



Mendelian Randomization Causal Analysis

Genetically predicted testosterone and electrocardiographic QT interval duration in Chinese: a Mendelian randomization analysis in the Guangzhou Biobank Cohort Study

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Abstract

Background QT interval prolongation, a predictor of cardiac arrhythmias, and elevated heart rate are associated with higher risk of cardiovascular mortality. Observationally testosterone is associated with shorter corrected QT interval and slower heart rate; however, the evidence is open to residual confounding and reverse causality. We examined the association of testosterone with electrocardiogram (ECG) parameters using a separate-sample instrumental variable (SSIV) estimator.

Methods To minimize reverse causality, a genetic score predicting testosterone was developed in 289 young Chinese men from Hong Kong, based on a parsimonious set of single nuclear polymorphisms (rs10046, rs1008805 and rs1256031). Linear regression was used to examine the association of genetically predicted testosterone with QT interval, corrected QT interval [using the Framingham formula (QTf) and Bazett formula (QTb)] and heart rate in 4212 older (50+ years) Chinese men from the Guangzhou Biobank Cohort Study.

Results Predicted testosterone was not associated with QT interval [−0.08 ms per nmol/l testosterone, 95% confidence interval (CI) −0.81 to 0.65], QTf interval (0.40 ms per nmol/l testosterone, 95% CI −0.12 to 0.93) or heart rate (0.26 beats per minute per nmol/l testosterone, 95% CI −0.04 to 0.56), but was associated with longer QTb interval (0.66 ms per nmol/l testosterone, 95% CI 0.02 to 1.31).

Conclusions Our findings do not corroborate observed protective associations of testosterone with QT interval or heart rate among men, but potentially suggest effects in the other direction. Replication in a larger sample is required.

Key words: Mendelian randomization, testosterone, QT interval

Key Messages

- The role of androgens in cardiovascular disease is controversial.
- We used a Mendelian randomization analysis with a separate-sample instrumental variable to examine the causal effects of endogenous testosterone on electrocardiogram parameters.
- Our findings do not corroborate observed protective associations of testosterone with cardiac arrhythmias or heart rate among men, but instead suggests testosterone could lengthen corrected QT interval.

Introduction

Cardiovascular disease is the leading cause of mortality globally.¹ Prolonged QT interval is associated with higher risk of cardiac arrhythmias,² and a longer QT interval, even within the normal physiological range, is associated with higher risk of cardiovascular disease (CVD) mortality with a graded association.³ Men have a shorter QT interval than women, with the difference emerging at puberty⁴ and potentially implicating sex hormones, although this difference in QT interval may also be related to a slower heart rate (HR) among men.⁵ Heart rate is another indicator of cardiovascular health, which is positively associated with CVD mortality.⁶

Observational studies generally show testosterone associated with shorter corrected QT interval^{5,7} and slower heart rate,⁵ suggesting a beneficial effect of androgens on cardiac arrhythmias and/or heart rate among men. However, the observed association of endogenous testosterone with a shorter corrected QT interval and a lower heart rate is not evident for a different androgen biomarker (androstenediol glucuronide).⁸ Additionally, these observations are somewhat inconsistent with the experimental evidence.^{9–15} In animal experiments, increased testosterone shows varied effects on QT interval, with reports of both shortening^{16,17} and prolongation,^{9,10} and also varied effects on HR, with reports of increases,¹⁶ no effect¹⁰ and decreases.¹⁷ Small randomized controlled trials (RCTs) have reported that testosterone has no effect on QT interval^{11–13} or shortens QT interval,¹⁸ and has no effect on HR^{11–13} or increases HR.¹⁴ One single-arm trial of an anti-androgen also reported no effect on QT interval or HR.¹⁵ These discrepancies within observational studies and by study design may have occurred for several reasons. Trials examining the effect of exogenous androgens may be too small to detect minor differences.^{11–13,15} Observations concerning endogenous testosterone,^{5,7} although well conducted, are open to reverse causality arising from testosterone falling with age and being affected by ill health.^{19,20} Moreover, numerous factors may affect CVD risk, such as lipids, blood pressure and diabetes. However, observations of serum testosterone associated with a healthier CVD risk factor profile have not been

substantiated by Mendelian randomization (MR) studies.^{21,22} A large-scale RCT to examine the effects of testosterone has been suggested,²³ but the Institute of Medicine (USA) recommended such RCTs only after the benefits of testosterone had been established;²⁴ concerns about the cardiovascular safety of androgen replacement²⁵ have also raised questions about such RCTs.

Given the widespread prescription of testosterone for men, particularly in North America,²⁶ the cardiovascular effect of testosterone is an important question relevant to clinical practice and policy, where evidence is limited. Health Canada recently issued a warning about cardiovascular disease, including risk of irregular heart rate.²⁷ The Food and Drug Administration (FDA) advisory committee also recommended restricting testosterone prescription, and investigation of cardiovascular risk.²⁸ Using naturally occurring testosterone-related genetic variants to predict peak testosterone, unaffected by ageing or ill health, in an MR analysis with a separate-sample instrumental variable (SSIV), enables examination of the causal effects of endogenous testosterone, without any intervention. Here we used a separate-sample two-stage MR analysis to assess the effects of endogenous testosterone on QT interval, corrected QT interval and heart rate among older men, to minimize reverse causality.

Methods

Study design

A separate-sample two-stage MR analysis was used. First, a genetic score predicting serum testosterone was developed in young Chinese men from Hong Kong, as described previously.²² Second, the association of predicted testosterone with QT interval, corrected QT interval and HR was examined in older Chinese men from the Guangzhou Biobank Cohort Study.

Sources of data

To generate a prediction rule for testosterone, a sample of students was recruited from the University of Hong Kong,

restricted to those with both parents and at least three grandparents born in Hong Kong or Guangdong, and who were not taking medication affecting hormones. Morning blood samples were collected for testosterone assessment and DNA extraction. Testosterone was assessed by radioimmunoassay (Roche Diagnostics GmbH, Mannheim, Germany). DNA was extracted and analysed at the Centre for Genomic Sciences of the University of Hong Kong for selected single nucleotide polymorphisms (SNPs) including SNPs from *ESR1* (rs722208 and rs2175898), *CYP19A1* (rs10046 and rs1008805) and *ESR2* (rs1256030 and rs1256031) using a Mass ARRAY system (Sequenom, San Diego, CA). Stepwise linear regression with all candidate SNPs (excluding those deviating from Hardy–Weinberg equilibrium or in linkage disequilibrium) was used to find a parsimonious set of SNPs which best predicted log testosterone in the sample of young men (as shown in the flow chart in Appendix Figure 1 and for each selected SNP in Appendix Table 1, available as Supplementary data at *IJE* online), as described previously.²² A self-administered questionnaire was used to collect data on socioeconomic position and health status.

To assess the effects of genetically predicted testosterone on electrocardiogram (ECG) parameters, we used a large sample of older people (50+ years) from the Guangzhou Biobank Cohort Study (GBCS). GBCS is an ongoing collaboration between Guangzhou Number 12 Hospital and the Universities of Hong Kong and of Birmingham, UK.²⁹ Recruitment of participants was in three phases. All participants were permanent residents of Guangzhou and members of the ‘The Guangzhou Health and Happiness Association for the Respectable Elders’ (GHHARE), a community social and welfare association unofficially aligned with the municipal government. Membership is open to older people for a monthly fee of 4 Yuan (50 US cents). About 7% of permanent Guangzhou residents aged 50+ years are members of GHHARE, of whom 11% (about 10 000 participants) enrolled for each of phases one, two and three of the study. Inclusion criteria were that they were capable of consenting, ambulatory and not receiving treatment modalities which if omitted might result in immediate life-threatening risk, such as chemotherapy or radiotherapy for cancer, or dialysis for renal failure. Fasting blood samples were collected for storage at recruitment in phase 3 and at follow-up for participants recruited in other phases. Samples were stored, as whole blood or as buffy coat and sera, at -80°C for all apart from a subset of phase 3 participants whose DNA was extracted from fresh blood and stored at -80°C . Selected SNPs were analysed by a commercial company (Beijing CapitalBio Corporation³⁰) in Beijing using a Mass ARRAY system (Sequenom, San Diego, CA). The University of

Hong Kong-Hospital Authority Hong Kong West Cluster Joint Institutional Review Board approved the study. The Guangzhou Medical Ethics Committee of the Chinese Medical Association approved GBCS, including the use of genetic data, and all participants gave written, informed consent prior to participation.

Electrocardiogram (ECG)

In GBCS a standard ECG was performed in the supine position after resting for 5 min, using a 3-channel, 12-lead ECG (Marquette MAC-500) in phase 1 and at the start of phase 2, and a synchronous 12-lead ECG (Marquette Cam-14 acquisition module) in the rest of phase 2 and in phase 3.³¹ The ECG tracings obtained by the Marquette MAC-500 electrocardiograph were evenly distributed to two qualified physicians blinded to other information and measured independently.³² QT interval was examined from the earliest QRS onset to the end of T-wave. Any uncertainties were resolved through discussion and consensus. In the rest of phase 2 and in phase 3, the QT interval and HR were measured automatically by the ECG machine.

Exposure

The primary exposure was predicted testosterone estimated as the anti-log of genetically predicted log testosterone. Testosterone, instead of log testosterone, was used as the exposure for ease of interpretation, as they both gave the same pattern of results.

Outcomes

The outcomes were QT interval, HR-corrected QT intervals using the Framingham formula (QTf) calculated as $\text{QT} + 154 \times (1 - 60/\text{HR})$ ³³ and the Bazett formula (QTb) calculated as $\text{QT}/\{\text{HR}/60\}^{1/2}$,³⁴ as well as HR. QT interval is influenced by HR, so HR correction is required for the analysis of repolarization duration.³⁵ The Bazett formula is the most widely used method for HR correction, but tends to underestimate or overestimate the duration of cardiac repolarization at extremely low or high HRs.³⁶ The Framingham correction performs better at extreme values of HR.³³

Statistical analysis

We used an SSIV estimate from two separate samples.³⁷ Genetically predicted log testosterone among men was calculated as $-0.07 \times \text{rs1008805} + 0.07 \times \text{rs10046} - 0.07 \times \text{rs1256031} + 3.0$ (Appendix Table 1, available as Supplementary data at *IJE* online), as previously.²²

The F-statistic for the regression of log testosterone on genetically predicted log testosterone was obtained; an F-statistic >10 suggests a reliable genetic instrument.³⁸

ANOVA was used to compare genetically predicted testosterone by key characteristics. Linear regression was used to assess the association of genetically predicted testosterone with QT, QTf and QTb intervals and HR. Model 1 had no covariates, because the genetic associations are unlikely to be confounded in a homogeneous population; Model 2 used bootstrapping with 1000 replications for internal validation. We assessed whether the association of genetically predicted testosterone with QT intervals and HR varied with ECG measurement methods from the relevant interaction term. As a sensitivity analysis, men with a major intraventricular conduction defect, as indicated by $QRS \geq 120$ milliseconds (ms), were excluded because the repolarization abnormalities in these men could be secondary to the conduction defects. To account for variations due to health status, a sensitivity analysis was also performed among a subgroup of healthy men (without self-reported CVD or hypertension and not taking medication to reduce blood pressure). All statistical analyses were conducted using Stata 10.1 (StataCorp LP, College Station, TX).

Results

Among the 8450 men in all three phases of GBCS, DNA with relevant SNP testing was available for 4262 men, with availability depending on the phase of recruitment and other logistical concerns, but not on ECG parameters. Among the 4262 men, 4212 (98.8%) had all relevant SNPs and of these, 3864 (91.7%) had complete data on QT interval and HR.

The proportion of variance in log testosterone explained by the genetic score was 4.1%. The F-statistic was 13.3, suggesting a reliable genetic instrument. As would be expected, age, socioeconomic position and lifestyle, including smoking status and use of alcohol, were not associated with genetically predicted testosterone (Table 1).

Table 2 shows that genetically predicted testosterone was not associated with QT interval among men. The estimates were close to null. Predicted testosterone was associated with longer QTb and QTf intervals as well as faster HR, but the confidence intervals for QTf and HR included the null. The results remained unchanged after bootstrapping replication (Model 2). The association of genetically predicted testosterone with QT, QTb and QTf intervals and HR did not vary with ECG measurement method (*p*-values for interaction 0.27, 0.73, 0.48 and 0.55, respectively). Sensitivity analysis excluding those with

$QRS \geq 120$ ms and among healthy men both showed similar results (Appendix Table 2, available as Supplementary data at *IJE* online), although the confidence interval for QTb interval among healthy men included the null whereas the confidence interval for HR among healthy men no longer included the null.

Discussion

Using an MR analysis with an SSIV estimator to minimize reverse causality, our study does not show any negative association of endogenous testosterone with QT interval or HR in men. Instead, predicted testosterone was associated with longer corrected QT interval. Our novel study provides no support for protective effects of endogenous testosterone on QT interval or HR among men.

To our knowledge, this is the first MR analysis using an SSIV estimator to provide an unbiased estimation of the effects of testosterone on QT interval or HR. SSIV is useful and cost-efficient when the phenotype of interest was either not measured or was measured with substantial error in the sample with the outcome.^{37,39} It is infeasible to obtain lifetime sex hormones for older people; neither biomaterials nor peak sex hormones dating back to the 1960s are available. The MR design makes it feasible to use testosterone in early adulthood as a marker of lifetime exposure. Thus, we can minimize reverse causality and avoid the imprecision in MR estimates that could arise from assessing the genetic association with testosterone at older ages, which may reflect ill health rather than lifelong exposure, inducing an underestimation of the genetic association with exposure thereby inflating the MR estimates.⁴⁰ SSIV also remedies weak-instruments bias, reducing concerns about using multiple polymorphisms as IVs.³⁷ Any correlation of the genetic variants with unmeasured confounders in the sample with the phenotype is unlikely to be replicated in the sample with the outcome, due to the different data structures.³⁷ We also restricted the sample of young people to those with both parents and at least three grandparents born in Hong Kong or Guangdong province, to ensure genetic homogeneity.

Although we used MR which can mimic the randomized treatment allocation in RCTs,⁴¹ several limitations exist. First, QT interval was measured using two methods. Assessing QT interval manually added some measurement error and thereby reduced differences between groups. However, any measurement error is likely to be random, which is compensated for by our large sample size, and the effect of genetically predicted testosterone did not vary with measurement method. QT interval and HR had the expected associations with age and sex (data not shown).^{42,43}

Table 1. Predicted testosterone by socio-demographic characteristics among 4212 men (aged 50+), Guangzhou Biobank Cohort Study, 2003–08

Characteristic	No.	%	Genetically predicted testosterone (nmol/l)		
			Mean	SD	<i>p</i> -value*
Age group, years	4212				0.94
50–54		8.1	18.05	1.10	
55–59		20.2	18.01	1.22	
60–64		25.4	18.01	1.24	
65–69		24.1	18.01	1.27	
70–74		16.5	17.99	1.19	
75–79		4.2	17.95	1.28	
≥80		1.5	18.15	1.52	
Education	4209				0.91
Less than primary school		2.6	18.11	1.36	
Primary school		26.9	18.01	1.22	
Junior middle school		30.0	18.01	1.19	
Senior middle school		24.2	18.01	1.23	
Junior college		8.9	17.99	1.34	
College		7.4	17.96	1.24	
Smoking status	4192				0.59
Never smoker		40.8	17.99	1.22	
Ex-smoker		27.9	18.02	1.25	
Current smoker		31.3	18.03	1.22	
Use of alcohol (frequency)	4212				0.49
Never		51.8	18.02	1.25	
<1 time/month		19.9	18.03	1.19	
<1 time/week		4.3	18.04	1.20	
1–4 times/week		6.0	18.03	1.30	
5+ times/week		11.9	17.90	1.19	
Ex-drinker		4.4	18.01	1.20	
Unknown		1.8	18.08	1.27	

SD, standard deviation.

*Two-sided *p*-value from ANOVA.**Table 2.** Effect of genetically predicted testosterone on QT interval, corrected QT interval and heart rate among men (aged 50+ years), Guangzhou Biobank Cohort Study, 2003–08

Outcome (n = 3864)	Mean (SD)	Model ^a	β coefficient ^b	95% CI	<i>p</i> -value
QT interval (milliseconds)	385.8(28.2)	1	–0.08	–0.81 to 0.65	0.83
QTf interval (milliseconds)	406.4(20.4)	1	0.40	–0.12 to 0.93	0.13
		2	0.40	–0.11 to 0.91	0.12
QTb interval (milliseconds)	416.7(24.9)	1	0.66	0.02 to 1.31	0.04
		2	0.66	0.04 to 1.29	0.04
Heart rate (beats per minute)	71.0(11.5)	1	0.26	–0.04 to 0.56	0.09
		2	0.26	–0.04 to 0.56	0.09

SD, standard deviation; CI, confidence interval.

^aModel 1 had no covariates; Model 2 used bootstrapping with 1000 replications for internal validation for Model 1.^b β coefficient refers to the average change in QT, QTf and QTb intervals and heart rate with each unit (nmol/l) increase in genetically predicted testosterone.

Second, the estimates for QTf and QTb are different, although they are in the same direction. Different correction methods may provide discordant results, as observed previously.⁴⁴ The wide range of HR in our study may account for the differences in the two correction methods; as

such, the Framingham correction may be more reliable. However, all our corrected estimates were in the same direction with only a difference in the level of variability. The estimates in the sensitivity analysis were also in the same direction, but sometimes had confidence intervals

including the null, perhaps because of the smaller sample size.

Third, this study is not totally representative, although the QT interval is similar to that in a nationally representative study.⁴⁵ The results would only be invalidated if the relation of genetic variants with testosterone or with ECG parameters in our sample differed from that in the general population, which is unlikely as the relevant genetic variants were not associated with socioeconomic position or lifestyle.

Fourth, only Chinese men were included, which could restrict generalizability. However, the association of genetic variants with testosterone or ECG parameters is unlikely to vary by setting or ethnicity.

Fifth, the selected SNPs may affect ECG parameters directly rather than via testosterone. To our knowledge, no study indicates a direct association of these SNPs with QT interval or HR.

Sixth, our SNP selection is restricted by the limited availability of genome-wide association studies (GWAS) of testosterone; also these are among older men, and so might reflect the factors causing testosterone to fall with age rather than testosterone. Moreover, several SNPs identified in GWAS of testosterone among Caucasian men have not replicated in Chinese men;⁴⁶ one SNP that did replicate (rs2075230) was associated with both testosterone and SHBG,⁴⁶ making it unsuitable as a genetic instrument for testosterone because of pleiotropy. In addition, genetic structure might vary by ethnicity; however, only Chinese originating from Guangdong province were included. We also tested for linkage disequilibrium in the young men, and discarded any SNPs in linkage disequilibrium.²² To avoid these issues, we generated a genetic prediction rule for testosterone from genes functionally relevant to testosterone in a sample of young men with the same genetic origin as the sample of older men with ECG data. Using the same genetic prediction rule in the same sample of older men, we have previously shown an association of genetically predicted testosterone with lower HDL-cholesterol,²² as would be expected from meta-analysis of RCTs,⁴⁷ which suggests some validity.

Seventh, genetic variants typically have small effects. MR estimates are less precise than conventional regression estimates.⁴⁸ However, MR avoids the confounding which occurs in traditional observational studies.⁴⁹ We used a weighted genetic score instead of one SNP as instrument, thereby reducing variability in MR estimation.⁵⁰ MR studies require large sample sizes: a sample size of ~4000 had 0.8 power to detect a relatively small effect size of 0.22. Meta-analysis combining our findings with previous MR estimates is required, but to our knowledge no other MR study exists.

Eighth, correction for multiple testing might be required. Such correction is widely used in agnostic or ex-

ploratory studies, such as GWAS,⁵¹ to control for type 1 error, whereas it may increase the risk of type 2 error.^{52,53} However, our study aims at confirmation and, given our negative findings, controlling for type 2 error is important from a public health perspective.

Ninth, we cannot determine the clinical significance of the small effect size, because MR estimates should be interpreted as hypothesis testing for causation, rather than indicating the size of causal effects.⁴⁰ However, small effects that may not be clinically significant may still be an important determinant of population health.

Tenth, serum testosterone might not be a good indicator for androgen activity. Successful use of anti-androgens at castrate levels of serum testosterone in prostate cancer trials has challenged the extent to which serum testosterone reflects all androgen activity.⁵⁴ The glucuronide derivatives of androgens, such as androsterone glucuronide or androstenediol glucuronide, are obligatory for elimination of androgens, and provide a measure or correlate of total androgen activity.^{55,56} Observationally, androstenediol glucuronide is poorly correlated with serum testosterone.⁵⁷ Replication using a measure of androgen activity is required.

Our study did not corroborate observations of endogenous testosterone associated with shorter corrected QT interval^{5,7} and slower HR.⁵ Serum testosterone is associated with health status, and falls in response to obesity or ill health.²⁰ Our study is more consistent with small RCTs which generally report no effect on QT interval or HR.¹¹⁻¹³ Notably, androgenic steroid users have also been reported to have prolonged QT interval and potentially higher risk of cardiac arrhythmias,⁵⁸ although the dose is much higher than that in RCTs. Previous MR studies have similarly not corroborated the associations of endogenous testosterone with healthier values of CVD risk factors,^{21,22} but suggest adverse effects on lipids.²² A meta-analysis of RCTs among men suggests testosterone replacement increases the risk of CVD-related events.²⁵

From a public health perspective, the benefits and the CVD safety of androgens have important clinical implications for the increasing numbers, particularly of older men, using testosterone replacement.²⁶ Our novel study adds to the limited evidence concerning testosterone and ECG parameters, with corresponding implications for the role of testosterone in cardiovascular health.

Conclusions

The study did not corroborate observations of an association of higher endogenous testosterone with lower QT interval or slower HR, and correspondingly a potentially protective effect of testosterone. Replication in a larger sample is required.

Supplementary Data

Supplementary data are available at *IJE* online.

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