# The Genetic Architecture of T-wave Morphology Restitution

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## 1. Introduction

Cardiovascular (CV) mortality is the main cause of death in the general population[1]. The analysis of the electrocardiogram (ECG) has potential for non-invasive diagnosis and prediction of CV risk. ECG markers are heritable and statistical genetic methods are available to estimate the cumulative contribution of genetic factors to CV events via genetic risk scores (GRSs)[2]. The T-wave morphology restitution (TMR)[3] is an ECG marker that quantifies the rate of variation of the T-wave morphology with heart rate and has shown to be a strong predictor of sudden cardiac death in chronic heart failure patients[3]. We hypothesize that the interaction between repolarization dynamics and CV risk has a genetic component and that TMR can be used to capture it.

The objective was to identify single-nucleotide variants (SNVs) significantly associated with TMR using genome-wide association studies (GWASs) and to develop genetic risk scores (GRSs) to evaluate their association with CV risk.

## 2. Methods

ECG recordings were obtained from 55,222 individuals who participated in the exercise stress test from the UK Biobank study (EST-UKB cohort). For each subject, TMRs were automatically computed for exercise ( $TMR^{ex}$) and recovery ( $TMR^{rec}$) using the algorithm presented in Figure 1.

We estimated the heritability of each TMR marker using a variance components method (BOLT-REML) and subsequently performed GWASs in discovery ($N$ = 29,393) and replication ($N$= 22,382) datasets separately using a linear mixed model method (BOLT-LMM)[4]. Both models included sex, age, body mass index (BMI) and a binary indicator variable for the genotyping array (UK Biobank versus UK BiLEVE). The $TMR^{ex}$ model included resting RR and the RR difference between peak exercise and resting ($\Delta RR^{ex}$) as well, while the $TMR^{rec}$ model included recovery RR and the RR difference between peak exercise and recovery ($\Delta RR^{rec}$). Replication was confirmed if SNV remained significant (with Bonferroni correction) with same direction observed in the discovery analyses. A full dataset GWAS for both markers was also conducted and additional loci reaching genome-wide significance ($P \leq 5 \times 10^{-8}$) were reported. Bioinformatics analyses were performed to annotate SNVs and identify candidate genes.

GRSs were derived and tested for association with the main endpoint (CV mortality or hospitalizations due to CV reasons) in the full cohort (FULL-UKB; N = 360,454, median follow-up of 8.4 years). Patients who died from causes not included in the primary endpoint were censored at the time of death. The GRSs were standardised to have a mean

of 0 and a standard deviation of 1. A value of $P<0.05$ was considered statistically significant. Statistical analyses were performed using R version 3.5.1. The UKB study has approval from the North West Multi-Centre Research Ethics Committee, and all participants provided informed consent[5].

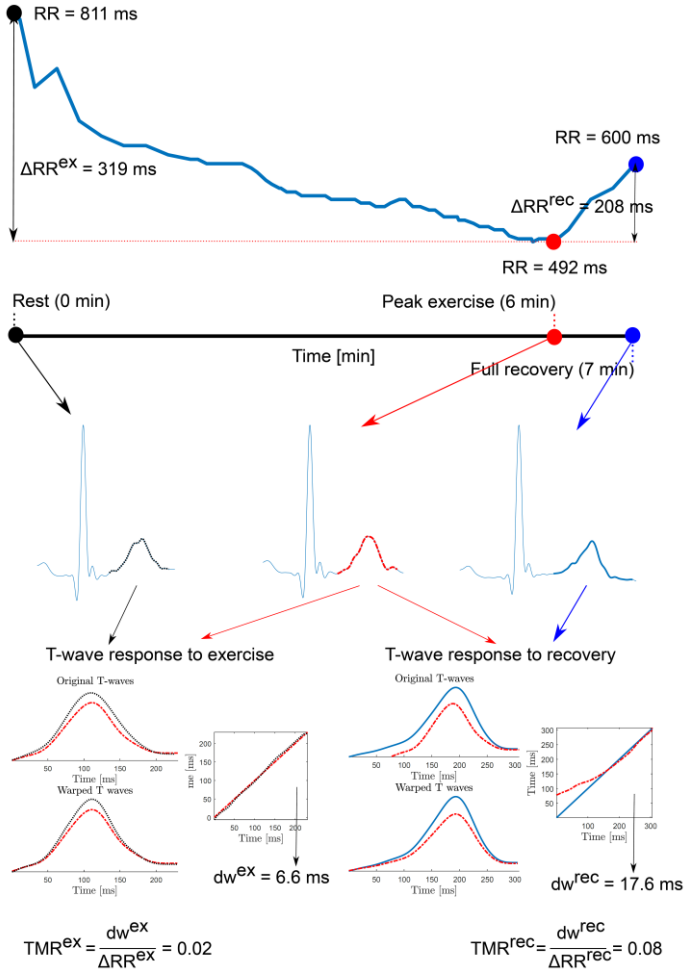


***Figure 1*** *Assessment of TMR. (Top) Illustration of the RR profile during the exercise stress test. (Middle) Three averaged heartbeats are derived at rest (black), peak exercise (red) and 50 s after peak exercise (full recovery, blue), respectively. (Bottom) After low pass filtering the T-waves,* $TMR^{ex}$ *is derived by quantifying the morphological change between the T-waves at rest (black T-wave) and at peak exercise (red T-wave), normalised by the RR change during this interval. Similarly,* $TMR^{rec}$ *is derived by quantifying the morphological change between the T-waves at peak exercise (red T-wave) and full recovery (blue T-wave), normalised by the subsequent RR change.*

## 3. Results

A total of 51,574 subjects were taken forward for genetic analyses after applying genetic QC and excluding individuals of non-European ancestry. The heritability estimations of $TMR^{ex}$ and $TMR^{rec}$ were 3.5% and 4.9%, respectively. In total, 12 loci were identified, eight loci for each trait (Figure 2). The discovered SNVs for $TMR^{ex}$ explained 0.63% of trait variance, and the 8 SNVs identified for $TMR^{rec}$ explained 1.14% variance.

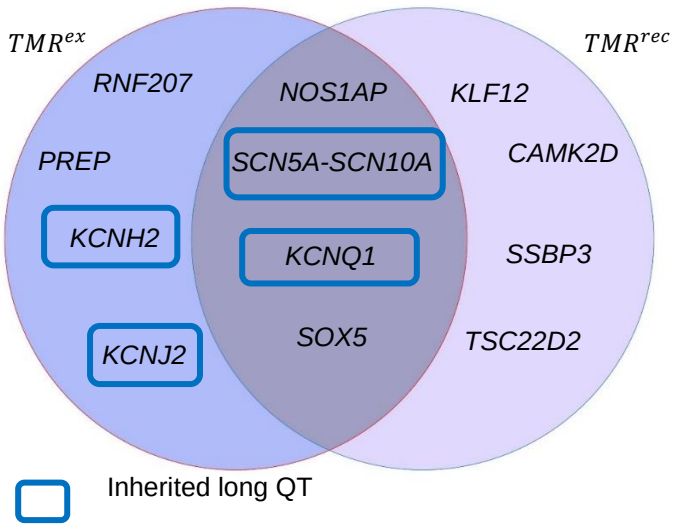


***Figure 2*** *Overlap of loci for TMR during exercise and TMR during recovery. The loci names indicate the nearest annotated genes.*

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*). Look ups in PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk/about/) indicated nine of the 12 SNVs have associations with other CV markers including pulse rate, QT interval, PR interval, QRS duration, P-wave duration, cardiac arrhythmias and heart function. None of the lead variants or their close proxies ($r^2$>0.8) were annotated as missense variants. Variants at two loci *NOS1AP* and *SSBP3* were associated with expression levels of nearby genes (*c1orf226* and *SSBP3*, respectively) in heart atrial appendage samples. The top three biological pathways for $TMR^{ex}$ were cardiac muscle cell action potential ($P = 4 \times 10^{-10}$), regulation of ventricular cardiac muscle cell membrane repolarization ($P = 4.7 \times 10^{-10}$) and ventricular cardiac muscle cell membrane repolarization ($P = 1 \times 10^{-9}$). The analyses for $TMR^{rec}$ indicated similar pathways including cardiac muscle cell action potential ($P = 6.6 \times 10^{-8}$), regulation of cardiac muscle contraction ($P = 1.2 \times 10^{-7}$) and regulation of striated muscle contraction ($P = 3 \times 10^{-7}$). The optimal GRS for $TMR^{ex}$ was not significantly different between individuals with a CV event and those without ($P = 8.6 \times 10^{-2}$). The optimal GRS for $TMR^{rec}$ was significantly higher in individuals with a CV event than those that did not have an event ($P = 1.6 \times 10^{-2}$). Univariate Cox analysis showed that individuals in the top 20% of the GRS for $TMR^{rec}$ were significantly more likely to have a CV event than those in the bottom 20% (HR 1.06, confidence interval 1.02 - 1.11, $P = 8.0 \times 10^{-3}$).

## 4. Discussion

TMR is an ECG marker that measures the rate of variation of the T-wave morphology due to heart rate changes. TMR is associated with spatio-temporal heterogeneity of ventricular repolarization that in this cohort was exposed by exercise and recovery from exercise. The main findings from this study are that $TMR^{rec}$ has a genetic predisposition and its GRS is significantly associated with CV events.

Genetic variants at 50% of the loci identified for $TMR^{ex}$ are the same loci as long-QT syndrome loci: *KCNH2*, *KCNJ2*, *SCN5A* and *KCNQ1*. These channel proteins underlie the major repolarising ventricular potassium currents. Variations in these currents might lead to changes in the T-wave morphology and this is entirely consistent with known physiology. 50% of the identified loci for $TMR^{rec}$ overlap with $TMR^{ex}$ loci, with the remaining loci specific for $TMR^{rec}$. 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, while variants at the three other loci have not been associated to 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. 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.

In conclusion, we have conducted a systematic investigation of the genetic basis of ventricular repolarization and its influence in modulating CV risk through the analysis of the T-wave morphology. We demonstrate that TMR has a genetic predisposition and reflects relevant biological mechanisms influencing the CV system.

## References


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