated with other ECG markers. Individuals having a cardiovascular event in a ≈500 000 cohort had a higher genetic risk score for TMR during recovery than unaffected individuals.
- We demonstrate that TMR is a heritable risk marker for cardiovascular risk in a UK middle-aged population.

Cardiovascular mortality is the main cause of death in the general population,[1] and it accounts for 31% of all deaths worldwide, with its estimated cost expected to be $1044 billion by 2030. Despite technological advances, prediction remains a critically important challenge.

The QT interval is the most recognized ECG index and reflects the duration of ventricular depolarization and repolarization. However, increasing evidence suggests that dispersion of repolarization and, in particular, its variations with heart rate, is a stronger marker for cardiovascular risk than the total duration of repolarization.[2,3] The T-wave morphology restitution (TMR)[4] is a recently proposed ECG marker that quantifies the rate of variation of the T-wave morphology with heart rate. This marker has shown to be a strong predictor of sudden cardiac death in chronic heart failure patients.[4,5] However, its performance as a potential cardiovascular risk marker in the general population has not been evaluated. Furthermore, the biological mechanisms underlying TMR are not known.

ECG markers are heritable[6] and statistical genetic methods are available to estimate the cumulative contribution of genetic factors to cardiovascular events via genetic risk scores (GRSs).[7] We hypothesize that the interaction between repolarization dynamics and cardiovascular risk has a genetic component and that TMR can be used to capture it.

Our primary objective was to validate the prognostic significance of TMR in a dataset of 55 222 individuals where exercise and recovery from exercise were used to expose spatio-temporal heterogeneity of ventricular repolarization. Our secondary objectives were to perform genome-wide association studies (GWASs) to identify single-nucleotide variants (SNVs) determining the genetic contribution of TMR and to develop GRSs to evaluate their association with cardiovascular events in an independent population of 360 631 individuals.

METHODS

Anonymized data and materials have been returned to UK Biobank (UKB) and can be accessed per request.

Study Population, Follow-Up, and End Points

UKB is a prospective study of 488 377 individuals (FULL-UKB cohort), comprising relatively even numbers of men and women aged 40 to 69 years old at recruitment (2006–2008). A total of 95 216 individuals were invited for an exercise test using a stationary bicycle in conjunction with a 1-lead ECG device (Methods in the Data Supplement). Complete ECG recordings from 58 839 individuals, who were considered fit to perform the exercise stress test (EST), were available (EST in UKB [EST-UKB] cohort; Figure 1). Individuals were excluded if they had existing medical conditions known to affect heart rate, if they had experienced a previous cardiovascular event (matching the codes from Table I in the Data Supplement), if they were on heart rate altering medications, had been diagnosed with bundle branch block, if the ECG had poor quality, or there was no heart rate change during the exercise test (Methods in the Data Supplement). This led to N=55 222 individuals included in the analyses. The UKB study has approval from the North West Multicentre Research Ethics Committee, and all participants provided informed consent.[8]

The primary end point of this study was cardiovascular events, defined as cardiovascular mortality or admission to hospital with a cardiovascular diagnosis. The exact *International Classification of Diseases, Tenth Revision* codes used to define cardiovascular events are presented in Table I in the Data Supplement. The secondary end points were all-cause mortality (excluding external causes), ventricular arrhythmic events (defined as arrhythmic mortality or admission to hospital with an arrhythmic diagnosis), and atrial fibrillation. Details on cause and date of death and diagnoses are available in the Methods in the Data Supplement. Follow-up was from the study inclusion date until March 31, 2017.

Derivation of TMR During Exercise and TMR During Recovery

The bicycle ergometer exercise test followed a standardized protocol: 15 s resting period, 2 minutes of constant load, 4 minutes of exercise during which the workload was gradually increased, and a 1-minute recovery period without pedaling (Figure 2A). Details of the preprocessing of

the ECG recordings are available in the Methods in the Data Supplement. Automatic quantification of TMR during exercise (TMR^{ex}) and recovery (TMR^{rec}; shown in Figure 2) was performed on every ECG recording in 3 steps:

1. Derivation of average T waves: signal averaging of all available heartbeats within a 15 s window at rest, peak exercise, and recovery was used to reduce noise (Figure 2B). The onset, peak, and offset timings of the waveforms were located using bespoke software.[9,10] Average T waves at rest, peak exercise, and recovery were selected using the T onset and T offset timings and were further low-pass filtered at 20 Hz.
2. T-wave morphology differences quantification: using a previously published algorithm based on time warping,[11] we derived the marker dw^{ex}, representing the average temporal stretching necessary to align each point of the average T wave at rest to the average T wave at peak exercise.[11] Figure 2C shows an example where 2 T waves have similar morphology and small dw^{ex}. Similarly, the marker dw^{rec} represents the average temporal stretching necessary to align each point of the average T wave at peak exercise and the average T wave at recovery. Figure 2C shows that the morphological difference between the 2 T waves has increased along with dw^{rec}.
3. TMR calculations: TMR^{ex} and TMR^{rec} were calculated by dividing dw^{ex} and dw^{rec} by the change in the RR interval (inverse of hearte rate) during exercise, ΔRR^{ex}, and during recovery, ΔRR^{rec}, respectively, and represent the T-wave morphological change per RR increment during exercise and recovery, respectively.[4]

Computation of Other ECG markers

The QT interval and QRS duration were measured as the interval between the QRS-onset and the T-wave end, and between the QRS-onset and the QRS-offset, respectively, from the averaged heartbeat at rest. Then, we corrected the QT interval using Bazett formula.[12] We additionally derived the marker T-wave inversion, which indicated a change in the polarity of the T waves between resting and exercise stages[13] (Methods in the Data Supplement).

Statistical Analyses

The 2-tailed Mann-Whitney and Fisher exact tests were used for univariate comparison of quantitative and categorical data, respectively. Correlation was evaluated with Spearman correlation coefficient. Receiver operator curves were derived using the pROC package[14] from R and C-indices were calculated for each marker. We estimated the optimal cutoff values for TMR^{ex} and TMR^{rec} in a training set (N=27 612) from the EST-UKB cohort (Methods in the Data Supplement) by means of log-rank statistics optimization with the aim of maximizing the predictive value. Kaplan-Meier curves were derived using the optimal cutoff values in the test set (N=27 610), with a comparison of cumulative events performed by using log-rank tests.

Univariate and multivariate Cox regression analyses were performed to determine the predictive value of the risk markers. The proportional hazard assumptions were checked when applying these analyses. Continuous variables were standardized to a mean of 0 and SD of 1 to allow for comparisons in the Cox models. Only the variables with a significant association with the end point in univariate analysis were included in the multivariate model. Individuals who died from causes not included in the primary end point were censored at the time of death. A value of $P<0.05$ was considered statistically significant. Statistical analyses were performed using R version 3.5.1.

Heritability and GWASs

Inverse-normal transformation of TMR^{ex} and TMR^{rec} was performed as the distributions were skewed and did not approximate a normal distribution (Figure I in the Data Supplement). Heritability was estimated using a variance components method (BOLT-REML).[15] GWAS for TMR^{ex} and TMR^{rec} were performed in a discovery (N=29 393) and replication (N=22 382) datasets separately using a linear mixed model method (BOLT-LMM).[16] The TMR^{ex} model included the following covariates: sex, age, body mass index (BMI), resting RR, ΔRR^{ex} and a binary indicator variable for the genotyping array (UKB versus UK BiLEVE). The TMR^{rec} model included covariates sex, age, BMI, recovery RR, ΔRR^{rec} and the genotyping array. After careful review of significant ($P<1\times10^{-6}$) SNVs from the discovery GWASs, 6 variants for TMR^{ex} and 7 variants for TMR^{rec} were taken forward into replication. Replication was confirmed if the SNVs remained significant (with Bonferroni correction) and with concordant direction of effects to the discovery analyses. A full dataset GWAS for both TMR^{ex} and TMR^{rec} was conducted and additional loci reaching genome-wide significance ($P<5\times10^{-8}$) were reported. Since TMR^{ex} and TMR^{rec} were genetically correlated ($\rho=0.58$), multitrait analysis of GWAS[17] was used to leverage additional loci discovery. Detailed information can be found in Methods in the Data Supplement.

To examine if there were independent secondary SNVs at TMR loci, we applied genome-wide complex trait analysis[18] for all reported loci from the full dataset GWAS. The percent variance of TMR^{ex} and TMR^{rec} explained by the identified loci was calculated with standard methods, detailed in the Methods in the Data Supplement. Bioinformatics analyses were performed to annotate SNVs and identify candidate genes, including Variant Effect Predictor,[19] GTEx (the Genotype-Tissue Expression project), and long-range chromatin interaction data.[20] We used PhenoScanner,[21] GWAS catalog (https://www.ebi.ac.uk/gwas/), and UKBiobank ICD PheWeb (http://pheweb.sph.umich.edu/SAIGE-UKB/) to determine SNV and gene associations with other traits. Pathway analyses were performed using g:profiler.[22] Further description of bioinformatics analyses can be found in the Methods in the Data Supplement. We downloaded the summary statistics for atrial fibrillation[23] to calculate its genetic correlation with TMR^{ex} and TMR^{rec} using LD score regression.[24]

Genetic Risk Score Analyses

We used PRSice v2[25] to construct the GRS for TMR^{ex} and TMR^{rec} using the effect sizes from the full-cohort GWASs (EST-UKB) and performed prediction for the primary end point in the full UKB cohort (FULL-UKB) dataset (after exclusions, Figure II and Methods in the Data Supplement). We

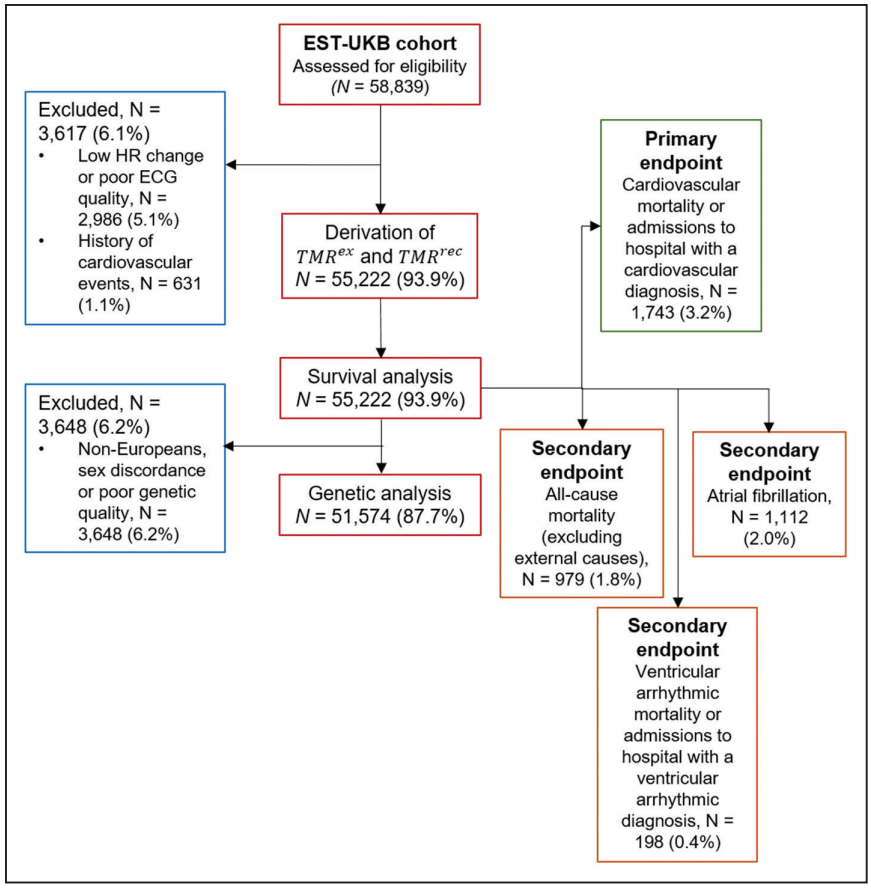

Figure 1. Flow diagram of analyses in the exercise stress test (EST; EST in UK Biobank [EST-UKB]) population.
HR indicates heart rate; TMR, T-wave morphology restitution; TMR^{ex}, TMR during exercise; and TMR^{rec}, TMR during recovery.

first removed individuals included in the GWASs (EST-UKB) and their relatives, then removed all individuals with a previous history of cardiovascular events and non-Europeans. The GRSs were standardized to have a mean of 0 and an SD of 1. Their association with the study end points was tested in the FULL-UKB cohort (after exclusions, Figure II in the Data Supplement) using Mann-Whitney and Univariate Cox regression analyses.

RESULTS

Predictive Value of TMR in a UK Middle-Aged Population

The EST-UKB population consisted of 55 222 individuals (25 669 males, 29 553 females) aged 40 to 73 years (mean 57±8 years) after exclusions. The demographic characteristics of this population are shown in Table II in the Data Supplement. During the follow-up, 1743 (3.2%) individuals had a cardiovascular event. The distributions of TMR^{ex} and TMR^{rec} are shown in Figure I in the Data Supplement.

Age, BMI, TMR^{rec} ($P<2\times10^{-16}$ for all), TMR^{ex} ($P=3\times10^{-8}$) and resting heart rate ($P=3\times10^{-4}$) were significantly higher in the cardiovascular events group than in the event-free group, whereas heart rate response to exercise and recovery were lower ($P<2\times10^{-16}$ for both). Also, there were more males, diabetics, hypertensives (stage 1 [130 mm Hg ≤ systolic blood pressure <140 mm Hg or 85 mm Hg ≤ diastolic blood pressure <90 mm Hg] and stage 2 [systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥ 90 mm Hg]), individuals with high cholesterol levels ($P<2\times10^{-16}$ for all), smokers ($P=1\times10^{-13}$), diagnosed with chronic kidney disease ($P=5\times10^{-2}$), or with T-wave inversions ($P=9\times10^{-3}$). QRS duration was not significantly different in individuals with and without cardiovascular events and thus was not included in the survival analyses (Table III and Figure III in the Data Supplement). Spearman correlation coefficient between TMR^{ex} and TMR^{rec} was 0.484; lower correlations were found between them and covariates (Table IV in the Data Supplement).

Individuals in the TMR^{ex} ≥ 0.082 group (stratified according to the optimal cutoff value—Figure IV in the Data Supplement) had 1.65 fold risk (95% CI, 1.38–1.98) of having a cardiovascular event than those in the TMR^{ex} <0.082 group ($P<10^{-3}$; Figure 3A). Similarly, individuals in the TMR^{rec} ≥0.115 group (Figure V in the Data Supplement) had 1.71 fold risk (95% CI, 1.43–2.05) of having a cardiovascular event than those in the TMR^{rec} <0.115 groups ($P<10^{-3}$; Figure 3B).

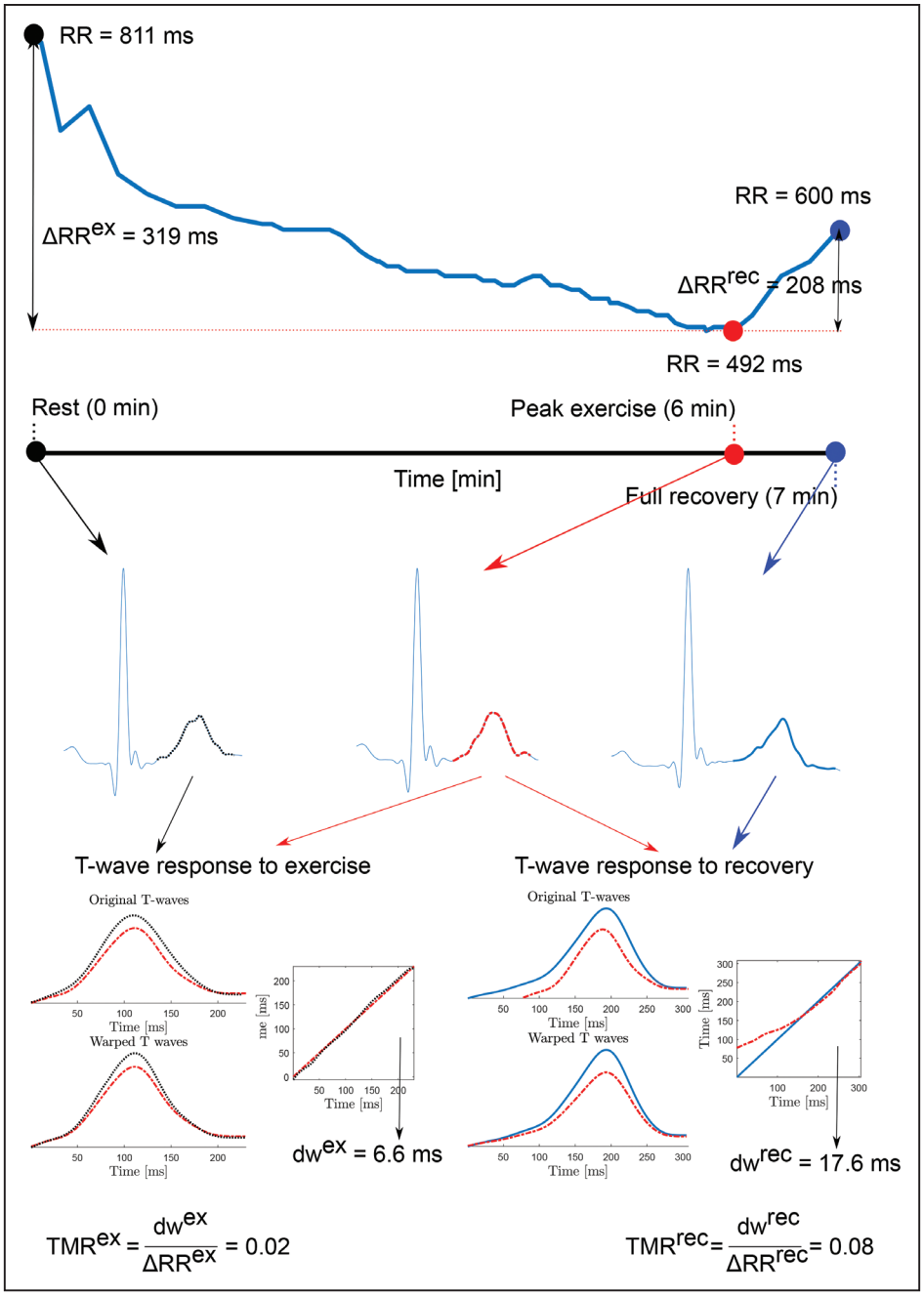

Figure 2. Assessment of T-wave morphology restitution (TMR).
A, Illustration of the RR profile during the exercise stress test. **B**, Three averaged heartbeats are derived at rest (black), peak exercise (red) and 50 s after peak exercise (full recovery, blue), respectively. **C**, TMR during exercise (TMR^{ex}) and TMR during recovery (TMR^{rec}) are derived by quantifying the morphological change between the T waves at rest (black T wave) and at peak exercise (red T wave), and between the T waves at peak exercise and full recovery (blue T wave), respectively, normalized by the corresponding RR change. ΔRR^{ex} indicates change in RR interval during exercise; and ΔRR^{rec}, change in RR interval during recovery.

To compare the hazard ratios (HRs) of TMR^{ex} and TMR^{rec} with those from other continuous markers, independently from cutoff thresholds, we included the continuous TMR^{ex} and TMR^{rec} markers into a multivariate Cox regression model. The following variables remained significantly associated with cardiovascular events (HR [95% CI] reported): chronic kidney disease (2.85 [1.07–7.62]), sex (2.82 [2.52–3.15]), T-wave inversion (2.21 [1.10–4.45]), age (1.73 [1.63–1.84]), diabetes mellitus (1.56 [1.32–1.84]), hypertension stage 2 (1.32 [1.15–1.51]), hypertension stage 1 (1.19 [1.02–1.39]), BMI (1.18 [1.13–1.25]), corrected QT interval (1.11 [1.06–1.17]), and TMR^{rec} (1.11 [1.07–1.16]; Table 1). Among ECG markers, resting heart rate, heart rate responses to exercise and recovery, and TMR^{ex} were no longer significant. Among all cardiovascular events, 81.7% were related to ischemic heart disease. TMR^{rec} was independently associated

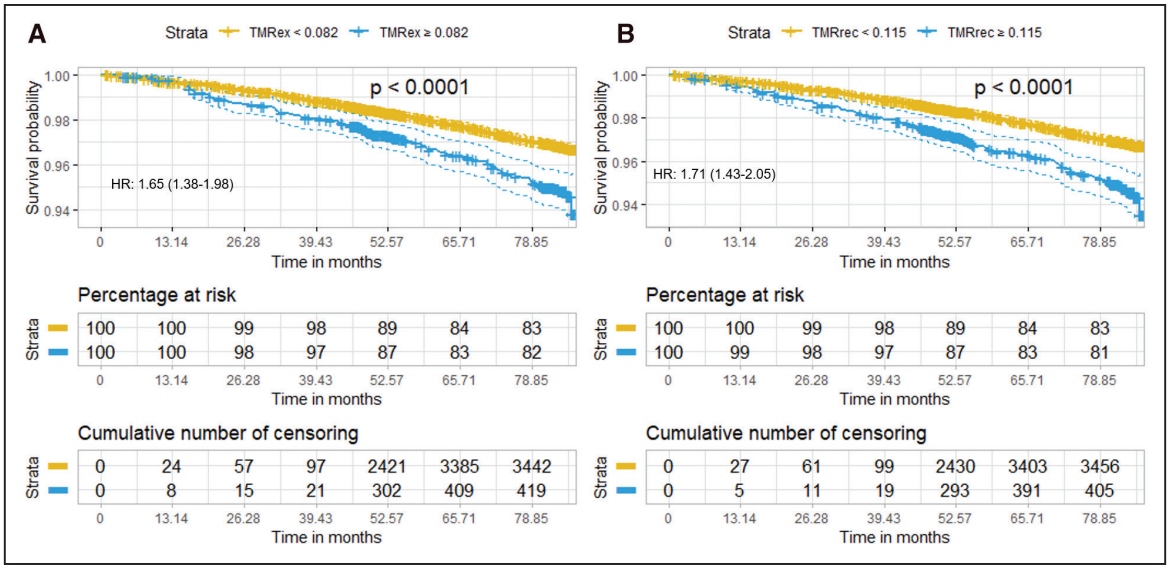

Figure 3. Kaplan-Meier survival curves.
Cumulative survival rates of individuals stratified by T-wave morphology restitution (TMR) during exercise (TMR^{ex}) of ≥0.082 (**A**) and by TMR during recovery (TMR^{rec}) of ≥0.115 (**B**). Dashed lines indicate the 95% confidence levels. HR indicates hazard ratio.

with both ischemic (HR [95% CI] of 1.08 [1.03–1.13]) and nonischemic (HR [95% CI] of 1.20 [1.11–1.30]) causes (Tables VA and VB in the Data Supplement). The assumption of proportional hazards was supported for all covariates.

For the secondary end points, there were 979 (1.8%) cases of all-cause mortality, 198 (0.4%) who had a ventricular arrhythmic event, and 1112 (2.0%) who had atrial fibrillation (Table II in the Data Supplement). In multivariate Cox analysis, TMR^{rec} remained significantly associated

Table 1. Association With Cardiovascular Risk

	Univariate		Multivariate	
	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Clinical variables				
Age (per 1 SD)	1.88 (1.78–2.00)	$<2\times10^{-16}$*	1.73 (1.63–1.84)	$<2\times10^{-16}$*
Sex (male)	3.01 (2.70–3.35)	$<2\times10^{-16}$*	2.82 (2.52–3.15)	$<2\times10^{-16}$*
Diabetes mellitus (yes)	2.71 (2.31–3.19)	$<2\times10^{-16}$*	1.56 (1.32–1.84)	2.20×10^{-7}*
High cholesterol (yes)	1.95 (1.72–2.20)	$<2\times10^{-16}$*	1.10 (0.97–1.25)	1.60×10^{-1}
BMI (per 1 SD)	1.28 (1.23–1.34)	$<2\times10^{-16}$*	1.18 (1.13–1.25)	3.00×10^{-11}*
Hypertensive stage 1	1.72 (1.48–2.01)	4.10×10^{-12}*	1.19 (1.02–1.39)	2.60×10^{-2}*
Hypertensive stage 2	2.43 (2.14–2.76)	$<2\times10^{-16}$*	1.32 (1.15–1.51)	4.70×10^{-5}*
Previous or current smoker (yes)	1.38 (1.25–1.53)	9.30×10^{-11}*	1.10 (0.99–1.21)	8.60×10^{-2}
CKD (yes)	3.62 (1.36–9.66)	1.00×10^{-2}*	2.85 (1.07–7.62)	3.70×10^{-2}*
ECG variables				
Resting heart rate (per 1 SD)	1.10 (1.05–1.15)	5.70×10^{-5}*	0.97 (0.91–1.03)	2.90×10^{-1}
Heart rate response to exercise (per 1 SD)	0.70 (0.66–0.74)	$<2\times10^{-16}$*	1.02 (0.94–1.10)	6.70×10^{-1}
Heart rate response to recovery (per 1 SD)	0.74 (0.71–0.76)	$<2\times10^{-16}$*	0.96 (0.90–1.03)	2.50×10^{-1}
Corrected QT (per 1 SD)	1.15 (1.10–1.20)	4.00×10^{-10}*	1.11 (1.06–1.17)	5.40×10^{-5}*
T-wave inversion (yes)	2.80 (1.40–5.60)	3.70×10^{-3}*	2.21 (1.10–4.45)	2.70×10^{-2}*
TMR during exercise (per 1 SD)	1.17 (1.12–1.22)	6.10×10^{-15}*	1.03 (0.98–1.08)	2.50×10^{-1}
TMR during recovery (per 1 SD)	1.23 (1.19–1.28)	$<2\times10^{-16}$*	1.11 (1.07–1.16)	4.90×10^{-7}*

Hypertensive stage 1 defined as 130 mm Hg ≤ SBP <140 mm Hg or 85 mm Hg ≤ DBP <90 mm Hg. Hypertensive stage 2 defined as SBP ≥140 mm Hg or DBP ≥90 mm Hg. Reference Hypertension group is Hypertensive stage 0, defined as SBP <130 mm Hg and DBP <85 mm Hg. BMI indicates body mass index; CKD, chronic kidney disease; DBP, diastolic blood pressure; HR, hazard ratio; SBP, systolic blood pressure; and TMR, T-wave morphology restitution.

*Indicates statistically significant.

Table 2. Loci Associated With TMR During Exercise

Locus	SNV	CHR	BP	EA	EAF	Discovery				Replication				Combined			
						P Value	N	β	SE	*P* Value	N	β	SE	*P* Value	N	β	SE
RNF207§	rs709208	1	6272137	A	0.679	2.60×10^{-7}	27939	−0.042	0.008	1.60×10^{-5}	20769	−0.040	0.009	1.80×10^{-11}	49203	−0.041	0.006
*NOS1AP**†	rs12143842	1	162033890	C	0.750	1.20×10^{-4}	29393	−0.033	0.008	3.40×10^{-3}	21850	−0.029	0.010	6.60×10^{-7}	51764	−0.032	0.006
*SCN5A–SCN10A**‡	rs7428232	3	38778618	T	0.416	5.20×10^{-6}	29352	−0.034	0.007	1.80×10^{-4}	21820	−0.032	0.008	3.70×10^{-9}	51692	−0.033	0.006
PREP†	rs4478445	6	105786660	C	0.943	2.50×10^{-5}	28913	−0.067	0.016	7.40×10^{-3}	21493	−0.049	0.018	8.00×10^{-7}	50919	−0.059	0.012
KCNH2	rs2072412	7	150647970	C	0.729	1.80×10^{-6}	28975	0.040	0.008	4.10×10^{-7}	21539	0.048	0.010	2.10×10^{-11}	51028	0.042	0.006
*KCNQ1**§	rs2074238	11	2484803	T	0.088	1.10×10^{-8}	29393	−0.073	0.013	1.20×10^{-3}	21850	−0.048	0.015	1.20×10^{-1}	51764	−0.062	0.010
*SOX5**§	rs7307613	12	24595192	C	0.505	1.80×10^{-7}	29359	0.038	0.007	3.50×10^{-6}	21825	0.039	0.008	2.80×10^{-12}	51704	0.039	0.006
KCNJ2§	17:68493468_GA_G	17	68493468	GA	0.674	7.60×10^{-7}	29318	0.039	0.008	3.70×10^{-7}	21794	0.046	0.009	2.90×10^{-13}	51632	0.043	0.006

The locus name indicates the gene that is in the closest proximity to the most associated SNV. BP indicates position, based on human genome build 19; CHR, chromosome; EA, effect allele; EAF, effect allele frequency from discovery data; LD, linkage dissequilibrium; MTAG, multitrait analysis of genome-wide association study; N, number of participants; SNV, single-nucleotide variation; and TMR, T-wave morphology restitution.

*SNV is the same or in high LD (r^2>0.8) with an SNV associated with the other index.

†Identified with MTAG.

‡Has a secondary signal.

§Replicated SNVs.

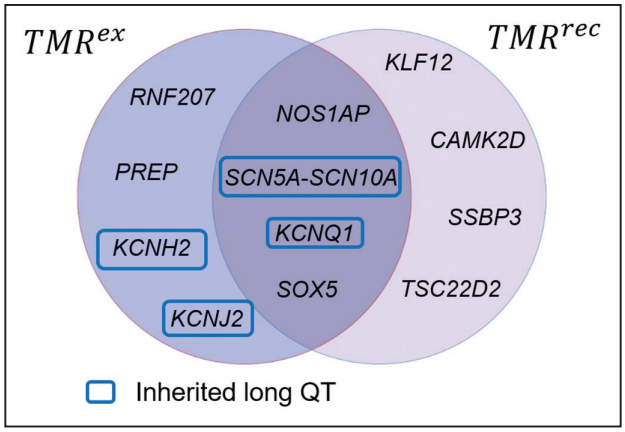

Figure 4. Overlap of loci for T-wave morphology restitution (TMR) during exercise (TMR^{ex}) and TMR during recovery (TMR^{rec}).
The loci names indicate the coding gene that is in the closest proximity to the most associated single-nucleotide variation.

with all-cause mortality (HR [95% CI] of 1.10 [1.04–1.17]) independently of age, sex, smoke, diabetes mellitus, resting heart rate, heart rate response to recovery, and heart rate response to exercise (Table VI in the Data Supplement). TMR^{rec} also remained significantly associated with ventricular arrhythmic events (HR [95% CI] of 1.16 [1.03–1.30]) independently of sex, age, and heart rate response to recovery (Table VII in the Data Supplement). Finally, TMR^{rec} was not independently associated with atrial fibrillation (Table VIII in the Data Supplement).

Twelve Genetic Loci Are Associated With TMR

A total of 51574 subjects were taken forward for genetic analyses after applying genetic quality control and excluding individuals of non-European ancestry (Figure 1). The heritability estimations of TMR^{ex} and TMR^{rec} were 3.5% and 4.9%, respectively, and their phenotypic correlation was 0.43.

In the discovery cohort GWAS (Methods), 1 genome-wide significant ($P\leq5\times10^{-8}$) locus was found for TMR^{ex}, and 3 for TMR^{rec} (Table IX in the Data Supplement). Four SNVs for TMR^{ex} and 3 for TMR^{rec} formally replicated in the independent validation cohort (Tables 2 and 3). In the full dataset analysis, 2 additional SNVs reached genome-wide significance for TMR^{ex} and 4 SNVs for TMR^{rec}, respectively, all with concordant directions of effect (Tables 2 and 3). Manhattan plots for the full dataset are shown in Figure VI in the Data Supplement. Visual inspection of the corresponding QQ plots from the discovery and full dataset GWASs did not show evidence of *P* value inflation or confounding (Figure VII in the Data Supplement). Analysis using multitrait analysis of GWAS[17] (Methods) indicated 2 additional loci were significantly associated with TMR^{ex} and 1 for TMR^{rec} (Tables XA and XB in the Data Supplement). Sex-stratified analyses did not identify sex-specific loci for TMR^{ex}

Table 3. Loci Associated With TMR During Recovery

Locus	SNV	CHR	BP	EA	EAF	Discovery				Replication				Combined			
						P Value	N	β	SE	*P* Value	N	β	SE	*P* Value	N	β	SE
SSBP3	rs562408	1	54742618	A	0.430	6.20×10^{-6}	28 299	0.030	0.007	7.40×10^{-3}	21 091	0.020	0.008	3.70×10^{-8}	49 895	0.027	0.005
*NOS1AP**§	rs12143842	1	162033890	C	0.750	8.10×10^{-9}	29 013	−0.043	0.007	1.60×10^{-8}	21 623	−0.048	0.009	5.10×10^{-16}	51 153	−0.045	0.006
*SCN5A-SCN10A**‡	rs7373065	3	38710315	T	0.019	2.00×10^{-6}	26 979	0.114	0.024	2.10×10^{-6}	20 107	0.132	0.028	1.60×10^{-11}	47 566	0.122	0.018
TSC22D2	rs112717154	3	149943115	G	0.863	1.40×10^{-6}	27 857	−0.046	0.010	5.30×10^{-3}	20 762	−0.031	0.011	9.30×10^{-9}	49 115	−0.041	0.007
CAMK2D	rs35408611	4	114423677	C	0.738	6.20×10^{-3}	28 362	−0.020	0.007	1.40×10^{-8}	21 138	−0.048	0.008	2.90×10^{-8}	50 006	−0.031	0.006
*KCNQ1**§	rs2074238	11	2484803	T	0.088	1.40×10^{-31}	29 013	−0.131	0.011	4.20×10^{-31}	21 623	−0.152	0.013	1.20×10^{-59}	51 153	−0.138	0.008
*SOX5**§	rs1396206	12	24576859	A	0.482	3.10×10^{-13}	28 318	0.048	0.007	4.00×10^{-5}	21 105	0.031	0.007	1.30×10^{-16}	49 927	0.040	0.005
KLF12†	rs7992314	13	74509346	G	0.631	2.50×10^{-6}	28 908	−0.032	0.007	6.00×10^{-3}	21 545	−0.021	0.008	6.40×10^{-8}	50 968	−0.027	0.005

The locus name indicates the gene that is in the closest proximity to the most associated SNV. BP indicates position, based on human genome build 19; CHR, chromosome; EA, effect allele; EAF, effect allele frequency from discovery data; LD, linkage disequilibrium; MTAG, multitrait analysis of genome-wide association study; N, number of participants; SNV, single-nucleotide variation; and TMR, T-wave morphology restitution.

*SNV is the same or in high LD (r^2>0.8) with an SNV associated with the other index.

†Identified with MTAG.

‡Has a secondary signal.

§Replicated SNVs.

or TMR^{rec}. Conditional analyses showed evidence for 2 secondary independent signals at the *SCN5A-SCN10A* locus, 1 for each trait (Tables 2 and 3).

In total, 12 loci were identified, 8 for each trait with SNVs at 4 loci associated with both markers (Figure 4). The lead SNVs at the shared loci at *NOS1AP*, *KCNQ1*, *SCN5A-SCN10A*, and *SOX5* were identical or in high linkage disequilibrium (r^2>0.8). The identified SNVs for TMR^{ex} explained 0.63% of its variance. Similarly, the 8 SNVs identified for TMR^{rec} explained 1.14% of its variance. This corresponds to 20% and 23% of the estimated heritability for each TMR marker, respectively.

Variants at 7 of the 12 TMR loci have previously been reported to be associated with resting QT (*RNF207*, *KCNH2*, *KCNJ2*, *NOS1AP*, *SCN5A-SCN10A*, *KCNQ1*, and *KLF12*). Regional plots are shown in Figure VIII in the Data Supplement. Look-ups in PhenoScanner indicated 9 of the 12 SNVs have associations with other cardiovascular markers, including pulse rate, QT interval, PR interval, QRS duration, P-wave duration, cardiac arrhythmias, and heart function (Tables XIA and XIB in the Data Supplement).

None of the lead variants or their close proxies (r^2>0.8) were annotated as missense variants. Variants at 2 loci *NOS1AP* and *SSBP3* were associated with expression levels of nearby genes (*c1orf226* and *SSBP3*, respectively) in heart atrial appendage samples (Table XII in the Data Supplement). We found 11 potential target genes whose promoter regions form significant chromatin interactions at 9 TMR loci (Table XIII in the Data Supplement). Using this information and literature review, we derived a list of candidate genes at each locus (Table XIV in the Data Supplement).

Table XV in the Data Supplement shows a lookup of all candidate genes in the GWAS catalog and in UKBiobank ICD PheWeb and indicate associations across different cardiovascular traits, including atrial fibrillation. Our LD Score regression analysis indicated there was no significant genetic correlation between TMR^{ex} or TMR^{rec} and atrial fibrillation. The top 3 biological pathways for TMR^{ex} were cardiac muscle cell action potential ($P=4\times10^{-10}$), regulation of ventricular cardiac muscle cell membrane repolarization ($P=4.7\times10^{-10}$), and ventricular cardiac muscle cell membrane repolarization ($P=1\times10^{-9}$; Figure IX in the Data Supplement). The analyses for TMR^{rec} indicated similar pathways including cardiac muscle cell action potential ($P=6.6\times10^{-8}$), regulation of cardiac muscle contraction ($P=1.2\times10^{-7}$), and regulation of striated muscle contraction ($P=3\times10^{-7}$, Figure X in the Data Supplement).

Predictive Value of GRSs for TMR

After excluding individuals from the EST-UKB cohort and applying the exclusion criteria defined in Methods, the FULL-UKB population consisted of 360 631 healthy

individuals (160 793 men, 199 838 women) aged 40 to 73 years (mean 57±8 years, Figure II and Table II in the Data Supplement). During the follow-up, 18 997 (5.3%) individuals had a cardiovascular event, and 12 081 (3.3%), 2040 (0.6%) and 14 517 (4.0%) were individuals of all-cause mortality, ventricular arrhythmic events, and atrial fibrillation, respectively.

The optimal GRS for TMR^{ex} was derived combining 3442 SNVs identified using a P value of 3.1×10^{-3} for thresholding (Figure XI in the Data Supplement). This GRS was not significantly different between individuals with a cardiovascular event and those without (P=5.5×10^{-2}). The optimal GRS for TMR^{rec} was derived combining 3281 SNVs with a P<2.9×10^{-3} (Figure XII in the Data Supplement). The TMR^{rec} GRS was significantly higher in individuals with a cardiovascular event than those that did not have an event (P=1.5×10^{-2}). Univariate Cox analysis showed that individuals in the top 20% of the GRS for TMR^{rec} were significantly more likely to have a cardiovascular event than those in the bottom 20% (HR [95% CI] of 1.07 [1.02–1.12]; P=5.9×10^{-3}). No significant associations were found with the secondary end points for the 2 GRSs.

DISCUSSION

TMR is a recently developed ECG marker to measure the rate of variation of the T-wave morphology due to heart rate changes. TMR is associated with spatiotemporal heterogeneity of ventricular repolarization,[11] exposed in this cohort by exercise and recovery from exercise. The main findings of this study are (1) TMR^{rec} is significantly associated with cardiovascular events, all-cause mortality, and ventricular arrhythmias in a UK middle-aged population and (2) the identified loci for TMR^{rec} show a significant association with cardiovascular events despite limited heritability.

TMR^{rec} was an independent predictor of cardiovascular risk, after adjustment for conventional predictors (age, sex, diabetes mellitus, BMI, smoking, chronic kidney disease, and hypertension) and other ECG markers, including heart rate, corrected QT interval, and T-wave inversions in a general UK middle-aged population (Table 1). In this population, the majority of cardiovascular events were related to ischemic heart disease, and TMR^{rec} was associated with cardiovascular events in both ischemic and nonischemic individuals (Tables VA and VB in the Data Supplement). Well-established predictors of cardiovascular risk, like resting heart rate,[26] chronotropic incompetence, or heart rate recovery,[27] did not remain significantly associated with cardiovascular events after adjustment for ECG markers of ventricular repolarization (corrected QT interval, T-wave inversion, and TMR^{rec}). This suggests that ventricular repolarization abnormalities may play a more important role in creating a substrate for malignant cardiovascular events than heart rate markers in a UK middle-aged population. The QRS duration was not associated with cardiovascular events in our population; this may be explained by our cohort being a low-risk population, and we had excluded individuals with previous cardiovascular events. We suggest that future analyses should incorporate additional ECG indices with similar proven findings in individuals undergoing an EST.[28]

In our previous work, TMR predicted sudden cardiac death in a population of 651 chronic heart failure patients.[4,5] In that work, TMR, derived from 24-hour ambulatory Holter recordings, was the strongest sudden cardiac death predictor compared with other markers, including left ventricular ejection fraction, QRS duration, or T-wave alternans.[4] Interestingly, although the prevalence of ventricular arrhythmic events in the current study is too small to infer any robust conclusions (0.4% in UKB-EST, compared with 8.4% in the published chronic heart failure study), our results seem to support an association of TMR with sudden cardiac death (Table VII in the Data Supplement). In this study, TMR^{rec} was not significantly associated with atrial fibrillation.

We observed the heritability of TMR^{ex} and TMR^{rec} to be 3.5% and 4.9%, respectively, in our data set, suggesting that the mechanisms underlying TMR are largely affected by environmental factors. Despite low heritability, we identified 12 loci associated with TMR^{ex} and TMR^{rec}, 4 of which were common to both markers (Figure 4). Genetic variations at 4 of the 8 loci identified for TMR^{ex} have previously been associated with long-QT syndrome and QT in the general population: *KCNH2*, *KCNJ2*, *SCN5A*, and *KCNQ1*,[29] all proven regulators of cardiac excitation through regulation of the action potential duration and cardiac repolarizing channels.[30] *KCNQ1*, *KCNH2*, and *KCNJ2* underlie the major repolarising ventricular potassium currents, I_{Ks}, I_{Kr}, and I_{K1}, respectively. Variations in these currents might lead to changes in the T-wave morphology is entirely consistent with the known physiology. The signal involved in both TMR^{ex} and TMR^{rec} at the *KCNQ1* locus is particularly significant as the modulation of this current by rate and sympathetic tone is one of the main mechanisms of adaptation of repolarization.[31] Candidate genes indicated at two of the TMR^{ex} loci were *PREP* and *SOX5* from Hi-C analyses, which have also been associated with heart rate response to exercise and to recovery.[32]

For TMR^{rec}, 4 of the identified loci overlapped TMR^{ex} loci (*NOS1AP*, *SCN5A-SCN10A*, *KCNQ1*, and *SOX5*). Regarding the remaining 4 loci, the variant at *KLF12* has previously been reported to be associated with the QT interval, the ST-T segment, and QRS duration. Variants at the 3 remaining loci (*CAMKD2*, *SSBP3*, and *TSC22D2*) have not been associated with an ECG marker previously. Candidate genes at these loci

include: *SSBP3*, which encodes single-stranded DNA binding protein 3, and the TMR^{rec} variant identified at this locus has been reported to be associated with P-wave parameters, with its putative function being the transcriptional regulation of the alpha 2(1) collagen gene.[33] In addition, *TSC22D2* encodes a DNA binding transcription factor. Finally, the protein *CAMK2D* regulates calcium dynamics, which is central in cardiac physiology, as the key event leading to the excitation-contraction coupling and relaxation processes.[34]

TMR was developed based on the hypothesis that it reflects changes in the dispersion of ventricular repolarization with heart rate.[4] Although this is the first study that attempts to investigate the biological mechanisms underlying TMR, our predictive and genetic results indicate that TMR reflects relevant electrophysiological information. Our prediction results indicate TMR is providing prognostic information independent to resting QT (reflecting total duration of ventricular repolarization) or T-wave inversions (reflecting variations in the T-wave amplitude not captured by TMR). However, genetic analyses indicate there is a substantial overlap of loci with other ECG markers, thus shared biological processes. Future studies will investigate the relation between TMR and intracardiac indices of dispersion of repolarization, which is paramount to confirm its cardiovascular predictive utility.

Cardiovascular mortality remains the most common cause of death, with >4 million victims across Europe every year.[1] Over the past 2 decades, numerous prediction models have been developed,[35] including the Framingham[36] and SCORE[37] models. This prediction can be further improved by including additional validated risk markers into the models. Table XVI in the Data Supplement shows the reclassification results for the addition of TMR^{rec} ≥0.115 to the SCORE model (Methods in the Data Supplement), indicating that TMR adds information on risk prediction beyond traditional risk factors. In addition, the significant association between the GRS for TMR^{rec} and cardiovascular events in the FULL-UKB cohort supports its potential as a cardiovascular risk predictor in high-risk populations, albeit with small HRs possibly due to the low number of events. Future work should combine ECG and genetic markers into one score (ECG markers could only be derived from EST-UKB in this study), which may show complementary cardiovascular predictive value of both TMR^{rec} and its GRS.

CONCLUSIONS

We have conducted a systematic investigation of the genetic basis of ventricular repolarization and its influence in modulating cardiovascular risk through the analysis of the T-wave morphology. We demonstrate that TMR and the GRS for TMR^{rec} are significantly associated with cardiovascular risk in a UK middle-aged population and that TMR reflects relevant biological mechanisms influencing the risk of cardiovascular events.

ARTICLE INFORMATION

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Disclosures

None.

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SUPPLEMENTAL MATERIAL

Supplemental Methods

Study population

The exercise protocol was adapted according to participants' risk factors. Participants were only included in the study if they were allowed to cycle at 50% or 30% of their maximum workload (no risk to minimum risk). If the heart rate reached the pre-set maximum heart rate level (75% of age-predicted maximum heart rate), the test was stopped. Also, if the participant reported chest pain, felt faint, dizzy or unwell, the test was also stopped (https://biobank.ctsu.ox.ac.uk/crystal/docs/Cardio.pdf). We only included participants who terminated the exercise stress test with any discomfort and with a heart rate lower than the pre-set maximum heart rate level.

Exclusions

Individuals were excluded based on existing medical conditions known to affect heart rate (atrial fibrillation, history of myocardial infarction or heart failure, (supra)-ventricular tachycardia, atrioventricular nodal re-entrant tachycardia, second or third degree atrioventricular block, bundle branch block and use of a pacemaker), individuals with a previous CV event (matching the codes from Supplemental Table 1) and/or individuals on heart rate altering medications (non-dihydropyridine calcium antagonists (Anatomic Therapeutic Chemical (ATC) code C08D, digoxin (ATC code C01AA5), and amiodarone (ATC code C01BD01)). Individuals with an RR interval (inverse of heart rate) change between resting and peak exercise, or between peak exercise and recovery, less than 10 ms or poor quality ECG recording were also excluded.

End points

The cause of death was defined according to the *ICD*-10 codes. Date of death was obtained from death certificates held by the National Health Service (NHS) Information Centre

and the NHS Central Register Scotland for participants from England and Wales and participants from Scotland, respectively. CV diagnoses were captured using the "Spell and Episode" category from the Hospital Episode Statistics records. This category contains main and secondary diagnoses, coded according to *ICD-10*, made during the hospital inpatient stay. The main diagnoses are those taken to be the main reason for hospital admission, while secondary diagnoses are more often contributory or underlying conditions. We used both the main and secondary diagnoses for recording prevalent and incident risk factors, conditions and events. Date of the event was defined as the date of the first diagnosis.

ECG lead placement during the exercise test

The cardio assessment involved a 1 lead (lead I) ECG recording (AM-USB 6.5, Cardiosoft v6.51) at a frequency of 500 Hz. The ECG was recorded using four electrodes placed on the right and left antecubital fossa and wrist (Figure R1) and stored in an xml-file of Cardiosoft (https://biobank.ctsu.ox.ac.uk/crystal/docs/Cardio.pdf).

Pre-processing of the ECG signals

Pre-processing of the ECG signals from the EST-UKB cohort included low-pass filtering at 50 Hz to remove electric and muscle noise but still allow QRS detection[1]. Baseline wander was removed by further high-pass filtering the ECG signals at 0.5 Hz.

Computation of other ECG markers

For the derivation of QTc, we first detected the heartbeats using a fully automated in-house developed delineation system[1, 2]. Then, we computed the averaged ECG waveform by collecting all heartbeats in a 15-s window at resting stage. Finally, the QT interval was measured as the interval between the QRS-onset and the T-wave end from the averaged ECG waveform at rest. We, then, corrected the QT interval using Bazett's formula[3].

The TWI marker reflects if the polarity of the T-wave changes between resting and peak exercise stages (i.e. goes from positive to inverted, or from inverted to positive). A

positive T-wave was determined if the amplitude of the T-wave peak was larger than the average amplitude values at T-wave onset and T-wave offset. An inverted T-wave was determined if the amplitude of the T-wave peak was more negative than the average amplitude values at T-wave onset and T-wave offset.

Statistical analyses

Based on previous papers reporting that the log-rank statistic optimization criteria is more robust than the AUC criteria for imbalanced datasets[4, 5], we derived the cut-off values for TMR^{ex} and TMR^{rec} in the training set using this method. The criteria was defined as the one that simultaneously verified the following criteria: (i) it was associated with a *P*-value < 10^{-3} (Supplemental Figures 4 and 5, panel A) (ii) it corresponded to a local maximum of the hazard ratio function from univariate Cox models (Supplemental Figures 4 and 5, panel B), and (iii) the proportion of individuals in the high-risk and low-risk groups was > 10% and > 50%, respectively (Supplemental Figures 4 and 5, panels C and D). If more than one cut-off value met these criteria, the one associated with the highest hazard ratio was used. The predictive value of the optimal cut-off values was then tested in the test group.

The net reclassification improvement (NRI[6] was used to quantify the added predictive value of $TMR^{rec} \geq 0.115$ beyond that from the model including all traditional risk factors for CV risk in the multivariate Cox analysis (age, sex, HTN, cholesterol and smoking status). We derived the NRI in the test set and we followed the same criteria as in our survival analyses, we predicted risk at 7 years (2,520 days) and individuals who died from causes not included in the primary endpoint were censored at the time of death. Bootstrapping (100 iterations) was performed to derive confidence intervals. The CV risk categories used for the NRI analysis were 1%, 1 to 5%, 5 to 10% and ≥10%[7]. A version of NRI appropriate for survival analyses was computed using the Kaplan–Meier method[6]. NRI was computed using the package "nricens" in R.

Genetic analyses

Individuals with poor genotype quality (high missingness or heterozygosity or discordance between the self-reported sex and the sex inferred from the genotypes) were excluded from the EST-UKB cohort and the FULL-UKB cohort (see later) [8]. All genetic analyses were restricted to individuals of European ancestry (Figure 1 and Supplementary Figure 2).

First, we selected model SNVs from the genotyped SNVs using PLINK 1.9[9]. This selection was based on previously published criteria[10]. Then, we estimated the proportion of TMR^{ex} and TMR^{rec} explained by additive genetic variation (heritability) using a variance components method (BOLT-REML)[11], with the model SNVs and ~ 9 million imputed variants with MAF ≥ 1% and imputation quality (INFO) > 0.3.

Next, we randomly divided the EST-UKB dataset into discovery (*N* = 29,393) and replication (*N* = 22,382) datasets. To ensure that there was no overlap with the discovery samples, we removed a total of *N* = 532 first- and second-degree related individuals (kinship coefficient > 0.88) from the replication cohort as indicated from UK Biobank[8]. Then, we performed a GWAS for TMR^{ex} and TMR^{rec} in the discovery dataset using a linear mixed model method (BOLT-LMM)[12] under the additive genetic model, including ~9 million imputed SNVs with MAF ≥ 1% and INFO > 0.3 from the latest release from UKB. For TMR^{ex} we included the following covariates: sex, age, body mass index (BMI), resting RR, RR difference between peak exercise and resting (ΔRR^{ex}) and a binary indicator variable for the genotyping array (UK Biobank versus UK BiLEVE). For TMR^{rec}, we included sex, age, BMI, recovery RR, RR difference between peak exercise and recovery (ΔRR^{rec}) and the genetic array.

A GWAS for both markers was also performed in the replication dataset. All SNVs with *P* < $1x10^{-6}$ from the discovery analysis for both markers were compiled and SNVs were mapped to individual loci based on a genomic distance of > 500 Kb to each side of another SNV and based on linkage disequilibrium (LD). If multiple SNVs fitted the selection criteria for a single region, only the SNV with the smallest *P* value was considered for follow up. We

reviewed each selected SNV to check for unrealistically high effect sizes, large standard errors, and none was observed. Locus Zoom plots were produced for all selected SNVs and these were carefully reviewed. Six variants for $\mathrm{TMR}^{\mathrm{ex}}$ and seven variants for $\mathrm{TMR}^{\mathrm{rec}}$ were taken forward into replication. Replication was confirmed if *P* (one-tailed) ≤ 0.05/6 = 8.3 × 10^{-3} for $\mathrm{TMR}^{\mathrm{ex}}$ and *P* (one-tailed) ≤ 0.05/7 = 7.1 × 10^{-3} for $\mathrm{TMR}^{\mathrm{rec}}$ and the effect was in the direction observed in discovery analyses for each marker in the replication cohort.

Finally, we performed a full dataset GWAS for each marker using BOLT-LMM in EST-UKB [12]. Additional loci for each marker reaching a genome-wide significance threshold (*P* ≤ 5 × 10^{-8}) were reported.

Multi-Trait Analysis of GWAS (MTAG)[13] enables the joint analysis of summary statistics from GWASs of correlated markers to boost the statistical power of each single-marker GWAS. We applied MTAG to the summary statistics for TMR^{ex} and TMR^{rec}, since the markers were correlated (ρ=0.56) to leverage additional loci discovery for each marker, loci with *P* ≤ 5 × 10^{-8} were reported.

A secondary signal would be declared if: (i) the newly identified SNV original *P* value was lower than 1x10^{-6}; (ii) there was less than a 1.5-fold difference between the lead SNV and secondary association *P* values on a $-\log_{10}$ scale, i.e., if $-\log_{10}(P_{lead})/-\log_{10}(P_{\mathrm{sec}}) < 1.5$; and (iii) if there was less than a 1.5-fold difference between the main association and conditional association *P* values on a $-\log_{10}$ scale, i.e., if $-\log_{10}(P_{sec})/-\log_{10}(P_{\mathrm{cond}}) < 1.5$.

The per cent variance explained of each marker was calculated by estimating the residuals from the regression model against the covariates used in each respective genetic model. We then fitted a second linear model for the marker residuals with all the identified variants plus the top ten principal components. The per cent variance explained was the difference between the adjusted R-squared parameters from each model.

Bioinformatic analyses

To explore shared mechanisms of disease, we assessed association of our identified SNVs (and their proxies, $r^2 \geq 0.8$) with other traits from published GWAS using PhenoScanner[14]. Using the University of California, Santa Cruz (UCSC) website, we annotated each TMR lead SNV to provide nearest genes and those located within 5kb. At the variant level, we used Variant Effect Predictor[15] to obtain comprehensive functional characterization of variants, including their gene location, conservation, and amino acid substitution impact based on a range of prediction tools including SIFT and PolyPhen-2.

We evaluated all SNVs in LD ($r^2 \geq 0.8$) with our validated lead SNVs for evidence of mediation of expression quantitative trait loci (eQTLs) using the GTEx database, focusing on loci with the strongest evidence of eQTL associations in brain, heart and adrenal tissue. In addition, genetic variants may have a causal effect through regulatory chromatin interactions. We investigated variants at the 12 independent loci associated with TMR^{ex} and TMR^{rec}. We identified variants with regulatory potential using RegulomeDB[16] and found genes whose promoter regions form significant chromatin interaction with them from a range of tissues, we report results from brain, heart and adrenal Hi-C data. We found the most significant promoter interactions for all potential regulatory SNVs (RegulomeDB score ≤ 5) in LD ($r^2 \geq 0.8$) with our sentinel SNVs and chose the interactors with the SNVs of highest regulatory potential to annotate the loci.

Subsequently, we performed pathway analyses using g:profiler[17] including our candidate genes. The National Center for Biotechnology Information (NCBI) Gene database and GeneCards®: The Human Gene Database were used to obtain official full names and, where relevant, common aliases for each candidate gene product. NCBI's PubMed was used to interrogate primary literature pertaining to gene function. We also reviewed gene-specific animal models using International Mouse Phenotyping Consortium[18] and the Mouse Genome Informatics database[19]. Finally, to investigate pleiotropy of our candidate genes, we queried them at the GWAS catalogue[20] and at the UKBiobank ICD PheWeb for case-control phenotypes.

Genetic risk score analyses

Variants with minor allele frequency < 0.05 and imputation quality ≤ 0.3 were removed from the calculation. PRSice clumped variants to obtain SNVs in linkage equilibrium ($r^2 < 0.1$) within a 250 kb window. Multiple GRSs were computed at a large number of GWAS *P*-value thresholds ranging from 1×10^{-4} to 0.5 with 5×10^{-5} increments. PRSice then performed a logistic regression analysis between each GRS and the primary endpoint, adjusting for age, sex, diabetes, cholesterol, BMI, systolic blood pressure (SBP), the genotyping array and the 10 first genetic principal components. The optimal GRS was then chosen as the one with the smallest *P*-value.

Supplemental Tables

Supplemental Table 1: ICD-10 codes used in follow-up analyses

Cardiovascular events

Code	Definition
I21	Acute myocardial infarction
I210	Acute transmural myocardial infarction of anterior wall
I211	Acute transmural myocardial infarction of inferior wall
I212	Acute transmural myocardial infarction of other sites
I213	Acute transmural myocardial infarction of unspecified site
I214	Acute subendocardial myocardial infarction
I219	Acute myocardial infarction, unspecified
I22	Subsequent myocardial infarction
I220	Subsequent myocardial infarction of anterior wall
I221	Subsequent myocardial infarction of inferior wall
I228	Subsequent myocardial infarction of other sites
I229	Subsequent myocardial infarction of unspecified site
I24	Other acute ischaemic heart diseases
I248	Other forms of acute ischaemic heart disease
I249	Acute ischaemic heart disease, unspecified
I25	Chronic ischaemic heart disease
I250	Atherosclerotic cardiovascular disease, so described
I251	Atherosclerotic heart disease
I253	Aneurysm of heart
I254	Coronary artery aneurysm
I255	Ischaemic cardiomyopathy
I256	Silent myocardial ischaemia
I258	Other forms of chronic ischaemic heart disease
I259	Chronic ischaemic heart disease, unspecified
I46	Cardiac arrest
I460	Cardiac arrest with successful resuscitation
I461	Sudden cardiac death, so described
I469	Cardiac arrest, unspecified
I470	Reentry ventricular arrhythmia
I472	Ventricular tachycardia
I490	Ventricular fibrillation and flutter
I499	Cardiac arrhythmia, unspecified
I50	Heart failure
I500	Congestive heart failure
I501	Left ventricular failure
I509	Heart failure, unspecified
I64	Stroke, not specified as haemorrhage or infarction

Ventricular arrhythmic events

Code	Definition

I460	Cardiac arrest with successful resuscitation
I461	Sudden cardiac death, so described
I469	Cardiac arrest, unspecified
I472	Ventricular tachycardia
I490	Ventricular fibrillation and flutter
I499	Cardiac arrhythmia, unspecified

Atrial fibrillation

I480	Paroxysmal atrial fibrillation
I481	Persistent atrial fibrillation
I482	Chronic atrial fibrillation
I489	Atrial fibrillation and atrial flutter, unspecified
I48	Atrial fibrillation and flutter

Supplemental Table 2: Patient characteristics in the EST-UKB and FULL-UKB cohorts

	EST-UKB cohort	**FULL-UKB cohort**
Study characteristics		
Number of subjects, N	55,222	360,631
Median follow-up (IQR), months	84 (3.3)	101.1 (15.1)
Cardiovascular events, n(%)	1,743 (3.2)	18,997 (5.3)
All-cause mortality events, n(%)	979 (1.8)	12,081 (3.3)
Arrhythmic events, n(%)	198 (0.4)	2,040 (0.6)
Atrial fibrillation events, n(%)	1,112 (2.0)	14,517 (4.0)
Patients characteristics		
Median age (IQR), years	58 (13)	58 (13)
Females, n(%)	29,553 (53.5)	199,838 (55.4)
Diabetes mellitus, n(%)	2,305 (4.2)	15,857 (4.4)
Median BMI (IQR), kg/m2	26.4 (5.4)	26.7 (5.7)
Hypertensive Stage 1	11,978 (21.7)	70,940 (19.7)
Hypertensive Stage 2	11,978 (43.3)	156,987 (43.5)
Previous or current smoker, n(%)	23,403 (42.4)	163,450 (45.3)
CKD, n(%)	44 (0.1)	467 (0.1)
High cholesterol, n(%)	6,297 (11.4)	40,431 (11.2)

*IQR, interquartile range; BMI, body mass index; CKD, chronic kidney disease

Hypertensive Stage 1 defined as 130 mmHg ≤ SBP < 140 mmHg or 85 mmHg ≤ DBP < 90 mmHg
Hypertensive Stage 2 defined as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg

Supplemental Table 3: Characteristics of the study population in the cardiovascular events and in the cardiovascular event-free groups

Characteristics	**Cardiovascular events group** N = 1,743	**Cardiovascular event-free group** N = 53,479	**P-value**
Median age (IQR), years	62 (9)	58 (13)	**2.20E-16**
Males, n(%)	1,243 (71.3)	24,426 (45.7)	**2.20E-16**
Diabetes mellitus, n(%)	178 (10.2)	2,127 (4.0)	**2.20E-16**
Median BMI (IQR), kg/m2	27.8 (5.6)	26.4 (5.3)	**2.20E-16**
Hypertensive Stage 1	364 (20.9)	11,614 (21.7)	**2.20E-16**
Hypertensive Stage 2	1,029 (59.0)	22892 (42.8)	
Previous or current smoker, n(%)	890 (51.1)	22,513 (42.1)	**1.20E-13**
CKD, n(%)	4 (0.2)	40 (0.1)	**5.00E-02**
High cholesterol, n(%)	336 (19.3)	5,961 (11.1)	**2.20E-16**
Median resting heart rate (IQR), bpm	71.3 (16.6)	70.3 (15.0)	**2.60E-04**
Median heart rate response to exercise (IQR), bpm	36.8 (15.4)	40.8 (16.5)	**2.20E-16**
Median heart rate response to recovery (IQR), bpm	23.7 (12.0)	27.5 (13.0)	**2.20E-16**
Median QTc (IQR), ms^-1	399.5 (33.4)	395.6 (30.4)	**4.70E-09**
Median QRS duration (IQR), ms	68 (17.3)	68 (18)	5.10E-01
T-wave inversions, n(%)	8 (0.5)	84 (0.2)	**8.50E-03**
Median TMR during exercise (IQR), d.u.	0.046 (0.034)	0.043 (0.029)	**2.60E-08**
Median TMR during recovery (IQR), d.u.	0.053 (0.060)	0.044 (0.044)	**2.20E-16**

*IQR, interquartile range; BMI, body mass index; CKD, chronic kidney disease; bpm, beats per minute; QTC, corrected QT interval; TMR, T-wave morphology restitution; d.u., dimensionless units

Hypertensive Stage 1 defined as 130 mmHg ≤ SBP < 140 mmHg or 85 mmHg ≤ DBP < 90 mmHg

Hypertensive Stage 2 defined as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg

Supplemental Table 4: Spearman correlation coefficient between TMR during exercise and recovery and covariates

	TMR during exercise	TMR during recovery	Age	sex	diabetes	high cholesterol	HTN	Smoker	CKD	BMI	Resting heart rate	QTc	QRS duration	T-wave inversion	Heart rate response to exercise	Heart rate response to recovery
TMR during exercise	1.000	0.484	0.061	-0.066	0.066	0.040	0.049	-0.010	0.008	0.070	0.438	0.382	-0.051	0.053	-0.219	-0.188
TMR during recovery	0.484	1.000	0.101	-0.014	0.103	0.074	0.079	0.031	0.026	0.104	0.367	0.265	-0.033	0.050	-0.117	-0.388

*BMI, body mass index; HTN, hypertension; CKD, chronic kidney disease; QTC, corrected QT interval; TMR, T-wave morphology restitution; d.u., dimensionless units

Supplemental Table 5A: Association with ischemic events

	Univariate		Multivariate	
	HR (95% CI)	p	HR (95% CI)	p
Clinical Variables				
Age [per 1 SD]	1.84 (1.72-1.96)	**<2x10-16**	1.65 (1.55-1.77)	**<2x10-16**
Sex (male)	3.32 (2.94-3.75)	**<2x10-16**	2.98 (2.62-3.38)	**<2x10-16**
Diabetes (yes)	2.87 (2.41-3.41)	**<2x10-16**	1.61 (1.34-1.93)	**3.20E-07**
High cholesterol (yes)	2.15 (1.89-2.46)	**<2x10-16**	1.23 (1.07-1.41)	**3.70E-03**
BMI [per 1 SD]	1.29 (1.23-1.35)	**<2x10-16**	1.18 (1.12-1.25)	**5.10E-09**
Hypertensive Stage 1	1.72 (1.45-2.04)	**5.00E-10**	1.19 (1.00-1.42)	**4.70E-02**
Hypertensive Stage 2	2.49 (2.17-2.87)	**<2x10-16**	1.36 (1.17-1.58)	**4.30E-05**
Previous or current smoker (yes)	1.43 (1.28-1.59)	**1.00E-10**	1.12 (1.00-1.25)	**4.70E-02**
ECG variables				
Resting heart rate [per 1 SD]	1.07 (1.01-1.13)	**1.60E-02**	0.96 (0.90-1.03)	2.80E-01
Heart rate response to exercise [per 1 SD]	0.71 (0.67-0.76)	**<2x10-16**	1.02 (0.93-1.11)	6.90E-01
Heart rate response to recovery [per 1 SD]	0.74 (0.71-0.78)	**<2x10-16**	0.98 (0.91-1.05)	5.60E-01
Corrected QT [per 1 SD]	1.11 (1.06-1.17)	**4.70E-05**	1.08 (1.02-1.14)	**1.10E-02**
T-wave inversion (yes)	3.41 (1.70-6.83)	**5.40E-04**	2.95 (1.46-5.94)	**2.60E-03**
TMR during exercise [per 1 SD]	1.14 (1.09-1.20)	**5.70E-09**	1.03 (0.97-1.09)	3.00E-01
TMR during recovery [per 1 SD]	1.21 (1.16-1.26)	**<2x10-16**	1.08 (1.03-1.13)	**1.40E-03**

*CI = Confidence interval; HR = Hazard ratio; SD = Standard Deviation; TMR = T-wave morphology restitution; CKD, Chronic kidney disease

Hypertensive Stage 1 defined as 130 mmHg ≤ SBP < 140 mmHg or 85 mmHg ≤ DBP < 90 mmHg

Hypertensive Stage 2 defined as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg

Reference Hypertension group is Hypertensive Stage 0, defined as SBP < 130 mmHg and DBP < 85 mmHg

The ischemic event group (N = 1,424) included individuals with an ICD 10 code I21-I25 for cause of death or admission to hospital.

The non-ischemic event group (N = 319) included individuals in the CV event group with no ischemic event

Supplemental Table 5B: Association with non-ischemic events

	Univariate		Multivariate	
	HR (95% CI)	p	HR (95% CI)	p
Clinical Variables				
Age [per 1 SD]	2.22 (1.92-2.56)	**<2x10-16**	2.08 (1.81-2.41)	**<2x10-16**
Sex (male)	1.98 (1.58-2.49)	**5.20E-09**	2.00 (1.59-2.53)	**5.50E-09**
Diabetes (yes)	2.35 (1.58-3.48)	**2.30E-05**	1.25 (0.82-1.88)	3.00E-01
BMI [per 1 SD]	1.27 (1.15-1.40)	**1.70E-06**	1.21 (1.09-1.35)	**6.00E-04**
Hypertensive Stage 1	1.73 (1.24-2.43)	**1.40E-03**	1.18 (0.84-1.66)	3.40E-01
Hypertensive Stage 2	2.21 (1.67-2.94)	**4.40E-08**	1.14 (0.84-1.53)	4.00E-01
CKD (yes)	9.53 (2.37-38.29)	**1.50E-03**	7.06 (1.75-28.49)	**6.00E-03**
ECG variables				
Resting heart rate [per 1 SD]	1.20 (1.10-1.31)	**2.70E-05**	1.01 (0.90-1.14)	8.50E-01
Heart rate response to exercise [per 1 SD]	0.66 (0.58-0.75)	**1.80E-11**	1.03 (0.88-1.21)	7.30E-01
Heart rate response to recovery [per 1 SD]	0.71 (0.66-0.75)	**<2x10-16**	0.88 (0.77-1.02)	9.00E-02
Corrected QT [per 1 SD]	1.19 (1.14-1.25)	**3.70E-13**	1.15 (1.08-1.22)	**9.60E-06**
TMR during exercise [per 1 SD]	1.26 (1.16-1.36)	**9.60E-09**	1.06 (0.96-1.18)	2.20E-01
TMR during recovery [per 1 SD]	1.33 (1.24-1.43)	**2.90E-14**	1.20 (1.11-1.30)	**4.10E-06**

*CI = Confidence interval; HR = Hazard ratio; SD = Standard Deviation; TMR = T-wave morphology restitution; CKD, Chronic kidney disease

Hypertensive Stage 1 defined as 130 mmHg ≤ SBP < 140 mmHg or 85 mmHg ≤ DBP < 90 mmHg

Hypertensive Stage 2 defined as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg

Reference Hypertension group is Hypertensive Stage 0, defined as SBP < 130 mmHg and DBP < 85 mmHg

The ischemic event group (N = 1,424) included individuals with an ICD 10 code I21-I25 for cause of death or admission to hospital.

The non-ischemic event group (N = 319) included individuals in the CV event group with no ischemic event

Supplemental Table 6: Association with all-cause mortality

	Univariate		Multivariate	
	HR (95% CI)	p	HR (95% CI)	p
Clinical Variables				
Age [per 1 SD]	2.07 (1.91-2.24)	**<2x10-16**	1.96 (1.81-2.13)	**<2x10-16**
Sex (male)	1.76 (1.54-2.00)	**<2x10-16**	1.60 (1.40-1.83)	**3.60E-12**
Diabetes (yes)	2.05 (1.62-2.58)	**1.70E-09**	1.30 (1.03-1.65)	**2.80E-02**
High cholesterol (yes)	1.52 (1.28-1.80)	**2.10E-06**	0.92 (0.77-1.10)	3.6x10-1
BMI [per 1 SD]	1.08 (1.02-1.15)	**9.70E-03**	0.99 (0.93-1.06)	8.10E-01
Hypertensive Stage 1	1.35 (1.12-1.64)	**2.10E-03**	1.00 (0.82-1.21)	9.90E-01
Hypertensive Stage 2	1.88 (1.61-2.20)	**1.20E-15**	1.12 (0.95-1.32)	1.80E-01
Previous or current smoker (yes)	1.88 (1.65-2.14)	**<2x10-16**	1.59 (1.39-1.81)	**3.80E-12**
ECG variables				
Resting heart rate [per 1 SD]	1.18 (1.12-1.24)	**7.30E-10**	1.15 (1.08-1.22)	**1.70E-05**
Heart rate response to exercise [per 1 SD]	0.77 (0.72-0.83)	**1.50E-12**	1.11 (1.03-1.20)	**7.00E-03**
Heart rate response to recovery [per 1 SD]	0.72 (0.68-0.76)	**<2x10-16**	0.89 (0.81-0.97)	**8.60E-03**
Corrected QT [per 1 SD]	1.13 (1.09-1.18)	**8.00E-09**	1.03 (0.96-1.10)	3.90E-01
QRS duration [per 1 SD]	1.07 (1.01-1.14)	**2.80E-02**	1.06 (0.99-1.12)	9.10E-02
TMR during exercise [per 1 SD]	1.16 (1.10-1.22)	**3.70E-08**	1.01 (0.94-1.08)	7.50E-01
TMR during recovery [per 1 SD]	1.26 (1.20-1.32)	**<2x10-16**	1.10 (1.04-1.17)	**1.40E-03**

*CI = Confidence interval; HR = Hazard ratio; SD = Standard Deviation; TMR = T-wave morphology restitution

Hypertensive Stage 1 defined as 130 mmHg ≤ SBP < 140 mmHg or 85 mmHg ≤ DBP < 90 mmHg

Hypertensive Stage 2 defined as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg

Reference Hypertension group is Hypertensive Stage 0, defined as SBP < 130 mmHg and DBP < 85 mmHg

Supplemental Table 7: Association with ventricular arrhythmic events

	Univariate		Multivariate	
	HR (95% CI)	p	HR (95% CI)	p
Clinical Variables				
Age [per 1 SD]	1.91 (1.61-2.27)	**1.00E-13**	1.75 (1.48-2.08)	**1.60E-10**
Sex (male)	2.37 (1.76-3.20)	**1.60E-08**	2.20 (1.62-2.97)	**3.10E-07**
Diabetes (yes)	1.81 (1.05-3.12)	**3.30E-02**	1.08 (0.62-1.90)	7.80E-01
BMI [per 1 SD]	1.17 (1.03-1.33)	**1.70E-02**	1.07 (0.92-1.24)	3.70E-01
Hypertensive Stage 1	1.32 (0.84-2.06)	2.20E-01	0.95 (0.61-1.50)	8.40E-01
Hypertensive Stage 2	2.20 (1.55-3.13)	**9.70E-06**	1.27 (0.88-1.84)	2.00E-01
ECG variables				
HR response to exercise [per 1 SD]	0.73 (0.62-0.85)	**6.80E-05**	1.08 (0.92-1.26)	3.50E-01
Heart rate response to recovery [per 1 SD]	0.71 (0.65-0.78)	**6.60E-14**	0.82 (0.69-0.97)	**2.10E-02**
Corrected QT [per 1 SD]	1.13 (1.03-1.25)	**1.10E-02**	1.08 (0.96-1.22)	2.00E-01
TMR during exercise [per 1 SD]	1.13 (1.00-1.27)	**4.70E-02**	0.97 (0.84-1.13)	7.30E-01
TMR during recovery [per 1 SD]	1.28 (1.16-1.41)	**1.30E-06**	1.16 (1.03-1.30)	**1.40E-02**

*CI = Confidence interval; HR = Hazard ratio; SD = Standard Deviation; TMR = T-wave morphology restitution; CKD = Chronic kidney disease
Hypertensive Stage 1 defined as 130 mmHg ≤ SBP < 140 mmHg or 85 mmHg ≤ DBP < 90 mmHg
Hypertensive Stage 2 defined as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg
Reference Hypertension group is Hypertensive Stage 0, defined as SBP < 130 mmHg and DBP < 85 mmHg

Supplemental Table 8: Association with atrial fibrillation

	Univariate		Multivariate	
	HR (95% CI)	p	HR (95% CI)	p
Clinical Variables				
Age [per 1 SD]	2.72 (2.50-2.95)	**<2x10-16**	2.58 (2.37-2.80)	**<2x10-16**
Sex (male)	2.35 (2.07-2.66)	**<2x10-16**	2.19 (1.92-2.49)	**<2x10-16**
Diabetes (yes)	1.69 (1.33-2.14)	**1.40E-05**	0.92 (0.72-1.18)	5.10E-01
High cholesterol (yes)	1.68 (1.44-1.96)	**7.90E-11**	0.90 (0.77-1.05)	1.90E-01
BMI [per 1 SD]	1.28 (1.21-1.35)	**<2x10-16**	1.24 (1.17-1.32)	**1.60E-12**
Hypertensive Stage 1	1.38 (1.15-1.67)	**6.10E-04**	0.89 (0.74-1.08)	2.30E-01
Hypertensive Stage 2	2.21 (1.91-2.57)	**<2x10-16**	1.05 (0.90-1.22)	5.70E-01
Previous or current smoker (yes)	1.40 (1.24-1.57)	**3.30E-08**	1.08 (0.96-1.22)	1.90E-01
CKD (yes)	3.92 (1.26-12.16)	**1.80E-02**	3.08 (0.99-9.57)	5.20E-02
ECG variables				
Heart rate response to exercise [per 1 SD]	0.65 (0.61-0.70)	**<2x10-16**	0.97 (0.88-1.06)	4.70E-01
Heart rate response to recovery [per 1 SD]	0.72 (0.69-0.75)	**<2x10-16**	0.92 (0.85-0.99)	**2.30E-02**
Corrected QT [per 1 SD]	1.15 (1.11-1.19)	**1.80E-15**	1.12 (1.07-1.17)	**1.70E-07**
TMR during exercise [per 1 SD]	1.13 (1.08-1.19)	**7.20E-07**	1.01 (0.95-1.08)	7.00E-01
TMR during recovery [per 1 SD]	1.19 (1.13-1.24)	**1.30E-12**	1.03 (0.98-1.09)	2.80E-01

*CI = Confidence interval; HR = Hazard ratio; SD = Standard Deviation; TMR = T-wave morphology restitution; CKD, Chronic kidney disease

Hypertensive Stage 1 defined as 130 mmHg ≤ SBP < 140 mmHg or 85 mmHg ≤ DBP < 90 mmHg

Hypertensive Stage 2 defined as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg

Reference Hypertension group is Hypertensive Stage 0, defined as SBP < 130 mmHg and DBP < 85 mmHg

Supplemental Table 9: Genome-wide significant SNVs for TMR during exercise and during recovery in the discovery sample

Trait	Chr	Pos	SNV	EA	AA	EAF	β	SE	*P*	N
TMR during exercise	11	2484803	rs2074238	T	C	0.090	-0.073	0.013	1.10E-08	29393
TMR during recovery	1	1620152 77	rs10918571	A	G	0.751	-0.042	0.007	1.40E-08	28846
TMR during recovery	1	1620157 40	rs12036340	A	G	0.751	-0.042	0.007	1.40E-08	28845
TMR during recovery	1	1620210 00	rs2010491	A	G A	0.758	-0.042	0.008	2.00E-08	28437
TMR during recovery	1	1620212 96	rs146475167	A	TT G	0.757	-0.042	0.008	2.30E-08	28369
TMR during recovery	1	1620231 84	rs60129000	T	C	0.756	-0.042	0.007	1.50E-08	28901
TMR during recovery	1	1620242 42	rs12042862	C	T	0.756	-0.042	0.007	1.80E-08	28900
TMR during recovery	1	1620338 90	rs12143842	C	T	0.752	-0.043	0.007	8.10E-09	29013
TMR during recovery	1	1621331 17	rs12029454	G	A	0.861	-0.051	0.009	3.80E-08	29013
TMR during recovery	1	1621341 07	rs12033217	C	A	0.862	-0.051	0.009	4.20E-08	29004
TMR during recovery	11	2478519	rs12271931	G	A	0.139	-0.087	0.010	7.70E-19	25900
TMR during recovery	11	2484803	rs2074238	T	C	0.090	-0.131	0.011	1.40E-31	29013
TMR during recovery	11	2505436	rs117903261	C	T	0.973	0.191	0.021	2.40E-19	24920
TMR during recovery	11	2510418	rs7115906	T	C	0.032	-0.114	0.019	8.00E-10	27987

TMR during recovery	11	2520974	rs79888274	G	A	0.904	0.077	0.012	3.70E-11	253.98
TMR during recovery	12	2456193 8	rs10771085	C	T	0.430	0.042	0.007	3.40E-10	273.68
TMR during recovery	12	2456434 4	rs7955427	T	C	0.621	0.041	0.007	1.20E-09	278.81
TMR during recovery	12	2456610 5	rs4309241	T	C	0.451	0.042	0.007	1.50E-10	277.55
TMR during recovery	12	2456611 4	rs4505147	C	A	0.649	0.041	0.007	4.10E-09	279.14
TMR during recovery	12	2456612 3	rs4517618	C	T	0.451	0.042	0.007	1.60E-10	277.41
TMR during recovery	12	2456768 3	rs11047427	C	G	0.649	0.041	0.007	2.70E-09	280.56
TMR during recovery	12	2456822 2	rs10771086	C	T	0.451	0.043	0.007	6.80E-11	279.34
TMR during recovery	12	2457156 1	rs11047430	G	A	0.635	0.040	0.007	2.50E-09	283.78
TMR during recovery	12	2457395 1	12:24573951_AG _A	AG	A	0.634	0.039	0.007	6.40E-09	283.64
TMR during recovery	12	2457685 9	rs1396206	A	T	0.480	0.048	0.007	3.10E-13	283.18
TMR during recovery	12	2457907 9	rs10842343	A	T	0.607	0.041	0.007	5.80E-10	285.49
TMR during recovery	12	2458140 4	rs4488295	T	C	0.462	0.043	0.007	5.80E-11	284.80
TMR during recovery	12	2458236 7	rs10842344	T	C	0.462	0.043	0.007	5.20E-11	285.03
TMR during recovery	12	2458565 4	rs1973564	A	G	0.480	0.043	0.006	5.10E-11	286.69
TMR during recovery	12	2458639 0	rs4963759	A	T	0.505	0.047	0.006	3.70E-13	288.52

TMR during recovery	12	2458749 1	rs11047436	T	A	0.665	0.039	0.0 07	1.50E- 08	288 75
TMR during recovery	12	2458870 9	rs10771089	C	G	0.480	0.042	0.0 06	6.90E- 11	287 15
TMR during recovery	12	2458874 9	rs10771090	A	G	0.523	0.047	0.0 07	5.90E- 13	287 17
TMR during recovery	12	2458915 1	rs2900532	C	A	0.480	0.042	0.0 06	6.40E- 11	287 20
TMR during recovery	12	2459040 5	rs11047438	G	A	0.540	- 0.043	0.0 06	4.30E- 11	289 35
TMR during recovery	12	2459208 7	rs11047439	C	T	0.662	0.040	0.0 07	4.70E- 09	289 42
TMR during recovery	12	2459362 4	rs11047441	C	G	0.660	0.040	0.0 07	6.20E- 09	289 61
TMR during recovery	12	2459519 2	rs7307613	C	T	0.503	0.047	0.0 06	3.70E- 13	289 80
TMR during recovery	12	2459639 1	rs7297742	T	G	0.540	- 0.043	0.0 06	3.70E- 11	290 13
TMR during recovery	12	2459900 5	rs11047444	A	G	0.661	0.040	0.0 07	4.10E- 09	289 67
TMR during recovery	12	2459957 8	rs10842350	A	G	0.504	0.047	0.0 06	3.30E- 13	289 76
TMR during recovery	12	2460104 8	rs11047447	A	G	0.661	0.040	0.0 07	3.70E- 09	289 70
TMR during recovery	12	2460181 8	rs11047449	T	C	0.462	0.043	0.0 06	3.50E- 11	289 78
TMR during recovery	12	2460242 1	rs1396197	G	A	0.663	0.040	0.0 07	4.00E- 09	289 59
TMR during recovery	12	2460287 3	rs1396198	T	C	0.662	0.040	0.0 07	4.10E- 09	289 64
TMR during recovery	12	2460313 0	rs11047451	A	T	0.663	0.040	0.0 07	4.40E- 09	289 54

TMR during recovery	12	2460722 5	rs1508224	C	T	0.539	- 0.043	0.0 06	3.20E- 11	289 48
TMR during recovery	12	2461294 4	rs2136019	C	T	0.463	0.043	0.0 06	3.30E- 11	289 63
TMR during recovery	12	2461436 5	rs4465447	C	A	0.494	- 0.047	0.0 06	3.80E- 13	289 55
TMR during recovery	12	2461448 6	rs4259904	A	G	0.494	- 0.047	0.0 06	3.60E- 13	289 52
TMR during recovery	12	2461511 4	rs10842353	G	A	0.320	- 0.038	0.0 07	4.80E- 08	289 37
TMR during recovery	12	2461620 9	rs7957437	C	A	0.508	0.046	0.0 06	8.70E- 13	289 40
TMR during recovery	12	2461725 7	12:24617257_ATT _A	ATT	A	0.291	- 0.041	0.0 07	4.60E- 08	265 20
TMR during recovery	12	2461765 9	rs11047460	T	C	0.494	- 0.047	0.0 06	3.70E- 13	289 27
TMR during recovery	12	2462079 1	rs4403889	A	G	0.462	0.044	0.0 06	1.10E- 11	290 13
TMR during recovery	12	2462096 9	rs4376999	A	G	0.462	0.044	0.0 06	1.10E- 11	290 00
TMR during recovery	12	2462134 8	rs10842356	A	T	0.485	0.042	0.0 06	1.00E- 10	289 93
TMR during recovery	12	2462203 6	rs4963761	C	T	0.514	0.045	0.0 06	2.30E- 12	289 67
TMR during recovery	12	2462216 0	rs4963762	T	C	0.462	0.044	0.0 06	1.00E- 11	289 92
TMR during recovery	12	2462361 8	rs7299141	A	G	0.462	0.044	0.0 06	1.10E- 11	289 90
TMR during recovery	12	2462843 3	rs10842357	T	G	0.461	0.043	0.0 06	2.10E- 11	289 29
TMR during recovery	12	2462893 2	rs146081992	A	A T	0.517	0.036	0.0 07	3.60E- 08	278 33

TMR during recovery	12	2462893 8	rs368370337	A	A T	0.517	0.036	0.0 07	3.60E- 08	278 33
TMR during recovery	12	2463031 5	rs4963764	A	G	0.461	0.043	0.0 06	3.20E- 11	289 03
TMR during recovery	12	2463253 8	rs10771091	C	T	0.461	0.042	0.0 06	5.90E- 11	288 94
TMR during recovery	12	2463333 2	rs4441108	A	T	0.461	0.042	0.0 06	5.60E- 11	288 94
TMR during recovery	12	2463430 6	rs10771092	T	C	0.489	0.044	0.0 06	1.70E- 11	288 96
TMR during recovery	12	2464085 5	rs7970266	T	C	0.461	0.041	0.0 06	1.70E- 10	289 05
TMR during recovery	12	2464203 4	rs10842358	C	T	0.461	0.041	0.0 06	2.00E- 10	288 95
TMR during recovery	12	2464398 6	12:24643986_CT CTT_C	CTC TT	C	0.462	0.041	0.0 07	3.70E- 10	286 71
TMR during recovery	12	2464901 3	rs7961520	T	C	0.451	0.039	0.0 06	1.50E- 09	288 86
TMR during recovery	12	2465637 5	rs10842362	C	T	0.450	0.039	0.0 06	1.90E- 09	288 81
TMR during recovery	12	2465754 7	rs7295036	A	G	0.436	0.041	0.0 07	4.40E- 10	288 62
TMR during recovery	12	2465880 6	12:24658806_TG C_T	TGC	T A A G	0.448	0.044	0.0 07	2.70E- 11	284 03
TMR during recovery	12	2465935 2	rs559214768	A	A G	0.439	0.042	0.0 07	1.30E- 10	286 22
TMR during recovery	12	2465996 2	rs7304608	G	A	0.451	0.039	0.0 07	1.60E- 09	286 67
TMR during recovery	12	2465997 5	rs7304397	C	T	0.433	0.041	0.0 07	2.60E- 10	287 71

TMR during recovery	12	2465998 2	rs7304404	C	T	0.433	0.041	0.0 07	2.60E- 10	287 71
TMR during recovery	12	2466603 9	rs10743500	T	C	0.437	0.041	0.0 06	1.70E- 10	290 13
TMR during recovery	12	2466615 5	rs4275708	C	T	0.449	0.039	0.0 06	2.10E- 09	289 50
TMR during recovery	12	2466716 4	rs4614552	T	G	0.616	0.037	0.0 07	3.90E- 08	288 07
TMR during recovery	12	2466760 1	rs10743501	C	T	0.434	0.041	0.0 06	3.20E- 10	288 89
TMR during recovery	12	2466910 1	rs4439602	C	T	0.554	- 0.039	0.0 07	2.80E- 09	286 43
TMR during recovery	12	2466910 2	rs2970418	G	A	0.448	0.039	0.0 07	2.70E- 09	286 55
TMR during recovery	12	2467100 7	rs374287296	CT	C	0.466	0.038	0.0 07	5.60E- 09	276 09
TMR during recovery	12	2467209 4	rs2955487	G	C	0.441	0.040	0.0 07	7.70E- 10	285 54
TMR during recovery	12	2467343 7	rs11397830	T	T A	0.544	- 0.039	0.0 07	3.00E- 09	281 59
TMR during recovery	12	2467522 2	rs2970419	C	A	0.457	0.038	0.0 07	5.00E- 09	284 51
TMR during recovery	12	2467547 9	rs7296354	A	G	0.541	0.037	0.0 07	1.10E- 08	283 68

*TMR: T-wave morphology restitution, SNV: single-nucleotide variant, Chr: Chromosome, Pos: Position, based on HG build 18, EA: Effect allele, AA: Alternate allele, EAF: Effect allele frequency, β: Beta, SE: Standard Error, N: number of participants, *P*: P-value.

Supplemental Table 10A: Lookup of loci associated with TMR during exercise in the MTAG results

						Discovery				Replication				Combined				MTAG			
Locus	SNV	CHR	BP	EA	EAF	*P*	N	β	SE	*P*	N	β	SE	*P*	N	β	SE	*P*	N	β	SE
RNF207	rs709208	1	6272137	A	0.679	2.60E-07	27939	-0.042	0.008	1.60E-05	20769	-0.040	0.009	1.80E-11	49203	-0.041	0.006	9.05E-11	49203	-0.040	0.006
NOS1AP*"	rs12143842	1	162033890	C	0.750	1.20E-04	29393	-0.033	0.008	3.40E-03	21850	-0.029	0.010	6.60E-07	51764	-0.032	0.006	2.01E-13	51764	-0.048	0.006
SCN5A-SCN10A*^	rs7428232	3	38778618	T	0.416	5.20E-06	29352	-0.034	0.007	1.80E-04	21820	-0.032	0.008	3.70E-09	51692	-0.033	0.006	2.02E-11	51692	-0.038	0.006
PREP"	rs4478445	6	105786660	C	0.943	2.50E-05	28913	-0.067	0.016	7.40E-03	21493	-0.049	0.018	8.00E-07	50919	-0.059	0.012	4.27E-08	50919	-0.067	0.012
KCNH2	rs2072412	7	150647970	C	0.729	1.80E-06	28975	0.040	0.008	4.10E-07	21539	0.048	0.010	2.10E-11	51028	0.042	0.006	1.42E-11	51028	0.043	0.006
KCNQ1*	rs2074238	11	2484803	T	0.088	1.10E-08	29393	-0.073	0.013	1.20E-03	21850	-0.048	0.015	1.20E-10	51764	-0.062	0.010	7.02E-34	51764	-0.120	0.010
SOX5*	rs7307613	12	24595192	C	0.505	1.80E-07	29359	0.038	0.007	3.50E-06	21825	0.039	0.008	2.80E-12	51704	0.039	0.006	3.57E-19	51704	0.050	0.006
KCNJ2‡	17:68493468_GA_G	17	68493468	GA	0.674	7.60E-07	29318	0.039	0.008	3.70E-07	21794	0.046	0.009	2.90E-13	51632	0.043	0.006	9.97E-11	51545	0.039	0.006

SNV: single-nucleotide variation, CHR: Chromosome, BP: Position, based on HG build 19, EA: Effect allele, EAF: Effect allele frequency from discovery data, β: Beta, SE: Standard Error, N: number of participants, P: P-value. The locus name indicates the coding gene that is in the closest proximity to the most associated SNV. The loci labelled as TSC22D2 and KCNJ2 have a LncRNA and a pseudogene as the nearest. Replicated SNPs are indicated in bold type. * indicates the SNP is the same or in high LD (r^2>0.8) with a SNP associated with the other index. ^ indicates has a secondary signal. "indicates identified with MTAG. ‡ A proxy (rs1860452) was used for this SNV with MTAG (LD>0.95)

Supplemental Table 10B: Lookup of loci associated with TMR during exercise in the MTAG results

						Discovery				Replication				Combined				MTAG			
Locus	SNV	CHR	BP	EA	EAF	*P*	N	β	SE	*P*	N	β	SE	*P*	N	β	SE	*P*	N	β	SE
SSBP3	rs562408	1	54742618	A	0.430	6.20E-06	28299	0.030	0.007	7.40E-03	21091	0.020	0.008	3.70E-08	49895	0.027	0.005	1.85E-08	49895	0.035	0.006
NOS1AP*	**rs12143842**	**1**	**162033890**	**C**	**0.750**	**8.10E-09**	**29013**	**-0.043**	**0.007**	**1.60E-08**	**21623**	**-0.048**	**0.009**	**5.10E-16**	**51153**	**-0.045**	**0.006**	**5.29E-17**	**51153**	**-0.059**	**0.007**
SCN5A-SCN10A*^	rs7373065	3	38710315	T	0.019	2.00E-06	26979	0.114	0.024	2.10E-06	20107	0.132	0.028	1.60E-11	47566	0.122	0.018	6.35E-13	47566	0.165	0.023
TSC22D2‡	rs112717154	3	149943115	G	0.863	1.40E-06	27857	-0.046	0.010	5.30E-03	20762	-0.031	0.011	9.30E-09	49115	-0.041	0.007	6.69E-09	50439	-0.052	0.009
CAMK2D‡	rs35408611	4	114423677	C	0.738	6.20E-03	28362	-0.020	0.007	1.40E-08	21138	-0.048	0.008	2.90E-08	50006	-0.031	0.006	2.18E-07	50988	-0.036	0.007
KCNQ1*	**rs2074238**	**11**	**2484803**	**T**	**0.088**	**1.40E-31**	**29013**	**-0.131**	**0.011**	**4.20E-31**	**21623**	**-0.152**	**0.013**	**1.20E-59**	**51153**	**-0.138**	**0.008**	**5.06E-58**	**51153**	**-0.174**	**0.011**
SOX5*	**rs1396206**	**12**	**24576859**	**A**	**0.482**	**3.10E-13**	**28318**	**0.048**	**0.007**	**4.00E-05**	**21105**	**0.031**	**0.007**	**1.30E-16**	**49927**	**0.040**	**0.005**	**4.91E-19**	**49927**	**0.055**	**0.006**
KLF12"	rs7992314	13	74509346	G	0.631	2.50E-06	28908	-0.032	0.007	6.00E-03	21545	-0.021	0.008	6.40E-08	50968	-0.027	0.005	2.70E-08	50968	-0.035	0.006

SNV: single-nucleotide variation, CHR: Chromosome, BP: Position, based on HG build 19, EA: Effect allele, EAF: Effect allele frequency from discovery data, β: Beta, SE: Standard Error, N: number of participants, P: P-value. The locus name indicates the coding gene that is in the closest proximity to the most associated SNV. The loci labelled as TSC22D2 and KCNJ2 have a LncRNA and a pseudogene as the nearest. Replicated SNPs are indicated in bold type. * indicates the SNP is the same or in high LD (r^2>0.8) with a SNP associated with the other index. ^ indicates has a secondary signal. "indicates identified with MTAG. ‡ A proxy was used for the SNV at TSC22D2 (rs2867860, LD>0.95) and for the SNV at CAMK2D (rs4834342, LD>0.95) for MTAG.

Supplemental Table 11A: TMR during exercise loci associations with other traits using PhenoScanner v2

Locus	Lead SNV	Proxy SNV	Proxy Chr:Pos (hg19)	EA	r2	Type	Trait	*P*	PMID
NOS1AP	rs12143842	rs12036340	chr1:162015740	A/G	0.94	Proxy	QRS complex 12-leads	1.00E-09	27659466
	rs12143842	rs12143842	chr1:162033890	C/T	1	Lead	Arrhythmias cardiac	1.00E-83	19587794
	rs12143842	rs12143842	chr1:162033890	C/T	1	Lead	Electrocardiography	2.00E-78	19305408
	rs12143842	rs12143842	chr1:162033890	C/T	1	Lead	QT interval	1.00E-213	24952745
SCN5A-SCN10A	rs7373065	rs6773331	chr3:38684397	T/A	0.87	Proxy	Pulse rate	8.05E-09	100000001
	rs7373065	rs7373065	chr3:38710315	C/T	1	Lead	Cause of death: cardiomegaly	2.69E-08	100000001
	rs7428232	rs10428132	chr3:38777554	G/T	0.99	Proxy	Brugada syndrome	1.00E-68	23872634
	rs7428232	rs6599255	chr3:38796415	C/A	0.98	Proxy	Resting heart rate	2.00E-10	27798624
	rs7428232	rs6790396	chr3:38771925	G/C	0.99	Proxy	P wave duration	2.00E-39	28794112
	rs7428232	rs6795970	chr3:38766675	G/A	0.94	Proxy	Electrocardiographic conduction measures	5.00E-27	23463857
	rs7428232	rs6795970	chr3:38766675	G/A	0.94	Proxy	Electrocardiographic traits	1.00E-58	20062063
	rs7428232	rs6800541	chr3:38774832	T/C	0.97	Proxy	Electrocardiography	2.00E-74	20062060
	rs7428232	rs6800541	chr3:38774832	T/C	0.97	Proxy	PR interval	9.70E-82	20062060
	rs7428232	rs6801957	chr3:38767315	C/T	0.98	Proxy	Heart function tests	3.00E-14	21076409
	rs7428232	rs6801957	chr3:38767315	C/T	0.98	Proxy	Pulse rate	2.98E-13	100000001
	rs7428232	rs6801957	chr3:38767315	C/T	0.98	Proxy	QRS duration	7.00E-40	27659466
	rs7428232	rs6801957	chr3:38767315	C/T	0.98	Proxy	QT interval	1.00E-10	24952745
PREP	rs4478445	rs67125925	chr6:105780309	C/T	0.89	Proxy	Pulse rate	8.90E-10	100000001
KCNH2	rs2072412	rs2072413	chr7:150647969	T/C	1	Proxy	QT interval	1.00E-49	24952745
KCNQ1	rs2074238	rs2074238	chr11:2484803	C/T	1	Lead	Electrocardiography	3.00E-17	19305408
	rs2074238	rs2074238	chr11:2484803	C/T	1	Lead	QT interval	2.00E-28	24952745
KCNJ2	rs17779747	rs17779747	chr17:68494992	G/T	1	Lead	Electrocardiography	6.00E-12	19305409

	rs17779747	rs17779747	chr17:68494992	G/T	1	Lead	QT interval	6.00E-12	19305409

* The look-up results from the lead TMR SNV or proxy SNVs in high LD ($r^2 \geq 0.8$) from the 1000 Genome Project are indicated. SNVs are ordered by chromosomal position, and only results with P value ≤ 5×10^{-8} are included. If there were multiple results for the same trait, the variant with the lowest P-value is shown. Proxy variants with additional traits that were not associated with the lead variants are also included. If multiple proxy SNVs were available, the proxy SNV with the highest LD was chosen. EA: Effect allele; r^2: A measure of the linkage disequilibrium between the proxy and lead SNV; Type: Whether the variant is the lead or proxy variant; P: P-value for the association between the variant and the trait; PMID: PubMed ID. The locus name indicates the coding gene that is in the closest proximity to the most associated SNV. The loci labelled as TSC22D2 and KCNJ2 have a LncRNA and a pseudogene as the nearest. Phenoscanner v2 (PMID: 27318201).

Supplemental Table 11B: TMR during recovery loci associations with other traits using PhenoScanner v2

Locus	Lead SNV	Proxy SNV	Proxy Chr:Pos (hg19)	EA	r2	Type	Trait	*P*	PMID
NOS1AP	rs12143842	rs12036340	chr1:162015740	A/G	0.94 9	Prox y	QRS complex 12-leads	1.00E-09	27659466
	rs12143842	rs12143842	chr1:162033890	C/T	1	Lead	Arrhythmias cardiac	1.00E-83	19587794
	rs12143842	rs12143842	chr1:162033890	C/T	1	Lead	Electrocardiographic traits	4.00E-18	25055868
	rs12143842	rs12143842	chr1:162033890	C/T	1	Lead	Electrocardiography	2.00E-78	19305408
	rs12143842	rs12143842	chr1:162033890	C/T	1	Lead	QT interval	1.00E-213	24952745
SSBP3	rs562408	rs562408	chr1:54742618	A/G	1	Lead	P wave duration	3.00E-09	28794112
SCN5A-SCN10A	rs7373065	rs7373065	chr3:38710315	C/T	1	Lead	Cause of death: cardiomegaly	2.69E-08	100000001
	rs9311197	rs10428132	chr3:38777554	T/G	0.80 1	Prox y	Brugada syndrome	1.00E-68	23872634
	rs9311197	rs10428132	chr3:38777554	T/G	0.80 1	Prox y	Pulse rate	3.52E-13	100000001
	rs9311197	rs6790396	chr3:38771925	C/G	0.80 1	Prox y	PR interval	2.18E-08	21347284
	rs9311197	rs6790396	chr3:38771925	C/G	0.80 1	Prox y	P wave duration	2.00E-39	28794112
KCNQ1	rs2074238	rs2074238	chr11:2484803	C/T	1	Lead	Electrocardiography	3.00E-17	19305408
	rs2074238	rs2074238	chr11:2484803	C/T	1	Lead	QT interval	2.00E-28	24952745
KLF12	rs7992314	rs1886512	chr13:74520186	A/T	0.97 9	Prox y	Heart function tests	1.00E-08	21076409
	rs7992314	rs728926	chr13:74513122	T/C	0.98 7	Prox y	QRS duration	6.00E-11	27659466
	rs7992314	rs728926	chr13:74513122	T/C	0.98 7	Prox y	QT interval	2.00E-08	24952745

* The look-up results from the lead TMR SNV or proxy SNVs in high LD ($r^2 \geq 0.8$) from the 1000 Genome Project are indicated. SNVs are ordered by chromosomal position, and only results with P value ≤ 5×10^{-8} are included. If there were multiple results for the same trait, the variant with the lowest P-value is shown. Proxy variants with additional traits that were not associated with the lead variants are also included. If multiple proxy SNVs were available, the proxy SNV with the highest LD was chosen. EA: Effect allele; r^2: A measure of the linkage disequilibrium between the proxy and lead SNV; Type: Whether the variant is the lead or proxy variant; P: P-value for the association between the variant and the trait; PMID: PubMed ID. The locus name indicates the coding gene that is in the closest proximity to the most

associated SNV. The loci labelled as TSC22D2 and KCNJ2 have a LncRNA and a pseudogene as the nearest. Phenoscanner v2 (PMID: 27318201).

Supplementary Table 12: Expression quantitative trait locus (eQTL) analysis for TMR during exercise and recovery traits

Lead SNV	Marker	Locus	Proxy SNV	Proxy SNV Chr:Pos (hg19)	Top eQTL SNV	r2 (Lead SNV-proxy SNV)	r2 (Lead SNV-Top eQTL SNV)	SNV (*P*)	Top eQTL SNV (*P*)	Tissue	Transcript
rs12143842	TMRex and TMRrec	*NOS1AP*	rs12143842	chr1:162024242	rs12143842	1.000	1.000	4.70E-12	4.70E-12	Heart atrial appendage	*C1orf226*
rs562408	TMRrec	*SSBP3*	rs562408	chr1:54742618	rs562408	1.000	1.000	6.80E-23	6.80E-23	Heart atrial appendage	*SSBP3*

*TMR during exercise and recovery variants with significant eQTLs and their corresponding genes are indicated. The results from proxy variants, with high LD ($r^2 \geq 0.8$) with the lead variant in the UK Biobank study were included if there was tissue expression data in addition to the lead variant. Results were filtered to those reaching a *P* value ≤ 5 x 10-8. The source was Genotype-Tissue Expression (GTEx) Consortium, PubMed ID is 25954001. r^2: A measure for the linkage disequilibrium between the proxy and lead SNVs; *P*: P value for the association between the variant and RNA tissue expression. The locus name indicates the coding gene that is in the closest proximity to the most associated SNV. The loci labelled as *TSC22D2* and *KCNJ2* have a LncRNA and a pseudogene as the nearest.

Supplemental Table 13: Long-range interactors in heart, adrenal, brain tissue and neural progenitor cells

Locus	Lead SNV	Proxy SNV	r2	Marker	Score	Right Ventricle	Left Ventricle	Hippocampus	Neural Progenitor Cell	Aorta
SSBP3	rs562408	rs702496	0.835	TMRrec	5		*SSBP3*			
	rs562408	rs1537430	0.949	TMRrec	5		*SSBP3*			
	rs562408	rs3753410	0.812	TMRrec	5		*SSBP3*			
	rs562408	rs536684	0.983	TMRrec	4		*SSBP3*			
	rs562408	rs590041	0.991	TMRrec	5		*SSBP3*			
	rs562408	rs562408	1.000	TMRrec	5		*SSBP3*			
NOS1AP	rs12143842	rs12143842	1.000	Both	2b				*UHMK1;SH2D1B*	*SH2D1B*
SCN5A-SCN10A	rs7428232	rs6783110	0.865	TMRex	5					
	rs7428232	rs6795970	0.945	TMRex	5		*SCN5A*			*SCN5A*
	rs7428232	rs6801957	0.984	TMRex	4		*SCN5A*			*SCN5A*
	rs7428232	rs7433306	0.977	TMRex	5		*SCN5A*			*SCN5A*
	rs7428232	rs6790396	0.996	TMRex	4		*SCN5A*			*SCN5A*
	rs7428232	rs9809798	0.801	TMRex	5		*SCN5A*			*SCN5A*
	rs7428232	rs10428132	0.996	TMRex	5		*SCN5A*			*SCN5A*
	rs7428232	rs7428167	0.801	TMRex	2b		*SCN5A*			*SCN5A*
	rs7428232	rs6599250	0.976	TMRex	5		*SCN5A*			*SCN5A*
SCN5A-SCN10A	rs9311197	rs6790396	0.805	sec signal, TMRrec	4		*SCN5A*			*SCN5A*
	rs9311197	rs9809798	1.000	sec signal, TMRrec	5		*SCN5A*			*SCN5A*
	rs9311197	rs10428132	0.805	sec signal, TMRrec	5		*SCN5A*			*SCN5A*
	rs9311197	rs7428167	1.000	sec signal, TMRrec	2b		*SCN5A*			*SCN5A*
TSC22D2	rs112717154	rs11923657	0.865	TMRrec	4	*TSC22D2*	*EIF2A;TSC22D2*		*TSC22D2*	*TSC22D2*
	rs112717154	rs12634526	0.889	TMRrec	4	*TSC22D2*	*EIF2A;TSC22D2*		*TSC22D2*	*TSC22D2*
PREP	rs4478445	rs55759324	0.984	TMRex	5		*ATG5*			
	rs4478445	rs60847040	0.984	TMRex	5		*ATG5*			
	rs4478445	rs58971260	1.000	TMRex	4		*PREP*			
	rs4478445	rs67558059	0.934	TMRex	4		*PREP*			
	rs4478445	rs67125925	0.886	TMRex	5		*PREP*			
	rs4478445	rs66935099	0.934	TMRex	5		*PREP*			
	rs4478445	rs67092423	0.934	TMRex	5		*PREP*			
	rs4478445	rs67330396	0.934	TMRex	5		*PREP*			
	rs4478445	rs60127716	0.934	TMRex	5		*PREP*			

	rs4478445	rs55873742	0.934	TMRex	5	*PREP*		
SOX5	rs1396206	rs1396206	1.000	TMRrec	5			
	rs1396206	rs4403889	0.844	TMRrec	5			*SOX5*
	rs1396206	rs7970266	0.819	TMRrec	5		*SOX5*	*SOX5*
	rs1396206	rs10842358	0.819	TMRrec	5		*SOX5*	*SOX5*
KLF12	rs7992314	rs17061696	0.996	TMRrec	5	*KLF12*		*KLF12*
	rs7992314	rs728926	0.988	TMRrec	5	*KLF12*		*KLF12*
	rs7992314	rs9573330	0.992	TMRrec	5	*KLF12*		*KLF12*
	rs7992314	rs1886512	0.981	TMRrec	5	*KLF12*		*KLF12*
KCNJ2	17:68493468_GA_G	rs72868940	0.836	TMRex	5	*CDC42EP4*		
	17:68493468_GA_G	rs7218368	0.996	TMRex	5	*CDC42EP4*		
	17:68493468_GA_G	rs17780076	0.826	TMRex	5	*CDC42EP4*		

*Results are presented for all SNVs in LD $r^2 \geq 0.8$ with lead SNVs found in this study that have a functional score ≤ 5, and the locus has at least one significant Hi-C interaction. The locus name indicates the coding gene that is in the closest proximity to the most associated SNV. The loci labelled as *TSC22D2* and *KCNJ2* have a LncRNA and a pseudogene as the nearest.

Supplemental Table 14. Candidate Genes for g:profiler analysis

Locus	Marker	SNV	CHR	BP	Candidate genes within 5 kb	eQTL	Hi-C interactor genes	Mouse model with cardiovascular or nervous system	Candidate gene(s) at locus
RNF207	TMRex	rs709208	1	6272137	*RNF207*				*RNF207*
SSBP3	TMRrec	rs562408	1	54742618	*SSBP3*	*SSBP3*	*SSBP3*	*SSBP3* (http://www.informatics.jax.org/marker/MGI:1919725)	*SSBP3*
NOS1AP	TMRex and TMRrec	rs12143842	1	162033890		*C1orf226*	*UHMK1;SH2D1B*	*NOS1AP* (http://www.informatics.jax.org/marker/MGI:1917979; http://www.informatics.jax.org/marker/MGI:1341908)	*NOS1AP**
SCN5A-SCN10A	TMRex and TMRrec	rs7373065, rs9311197 and rs7428232	3	38710315, 38776603 and 38778618	*SCN10A*		*SCN5A*	*SCN10A* (http://www.informatics.jax.org/marker/MGI:108029; http://www.informatics.jax.org/marker/MGI:98251)	*SCN5A, SCN10A*
TSC22D2	TMRrec	rs112717154	3	149943115			*EIF2A;TSC22D2*		*TSC22D2*
CAMK2D	TMRrec	rs35408611	4	114423677	*CAMK2D*			*CAMK2D* (http://www.informatics.jax.org/marker/MGI:1341265)	*CAMK2D*
PREP	TMRex	rs4478445	6	105786660	*PREP*		*ATG5; PREP*	*ATG5* (http://www.informatics.jax.org/marker/MGI:1270863); *PREP* (http://www.informatics.jax.org/marker/MGI:1277186)	*PREP, ATG5*
KCNH2	TMRex	rs2072412	7	150647970	*KCNH2*			*KCNH2* (http://www.informatics.jax.org/marker/MGI:1341722)	*KCNH2*
KCNQ1	TMRex and TMRrec	rs2074238	11	2484803	*KCNQ1*			*KCNQ1* (http://www.informatics.jax.org/marker/MGI:108083)	*KCNQ1*
SOX5	TMRex and TMRrec	rs7307613 and rs1396206	12	24595192 and 24576859	*SOX5*		*SOX5*		*SOX5*
KLF12	TMRrec	rs7992314	13	74509346	*KLF12*		*KLF12*		*KLF12*
KCNJ2	TMRex	17:68493468_GA_G	17	68493468			*CDC42EP4*	*CDC42EP4* (http://www.informatics.jax.org/marker/MGI:1929760)	*CDC42EP4*

*Abbreviations: SNV: Single-nucleotide variant, CHR: chromosome, BP: base pair position, based in HG build 18; eQTL: expression quantitative trait locus.
Column I provides URL links to mouse models with cardiovascular and neural phenotypes.
NOS1AP* - this gene was selected as the likely candidate gene at this locus based on functional analyses and mouse models. C1orf226 is a paralogue of NOS1AP.
The locus name indicates the coding gene that is in the closest proximity to the most associated SNV. The loci labelled as *TSC22D2* and *KCNJ2* have a LncRNA and a pseudogene as the nearest.

Supplemental Table 15. Candidate gene look-up for pleiotropy for cardiovascular diseases

Candidate gene(s)	Marker	GWAS catalogue		UKBiobank ICD PheWeb	
		Disease	P-value	Disease	P-value
RNF207	TMRex				
SSBP3	TMRrec				
NOS1AP	TMRex and TMRrec				
SCN5A	TMRex and TMRrec	Atrial fibrillation	3.00E-16	Atrial fibrillation and flutter	1.30E-08
	TMRex and TMRrec	Brugada syndrome	1.00E-14	First degree AV block	4.60E-08
SCN10A	TMRex and TMRrec	Brugada syndrome	1.00E-68	Atrial fibrillation and flutter	1.30E-08
	TMRex and TMRrec	Atrial fibrillation	2.00E-20		
TSC22D2	TMRrec	Cardiovascular disease	2.00E-13	Hypertension	1.10E-08
CAMK2D	TMRrec	Atrial fibrillation	2.00E-13		
PREP	TMRex				
ATG5	TMRex				
KCNH2	TMRex	Atrial fibrillation	2.00E-11	Hypertension	1.20E-25
	TMRex			Ischemic heart disease	2.70E-10
	TMRex			Myocardial infarction	9.20E-10
	TMRex			Coronary atherosclerosis	1.40E-08
KCNQ1	TMRex and TMRrec	Long QT	1.00E-54	Diabetes mellitus	2.40E-17
SOX5	TMRex and TMRrec	Atrial fibrillation	2.00E-17		
KLF12	TMRrec	Sudden cardiac arrest	5.00E-20		
CDC42EP4	TMRex				

Results are presented for all candidate genes for TMR during exercise or during recovery, with a genome-wide significant association with a cardiovascular or neural disease in GWASs published in the GWAS catalogue or the UK Biobank ICD PheWeb.

Supplemental Table 16: Net reclassification improvement for CV events with estimates of the expected number of reclassifications per risk category for cases and controls

Reclassification Table for all subjects

	ESC SCORE + TMR during recovery ≥ 0.115				Reclassified up, n(%)	Reclassified down, n(%)	NRI (95% CI)
Standard	< 1%	1 to <5%	5 to <10%	≥ 10%			
< 1%	4,167	145	0	0	738 (2.7)	794 (2.9)	0.019 (0.015-0.022)
1 to <5%	173	16,366	298	0			
5 to <10%	0	368	4,633	295			
≥ 10%	0	0	253	799			

Reclassification Table for cases

	ESC SCORE + TMR during recovery ≥ 0.115				Reclassified up, n(%)	Reclassified down, n(%)	NRI (95% CI)
Standard	< 1%	1 to <5%	5 to <10%	≥ 10%			
< 1%	25	1	0	0	48 (5.7)	33 (3.9)	0.016 (0.012-0.019)
1 to <5%	2	371	19	0			
5 to <10%	0	13	300	28			
≥ 10%	0	0	18	72			

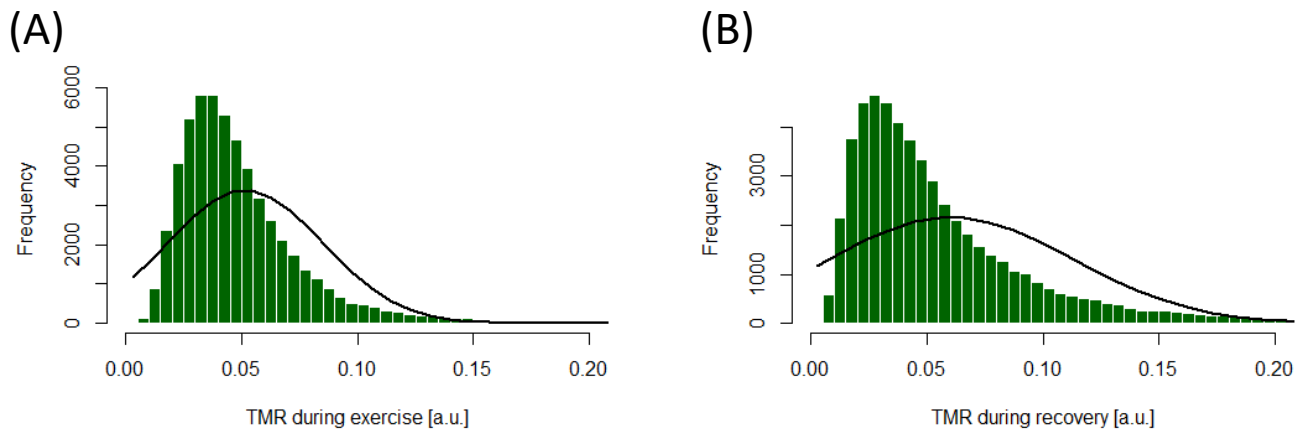
Reclassification Table for controls

	ESC SCORE + TMR during recovery ≥ 0.115				Reclassified up, n(%)	Reclassified down, n(%)	NRI (95% CI)
Standard	< 1%	1 to <5%	5 to <10%	≥ 10%			
< 1%	2,157	70	0	0	339 (2.6)	356 (2.7)	0.003 (0.002-0.004)
1 to <5%	85	7,955	139	0			
5 to <10%	0	160	2,037	130			
≥ 10%	0	0	111	289			

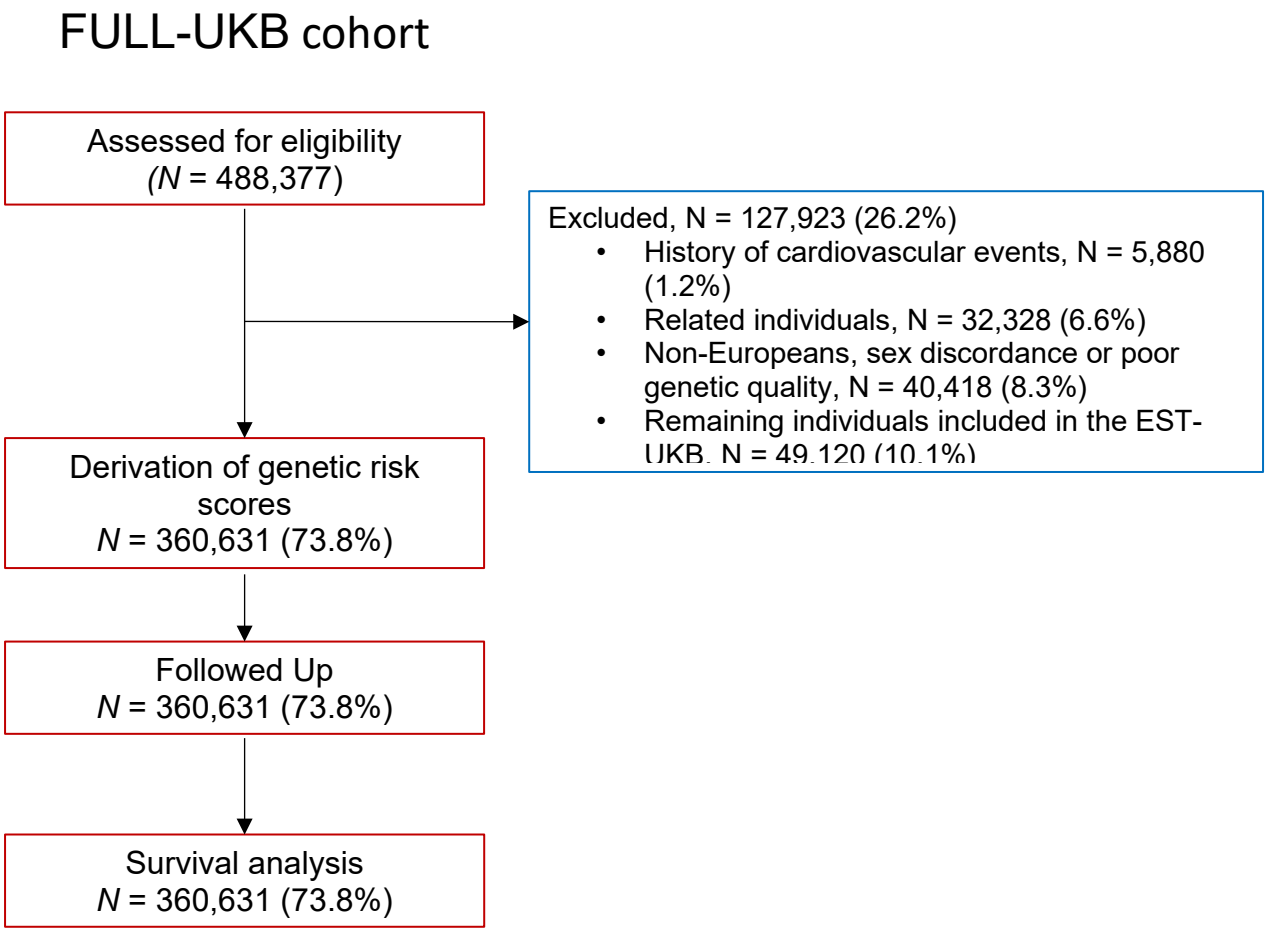
ESC SCORE includes HTN, cholesterol, smoke, sex and age

Abbreviations: ESC: European Society of Cardiology, TMR: T-wave morphology restitution, NRI: Net reclassification index, CI: confidence interval

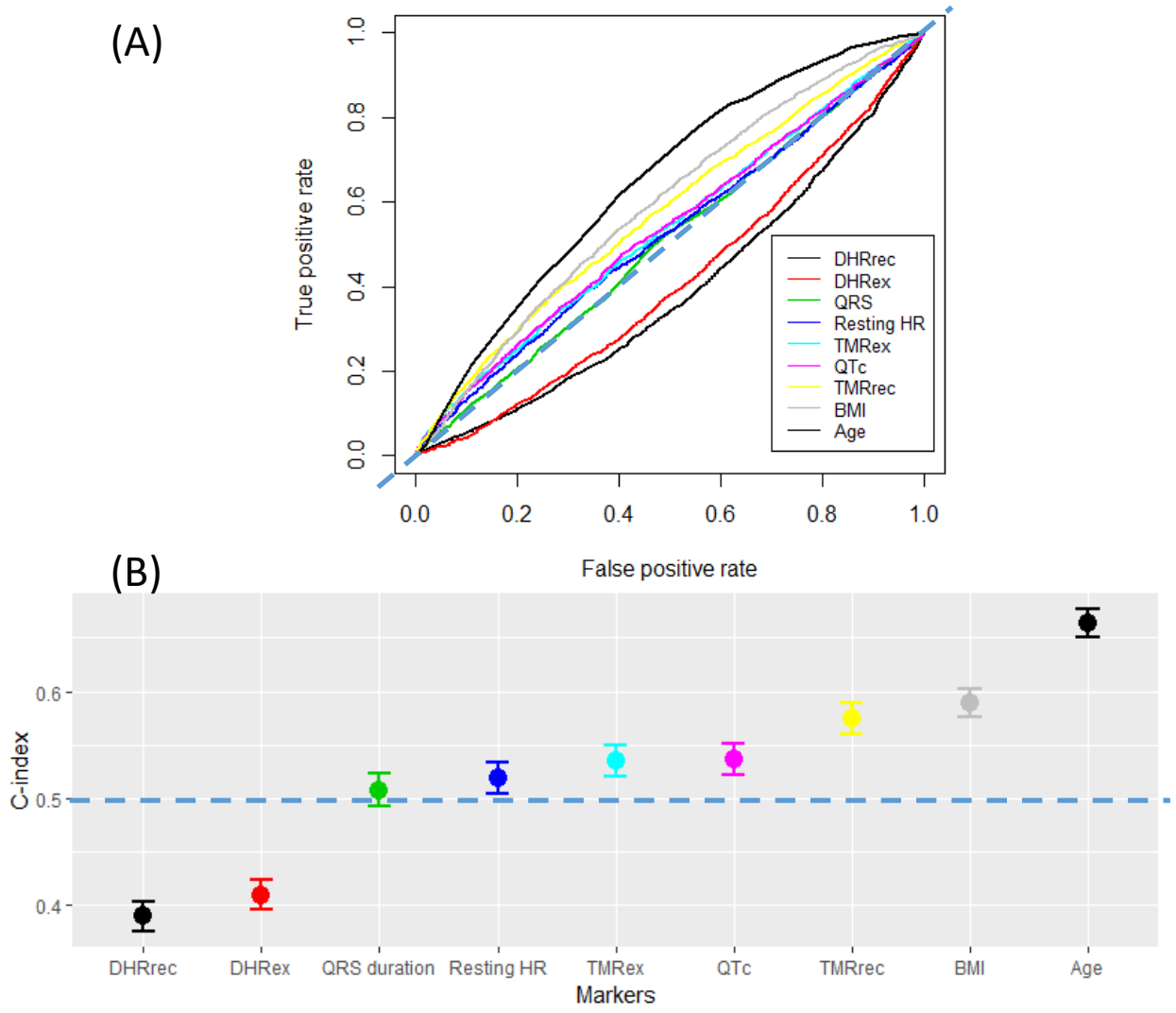
Supplemental Figures



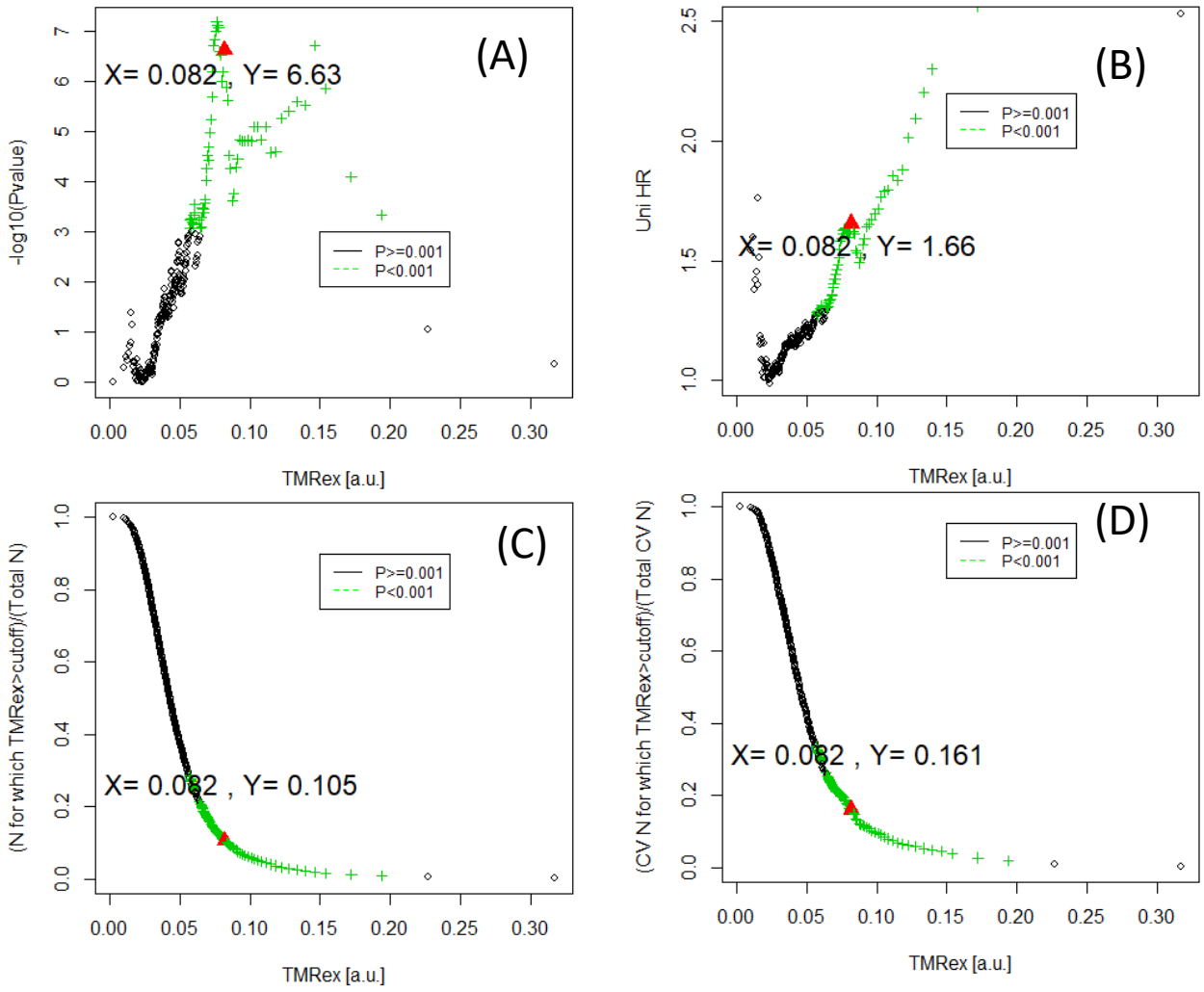
Supplemental Figure 1: Histograms of TMR during exercise (A) and TMR during recovery (B). The black curves indicate a normal distribution using the mean and standard deviation from each distribution.



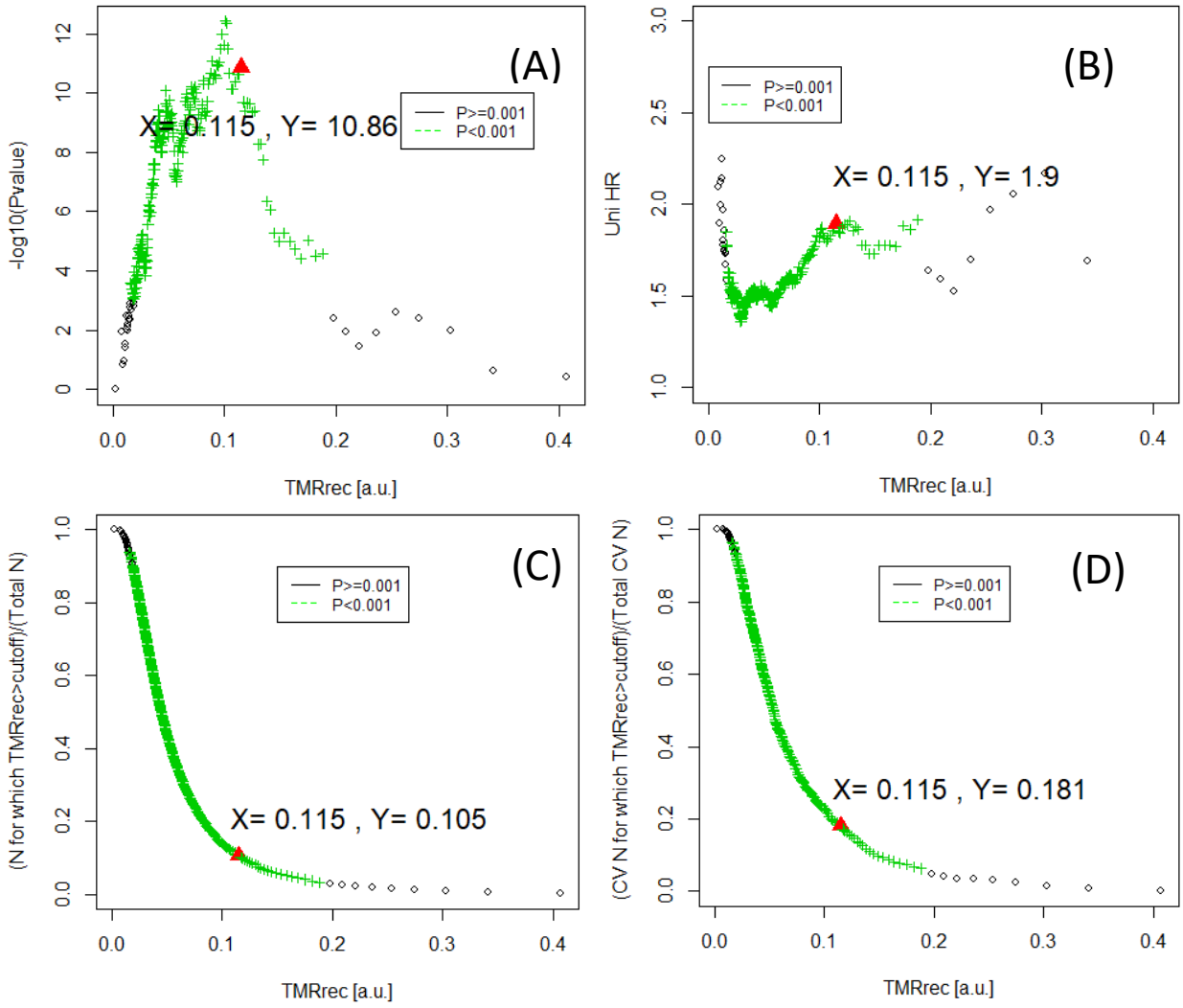
Supplemental Figure 2: Full cohort (FULL-UKB) study population flow diagram.



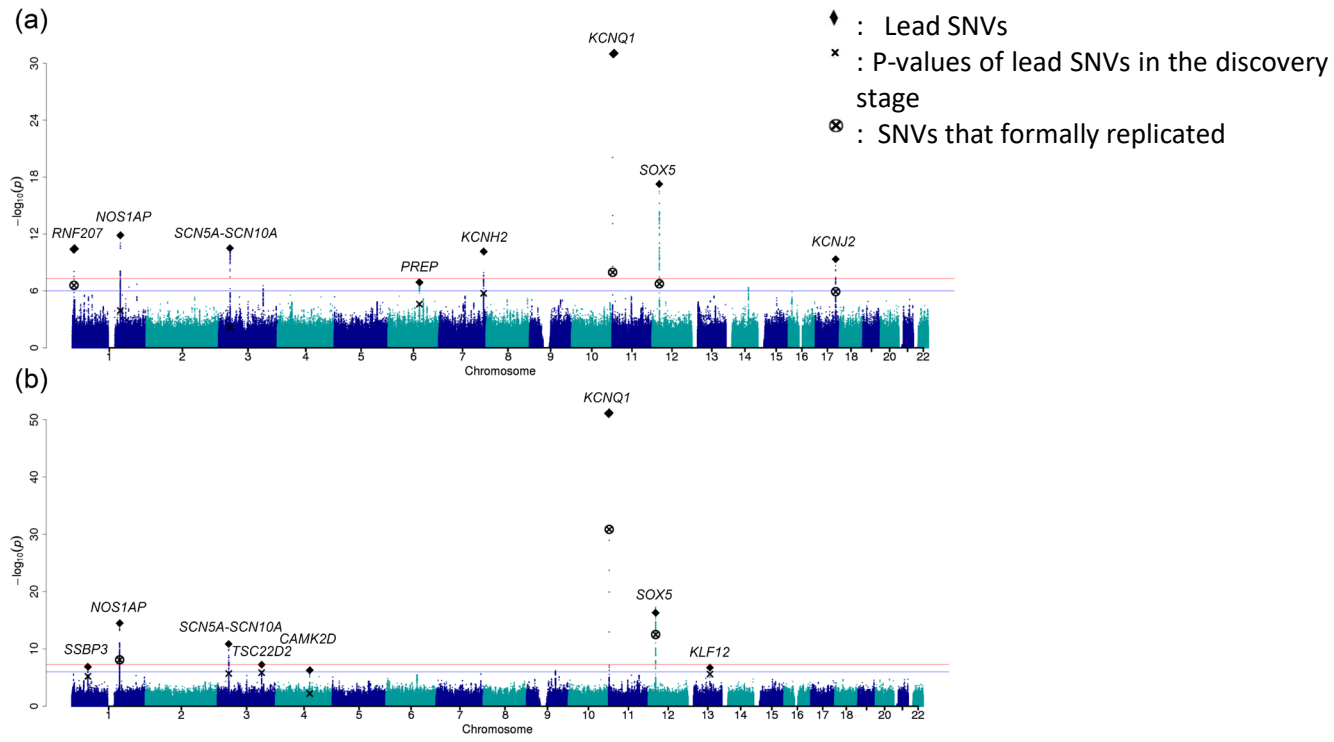
Supplemental Figure 3: Classification performance of clinical and ECG markers. (A) True positive rate versus false positive rate for the clinical and ECG markers, including TMR during exercise and recovery. (B) C-index for the clinical and ECG markers, including TMR during exercise and recovery. The dash-blue horizontal line indicates the C-index that would be obtained by chance.



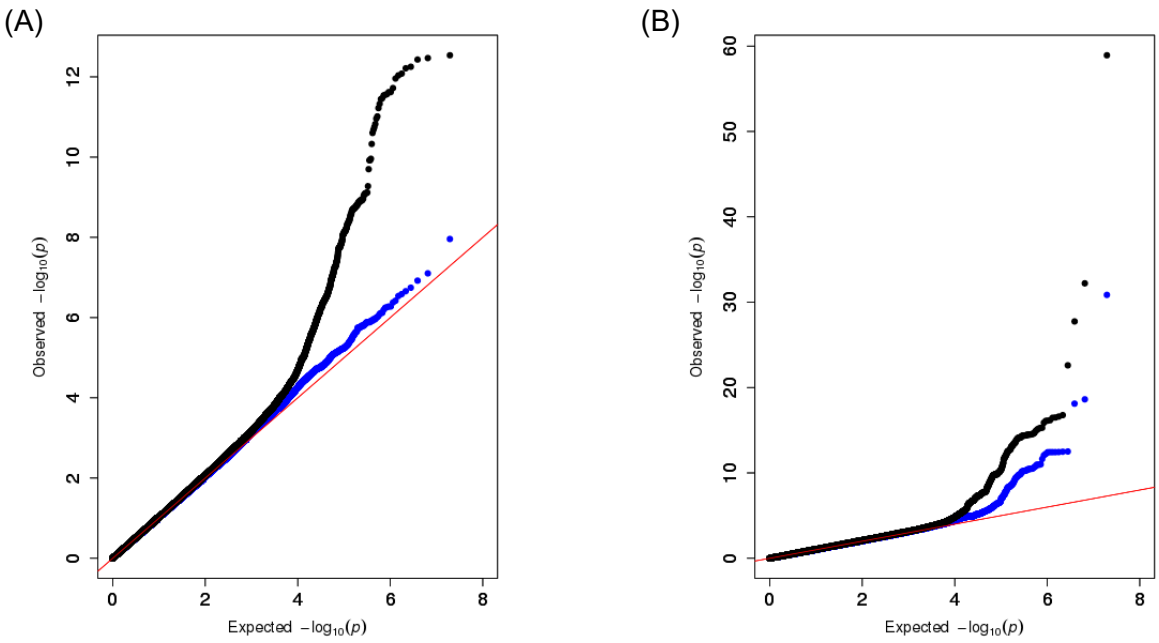
Supplemental Figure 4: Criteria to derive the optimal cut-off values for TMR during exercise. A: -log10(P-value) versus TMR during exercise. B: Univariate hazard ratio (HR) versus TMR during exercise. C: Proportion of individuals with values of TMR during exercise above the cut-off vs TMR during exercise. D: Proportion of individuals from the CV event group with values of TMR during exercise above the cut-off vs TMR during exercise. The red triangle indicates the optimal cut-off value for TMR during exercise. The optimal cut-off value was defined as the one that simultaneously verified the following criteria: (i) it corresponded to a local maximum of the hazard ratio function from binary univariate Cox models, (ii) it was associated with a *P*-value < 10^{-3} and (iii) the proportion of individuals in the high-risk and low-risk groups was > 10% and > 50%, respectively. If more than one cut-off value met these criteria, the one associated with the highest hazard ratio was used.



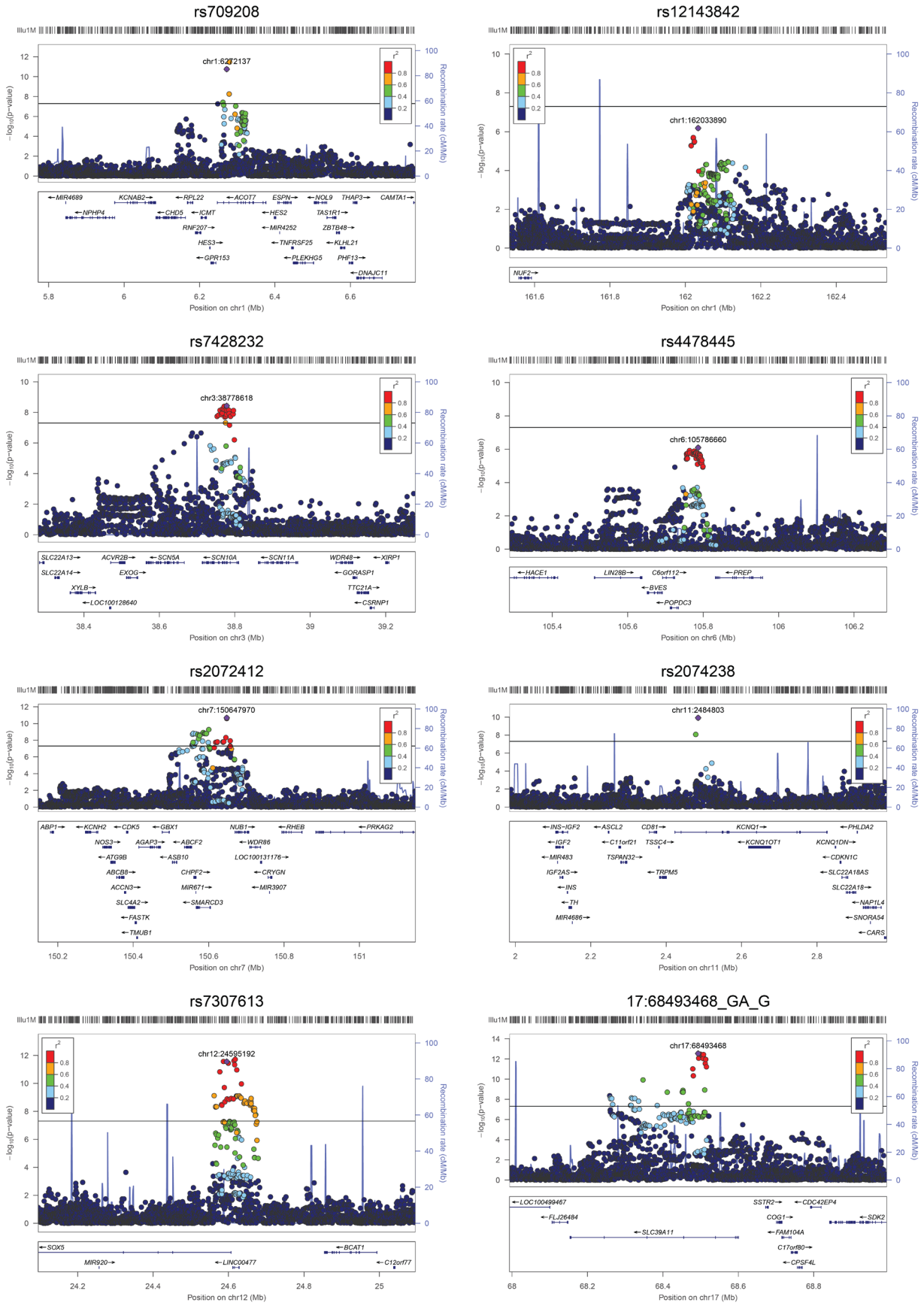
Supplemental Figure 5: Criteria to derive the optimal cut-off values for TMR during recovery. A: -log10(P-value) versus TMR during recovery. B: Univariate hazard ratio (HR) versus TMR during recovery. C: Proportion of individuals with values of TMR during recovery above the cut-off vs TMR during recovery. D: Proportion of individuals from the CV event group with values of TMR during recovery above the cut-off vs TMR during recovery. The red triangle indicates the optimal cut-off value for TMR during recovery. The optimal cut-off value was defined as the one that simultaneously verified the following criteria: (i) it corresponded to a local maximum of the hazard ratio function from binary univariate Cox models, (ii) it was associated with a *P*-value < 10^{-3} and (iii) the proportion of individuals in the high-risk and low-risk groups was > 10% and > 50%, respectively. If more than one cut-off value met these criteria, the one associated with the highest hazard ratio was used.



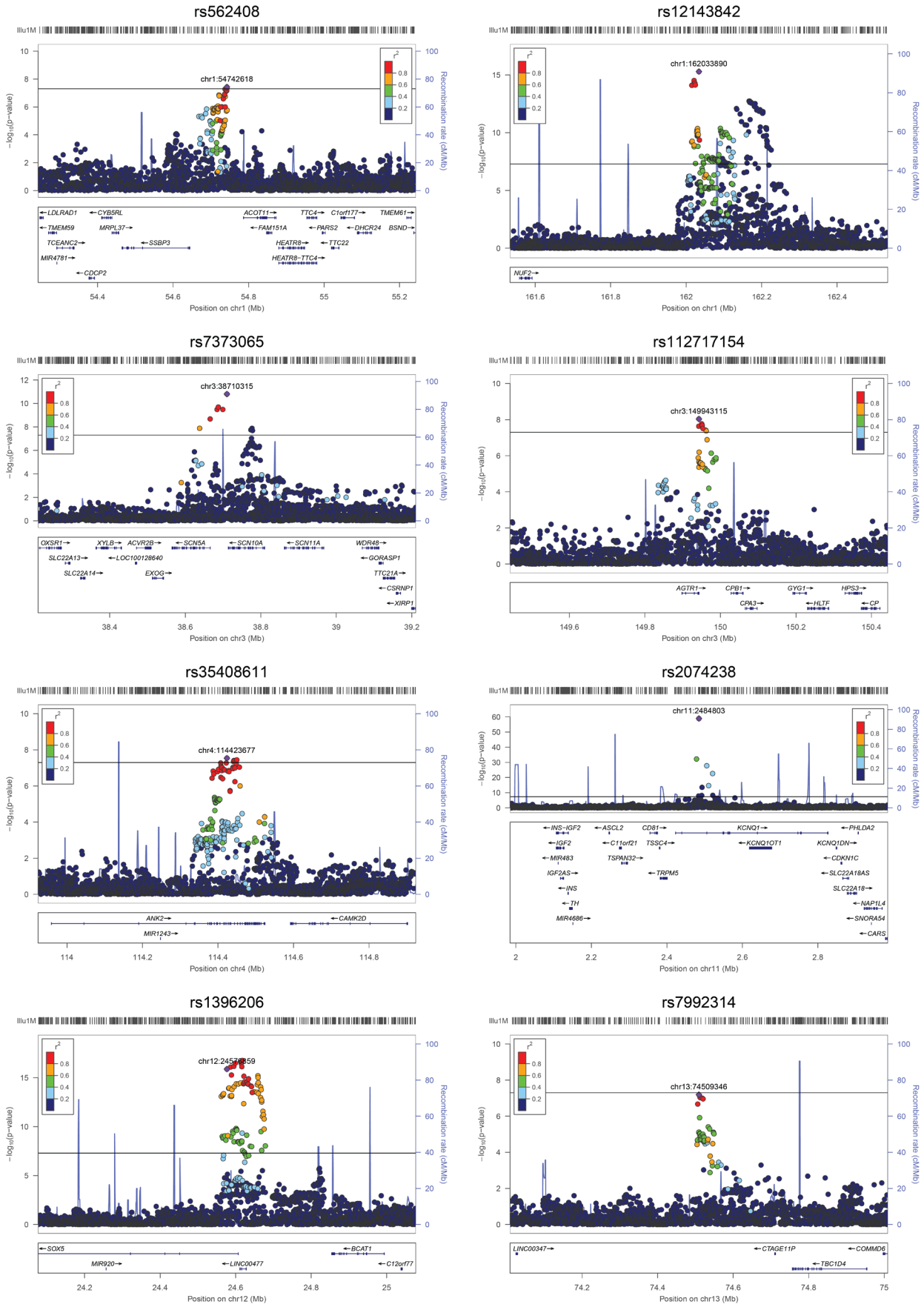
Supplemental Figure 6: Manhattan plots of TMR during exercise (a) and during recovery (b) in the full cohort analysis. P values, expressed as –log10(P), are plotted according to physical genomic locations by chromosome. Lead SNVs are marked by the diamonds. The crosses indicate the P values of these SNVs in the discovery data set. Crosses are encircled for SNPs that formally replicated. Locus names of the novel loci correspond to the nearest annotated gene. The blue horizontal line indicates a P value threshold of $1x10^{-6}$, corresponding to the lookup significance threshold. The red horizontal line indicates a P-value threshold of $5x10^{-8}$, corresponding to genome-wide significance.



Supplemental Figure 7: QQ plots for TMR during exercise (A) and during recovery (B) in the discovery (blue) and full (black) cohorts.

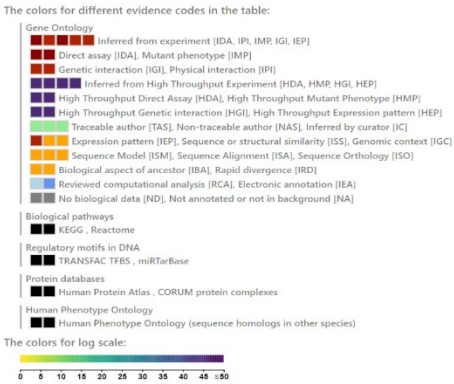


Supplemental Figure 8A: Locus Zoom plots for all the identified loci for TMR during exercise.

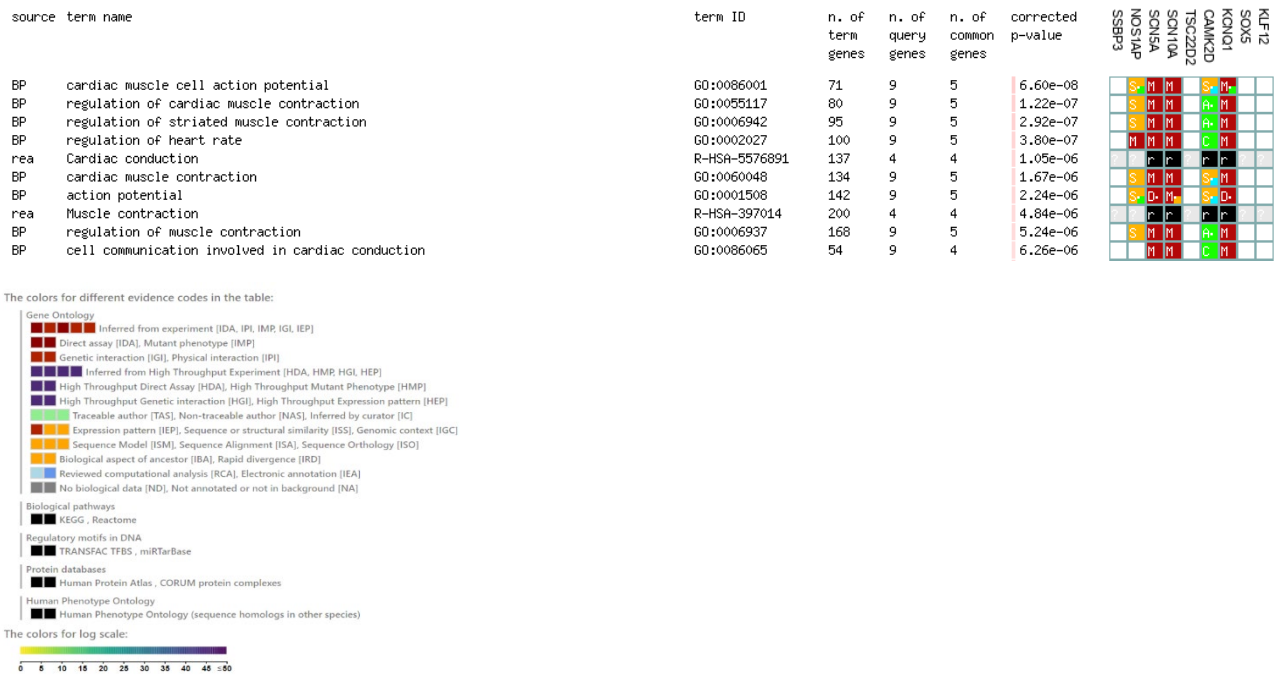


Supplemental Figure 8B: Locus Zoom plots for all the identified loci for TMR during recovery.

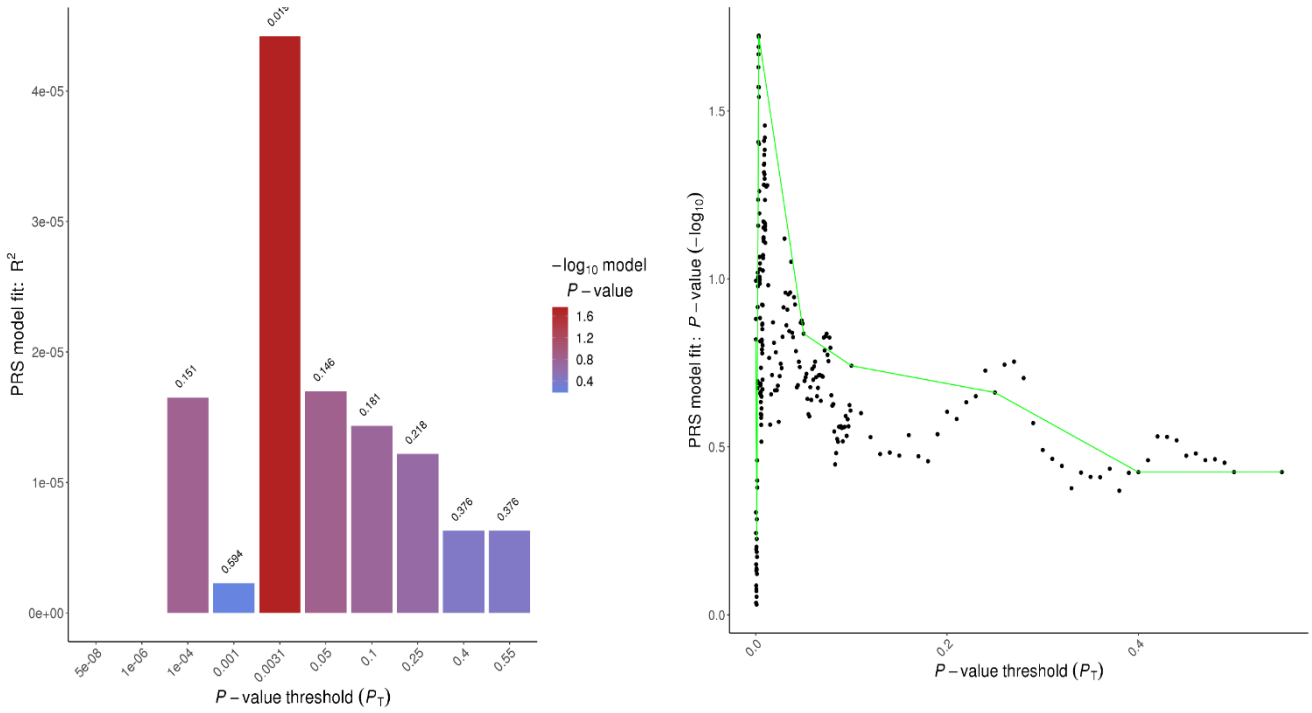
source	term name	term ID	n. of term genes	n. of query genes	n. of common genes	corrected p-value	RNF207	NOS1AP	SCN5A	SCN10A	PREP	ATG5	KCNH2	KCNQ1	SOX5	CDC42EP4
BP	cardiac muscle cell action potential	GO:0086001	71	10	6	4.02e-10	M	S	M	M			M	M		
BP	regulation of ventricular cardiac muscle cell membrane repolarization	GO:0060307	24	10	5	4.69e-10	M	M	M				M	M		
BP	ventricular cardiac muscle cell membrane repolarization	GO:0099625	28	10	5	1.08e-09	M	M	M				M	M		
BP	regulation of cardiac muscle cell membrane repolarization	GO:0099623	29	10	5	1.31e-09	M	M	M				M	M		
BP	ventricular cardiac muscle cell action potential	GO:0086005	36	10	5	4.15e-09	M	S	M				M	M		
BP	regulation of membrane repolarization	GO:0060306	37	10	5	4.79e-09	M	M	M				D	D		
BP	heart contraction	GO:0060047	245	10	7	5.44e-09	M	M	M	M		e	M	M		
BP	cardiac muscle cell membrane repolarization	GO:0099622	39	10	5	6.33e-09	M	M	M				M	M		
BP	heart process	GO:0003015	253	10	7	6.82e-09	M	M	M	M		e	M	M		
BP	membrane repolarization	GO:0086009	48	10	5	1.88e-08	M	M	M				D	D		



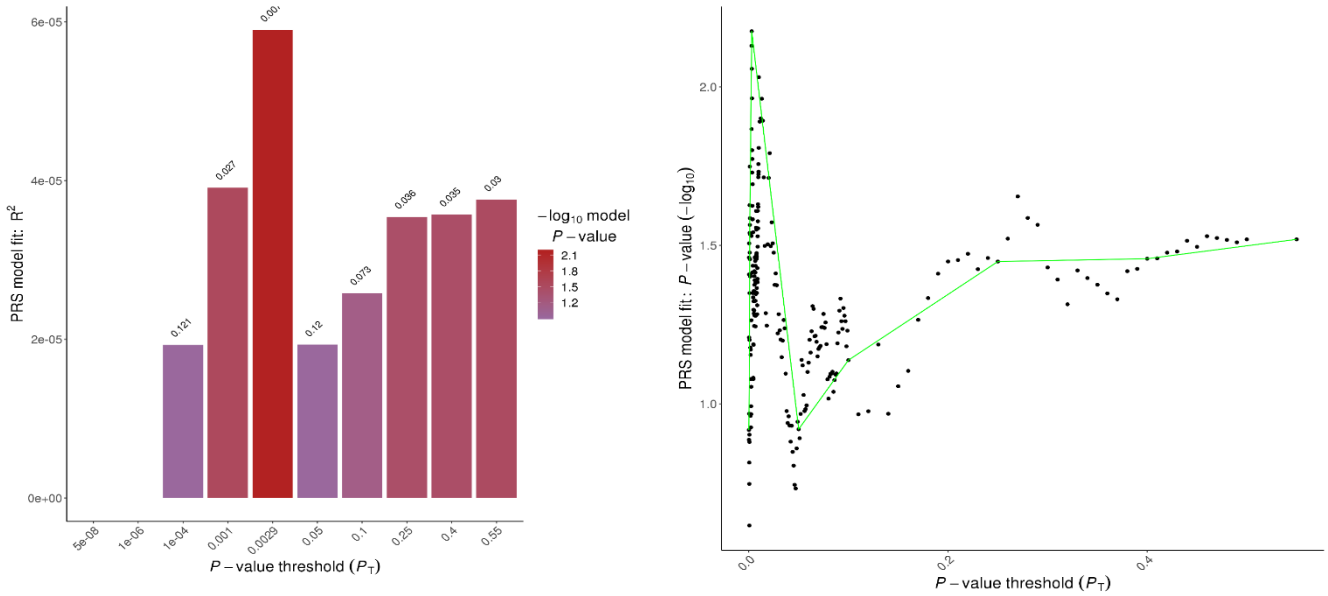
Supplemental Figure 9: Biological processes enrichment of candidate genes at TMR during exercise loci. g:profiler GO (gene ontology) term enrichment was performed using the candidate genes for TMR during exercise.



Supplemental Figure 10: Biological processes enrichment of candidate genes at TMR during recovery loci. g:profiler GO (gene ontology) term enrichment was performed using the candidate genes for TMR during recovery.



Supplemental Figure 11: Left, adjusted R-squared versus the P-value threshold. Right, -log10 of the P-value from each logistic regression versus the P-value threshold. The optimal P-value threshold is the one for which the adjusted R-squared is highest and the P-value is lowest.



Supplemental Figure 12: Left, adjusted R-squared versus the P-value threshold. Right, -log10 of the P-value from each logistic regression versus the P-value threshold. The optimal P-value threshold is the one for which the adjusted R-squared is highest and the P-value is lowest.

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