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**List of abbreviations**

ALDH2: Acetaldehyde dehydrogenase 2

CI: Confidence interval

CVD: Cardiovascular diseases

ECG: Electrocardiogram

GBCS: Guangzhou Biobank Cohort Study

HDL: High density lipoprotein

HEPA: Health-enhancing physical activity

HR: Heart rate

IPAQ: International Physical Activity Questionnaire

MET: Metabolic equivalent

RCT: Randomized controlled trial
Abstract

Western observational studies show moderate alcohol use associated with lower cardiovascular disease risk but these associations may be confounded by the healthier attributes of moderate users in these settings. Mendelian randomization analysis may help ascertain the causal effect of moderate alcohol use on specific factors related to cardiovascular disease and thereby clarify the role of alcohol. The authors used Mendelian randomization analysis with \textit{ALDH2} as an instrumental variable to examine the association of alcohol units (10g ethanol) per day with heart rate(HR)-corrected QT interval and HR assessed from electrocardiogram (ECG) among 4,588 older Southern Chinese men in the Guangzhou Biobank Cohort Study (2003-2008). The F-statistic was 77 for \textit{ALDH2} on alcohol use, suggesting little weak-instrument bias. Instrumental variable analysis showed alcohol units were not associated with corrected QT interval (1.04 milliseconds, 95% confidence interval -0.61, 2.70), but increased HR (0.98 beat per minute, 95% confidence interval 0.04, 1.92). This study suggests moderate alcohol use in men is not beneficial for heart function via QT interval or HR, but could be detrimental. Future studies using specific cardiovascular outcomes may elucidate how alcohol affects different aspects of the cardiovascular system, hence the overall effects of alcohol on cardiovascular disease can be estimated.
Observational studies, mainly concerning Western populations, consistently show moderate alcohol use associated with a lower risk of cardiovascular disease (CVD) (1). However, moderate users in the West tend to be systematically different from other and non-alcohol users with moderate users having more favourable health-related attributes, including healthier lifestyles and higher socioeconomic position, which may generate residual confounding (2). In contrast, in studies from other settings, such as China, where the social patterning of alcohol use is different from in the West, moderate alcohol use is less clearly associated with lower risk of CVD (3, 4). Discrepancies between settings may indicate confounding rather than a biological effect of alcohol or differences in the pattern of CVD. Alcohol may protect against ischemic CVD by raising high density lipoprotein (HDL) cholesterol (5), although randomized controlled trials (RCTs) raising HDL cholesterol have consistently shown no effect on CVD (6-8). In addition, RCTs suggest alcohol could have negative effects on CVD by raising blood pressure (9), which may be particularly relevant in a Chinese setting where hemorrhagic stroke is more common than in Western settings (10, 11). To date, RCTs to investigate the long-term effects of moderate alcohol use on CVD have not been conducted because of ethical and logistic concerns.

Mendelian randomization analysis is increasingly used to assess empirically derived hypotheses particularly where an RCT is infeasible. Mendelian randomization analysis takes advantage of naturally occurring genetically determined differences in exposure (12). As genetic differences are randomly allocated at conception, Mendelian randomization analysis is less susceptible to the confounding which often biases observational studies, but requires stringent assumptions (12). To date, three Mendelian randomization analyses have examined the effect of alcohol use on CVD or its risk factors. One very large study showed that a specific genetic variant in the alcohol
dehydrogenase gene associated with alcohol use was also associated with a higher risk of CVD events. However, the association of the alcohol dehydrogenase gene variant with CVD was evident for alcohol drinkers and for non-drinkers, perhaps because of misclassification of current or former uses as non-drinkers, which might inflate the estimates (13), or because of an effect independent of alcohol and thus casting doubt on the estimate because of the violation of the key “exclusion-restriction” assumption for Mendelian randomization analysis (14, 15). The “exclusion-restriction” assumption states that the instrument (i.e., the genetic variant) should only be associated with the outcome (CVD) via the exposure (in this case alcohol use), but not otherwise. The two smaller studies, both showed, as expected, that alcohol increased HDL cholesterol and blood pressure, but did not provide conclusive information about effects on CVD events (16, 17), so the role of moderate alcohol use in CVD, and the means by which alcohol affects CVD, remains a subject of intense debate highly relevant to public health policy.

In contrast to an RCT, which usually tests whether an intervention works, Mendelian randomization may help elucidate specific mechanistic pathways if variables on the mechanistic pathway and their genetic variants are available. Understanding how alcohol affects CVD is not only important from a policy perspective, but may also provide insight into overlooked harmful and protective risk factors for CVD. Moreover, in an increasingly globalized world where patterns and rates of CVD vary substantially among settings, understanding the mechanisms by which alcohol affects CVD will facilitate generalization across settings with different patterns of risk factors and diseases. Many unexplored pathways exist by which alcohol may affect the risk of CVD. For example, alcohol use may influence the electrical cycle in the heart or heart rate. Alcoholics have longer QT intervals (18), the period between the start of Q wave to the end of T
wave in a heart electrical cycle. Habitual alcohol use is also positively associated with heart rate (HR) (19). Both longer QT intervals and higher HR are associated with higher CVD mortality, providing another potential mechanism by which alcohol use may affect CVD events (20, 21).

Chinese men provide a particularly suitable setting for a Mendelian randomization analysis concerning the effect of moderate alcohol use. Among Chinese men alcohol use is low to moderate and influenced by the aldehyde dehydrogenase 2 (ALDH2) genetic polymorphism (22, 23). People with inactive alleles ALDH2 tend to drink less alcohol because slower acetaldehyde metabolism generates flushing and makes them feel unwell (24). In addition, we have shown that ALDH2 is a credible instrument for Mendelian randomization analysis in southern Chinese men (25). In the present study, we investigated the association of alcohol use with QT interval and HR, measured from an electrocardiogram (ECG), among Southern Chinese men using Mendelian randomization analysis.

METHODS

Ethics statement

The Guangzhou Medical Ethics Committee of the Chinese Medical Association approved the study and all participants gave written, informed consent before participation.

Participants

The Guangzhou Biobank Cohort Study (GBCS) is a collaboration between the Guangzhou No.12 Hospital and the Universities of Hong Kong and Birmingham (26). The participants were recruited from “The Guangzhou Health and Happiness Association for the Respectable Elders”, a
community social and welfare association unofficially aligned with the municipal government where membership is open to anyone aged 50 years or older for a monthly, nominal fee of 4 Yuan (50 US cents). Recruitment for phase 1 took place from September 2003 to November 2004, for phase 2 from April 2005 to May 2006, and for phase 3 from September 2006 to January 2008. Follow-up of the participants started in 2008. Approximately 7% of permanent Guangzhou residents aged 50 years or more are members of “The Guangzhou Health and Happiness Association for the Respectable Elders”, of whom 33% enrolled in all the 3 phases were included if they were capable of consenting, ambulatory, and not receiving treatment modalities that, if omitted, may result in immediate life-threatening risk, such as chemotherapy or radiotherapy for cancer, or dialysis for renal failure. Participants in GBCS are ethnic Chinese largely from southern China. Participants underwent a detailed interview and physical examination at baseline recruitment, including medical history and report of doctor diagnosed conditions. The methods of measurement have previously been reported (26). Alcohol use was recorded in terms of frequency, type of beverage and usual amount per occasion. A standard ECG was performed in the supine position after resting for 5 minutes using a 3-channel, 12-lead ECG (Marquette MAC-500; General Electric, Milwaukee, WI) in phase 1 and at the start of phase 2 and a synchronous 12-lead ECG (Marquette Cam-14 acquisition module; General Electric, Milwaukee, WI) in the rest of phase 2 and in phase 3 (27). The ECG tracings obtained by the Marquette MAC-500 electrocardiograph were evenly distributed to two qualified physicians and measured independently, blinded to other information (28). 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 phase 3 the QT interval and HR were measured automatically by the ECG machine.
DNA extraction and SNP analysis

Biological samples for DNA extraction used in the present study were obtained in GBCS phase 3 at recruitment and in phases 1 and 2 at follow-up. DNA was extracted at Guangzhou No. 12 Hospital either from fresh blood using a standard phenol-chloroform extraction procedure and stored at -80°C or from blood or buffy coat previously stored at -80°C using a standard magnetic bead extraction procedure, and the results of our MR studies on cognition and traditional CVD risk factors have been reported elsewhere (29). Genotyping was performed using the MassARRAY system (Sequenom, San Diego, CA, USA) and the iPLEX assay at a commercial company (Beijing CapitalBio Corporation, Beijing, China).

Instrument

SNP rs671 of ALDH2 was used as the genetic instrument.

Alcohol use

The main exposure was continuous alcohol units (10 gram (g) ethanol per day) based on total alcohol consumption obtained from the frequency, quantity and type recorded at recruitment, as previously (29). Specifically, we asked the participants how often they drank alcohol (once or twice per year, once every couple of months, <1 day/week, 1-2 days/week, 3-4 days/week, 5-6 days/week, daily or almost every day), the type of alcohol usually consumed, and how much of each type of alcohol (beer, western table wine, spirits, Chinese rice wine or Chinese rice wine (high strength)) usually consumed per occasion, from which we calculated units per day. Infeasible amounts (>30 alcohol units per day) were excluded (29). Former alcohol users were included as non-drinkers because former alcohol users may have abstained from alcohol because
of poor health unrelated to former alcohol use; excluding them could create a bias. Many former
users (58%) reported previously infrequent alcohol use, i.e., once or twice a year.

Outcomes
The outcomes were heart rate corrected QT interval, uncorrected QT interval, and HR. The QT
correction was made using the Framingham formula (corrected QT interval = uncorrected QT
+154x(1-HR)) (30).

Statistical analysis
We tested for Hardy-Weinberg equilibrium at the SNP locus on a contingency table of observed-
versus-predicted frequencies with an exact test. We used analysis of variance to assess the
associations of ALDH2 genotypes with alcohol consumption. We used chi-square tests to assess
whether ALDH2 genotypes were associated with potential confounders, including socioeconomic
position and lifestyle. To test the assumption that ALDH2 is only associated with the outcomes
via alcohol use (exclusion-restriction assumption), we used multivariable linear regression to
assess the adjusted association of ALDH2 with QT interval and HR in men who never used
alcohol. We adjusted for age, socio-economic position and lifestyle to control for potential
collider bias upon restriction on alcohol use status (31).

We implemented Mendelian randomization as instrumental variable analysis with genetic
instruments using 2 stage least square. Two stage least square first predicts the exposure from the
instrumental variable, from which we reported the F-statistic for ALDH2 on alcohol use, and the
second stage estimates the association of predicted exposure with the outcome. An F-statistic of
<10 indicates weak-instrument bias. We used instrumental variable analysis (2 stage least square) with $ALDH2$ genotype categories as an instrumental variable for alcohol units, because there was a non-linear association with alcohol consumption. We did not adjust for confounders in the instrumental variable analysis because $ALDH2$ genotypes randomly allocated at conception cannot be confounded by age or subsequent socio-economic position and lifestyle. For comparison, we also present the adjusted associations of alcohol units with corrected QT interval, uncorrected QT interval and HR under multivariable linear regression models in an observational design adjusted for potential confounders, i.e., age, education, physical activity, smoking status, tea consumption; tea consumption may be related to alcohol consumption and CVD (32).

Sensitivity analyses

In sensitivity analysis we excluded men with a major intraventricular conduction defect, as indicated by $\text{QRS} \geq 120$ milliseconds (ms) from corrected QT-interval, because the repolarization abnormalities in these men could be secondary to the conduction defects. We also excluded former alcohol users in a sensitivity analysis. To account for any potential U shaped relation of alcohol use with QT interval or HR, we excluded heavy alcohol users (weekly drinking of $>210g$ ethanol/week).

All statistical analyses were conducted using Stata version 13.1 (StataCorp LP, College Station, TX).

RESULTS

Of the 5,030 men with viable DNA, 4,588 had complete information on $ALDH2$ genotypes,
alcohol use and QT interval or HR. QT interval has a mean of 387ms with a standard deviation of 28ms. Heart rate had a mean of 71bpm with a standard deviation of 11bpm. Table 1 shows that men with two active ALDH2 alleles on average consumed more than 10 times as much alcohol per day (0.9 unit) as men with two inactive alleles (0.07 unit). ALDH2 satisfied the assumptions for being a credible instrument for alcohol use, including an association with alcohol use (F-statistic 77) (Table 1), little association with potential confounders (Table 1), and no association with corrected QT interval, uncorrected QT-interval, or HR in never users (Table 2). ALDH2 genotypes had the distribution consistent with Hardy Weinberg Equilibrium (p=0.75).

In the instrumental variable analysis, an increase of 1 unit of alcohol was not associated with QT interval but was associated with an increase in HR in the entire sample (Table 3). Similar results were seen in the multivariable linear regression but the estimates were smaller and the confidence intervals narrower.

In sensitivity analysis (Table 4), alcohol use was associated with higher corrected QT interval after excluding men with QRS≥120 ms. However, in the sensitivity analyses excluding former alcohol users or heavy alcohol users or both former and heavy alcohol users, alcohol use remained unassociated with QT interval in any form (uncorrected, corrected, or corrected excluding QRS≥120 ms) in the instrumental variable analysis and the observational analysis. In the sensitivity analysis excluding former users or heavy users did not change the direction of the association of alcohol use with HR in the instrumental variable analysis, but all the confidence intervals included no effect. In the sensitivity analysis excluding specifically heavy alcohol users
from the observational analysis also attenuated the association of alcohol use with HR from positive to the null.

DISCUSSION

This study is the first study to examine the association of alcohol use with QT interval and HR using Mendelian randomization analysis. It also takes advantage of an understudied population where alcohol use is mainly low to moderate. Consistent with previous studies, we found alcohol use was not clearly associated with shorter QT interval but associated with higher HR (19, 33). This study adds by showing that low to moderate alcohol use is not associated with shorter QT interval in a design better suited to establishing causality, and hence any potential beneficial effects of alcohol are unlikely to be mediated by effects on the electrical cycle in the heart. On the other hand, this study cannot rule out the possibility that alcohol lengthens corrected QT interval and suggests that alcohol increasing HR could be a potential pathway by which alcohol has effects on CVD that are not beneficial.

Low to moderate alcohol use is consistently associated with lower risk of CVD in observational studies (1), with the mechanisms thought to include increasing HDL cholesterol, increasing adiponectin and decreasing fibrinogen. However, the causal role of these mechanisms in protecting against CVD remains to be established. A meta-analysis of several HDL modifying drugs showed no effect on CVD against a background of statin treatment (8), whereas genetic markers associated with adiponectin, not fibrinogen, are associated with CVD (34, 35). Conversely, alcohol increases blood pressure but blood pressure does not necessarily increase HR (16). A biologically plausible explanation strengthens a theory but does not confirm or refute.
For example, the observed U shaped relation between alcohol use and CVD could indicate confounding by healthier attributes of moderate users than non-users and other alcohol users, which cannot be completely adjusted for in statistical analyses (2). Our study is consistent with this argument given the association of alcohol use with QT interval and HR did not suggest benefit, but possibly harm, for CVD. We have previously examined the association of the same exposure with cardiovascular disease and its risk factors (16), and cognitive function (29). So, we cannot rule out the possibility of false positives, due to multiple comparisons, concerning alcohol use and heart rate, albeit the association is biologically plausible. Hence, these associations should be verified in future studies. Other than confounding, methodological issues may also bias observational estimates in favour of moderate alcohol use. The recent European Prospective Investigation into Cancer and Nutrition (EPIC) study suggested that the association of alcohol use with health may be an artifact of selection bias and competing risks (36).

Excluding former users did not substantially change the estimate, suggesting little bias introduced by classifying formers users as non-drinkers although alcohol use was associated with higher HR in the observational analysis. Excluding heavy users made the estimates larger, possibly because of the narrower range of mean alcohol units by ALDH2 genotype. Nevertheless, the direction of the estimate remained the same. Exclusion of men with QRS≥120ms also did not change the direction of the estimates, suggesting the absence of potential cardio-protection via QT interval was not due to inclusion of men with wide QRS, which can also increase QT interval but is related to impaired ventricular conduction (37). The positive association of alcohol use with corrected QT interval was only evident after excluding men with QRS≥120ms (Table 4)
suggesting alcohol use may act via prolonging QT interval rather generating a major intraventricular conduction defect.

Although we used a Mendelian randomization analysis which is less susceptible to confounding, limitations exist. First, alcohol use was self-reported but we previously observed the known effects of alcohol use, i.e., higher HDL cholesterol and blood pressure (38). Although Mendelian randomization analysis can better capture the lifetime effect of alcohol use and the outcomes concerned (39), the use of only current alcohol use in our data may inflate the Mendelian randomization estimates (13). Second, we did not use CVD mortality because too few events have occurred for a meaningful Mendelian randomization analysis. Instead, this study focused on potential pathways by which alcohol may affect CVD, i.e. via QT interval or HR. However, our study cannot rule out the possibility that moderate alcohol use could still overall be protective for CVD via other pathways not examined in this study, although our previous, albeit underpowered, Mendelian randomization study, found no association of alcohol use with self-reported CVD (16). Third, GBCS is not population-representative, which would affect internal validity only if recruitment of participants had generated selection bias, which is unlikely (40). On the other hand, GBCS participants are mostly ethnically homogeneous Chinese, making confounding by population stratification very unlikely (26). Fourth, we did not explicitly consider non-linear effects, although this possible with in a Mendelian randomization design (41). We would expect causal factors to have a dose response effect on discrete aspects of cardiac function unless other moderating factors were involved. In contrast, the many factors affected by alcohol and involved in CVD might generate a U-shaped relation for a multifactorial disease, such as CVD, where consideration of non-linear effects of alcohol might be more appropriate. Lastly, the Mendelian
randomization analyses could be underpowered, as indicated by the wide confidence intervals, so our interpretation is cautious for QT interval. However, this study may contribute to future meta-analyses examining similar questions. Mendelian randomization relies on assumptions for credible estimates. Although violation of the exclusion restriction assumption was not evident (Table 2) estimates in non-drinkers were not exactly null, possibly due to misclassification of alcohol use and/or residual collider bias. On the other hand, although genes are usually not related to the confounders which commonly confound the association of alcohol use with cardiovascular disease, we cannot rule out the possibility that parental *ALDH2* may be an unmeasured confounder if it also affected QT interval and heart rate via a pro-drinking environment related to parental *ALDH2*. However, alcohol use was low in China during 1950s and 1960s, hence most people would have grown up in a non-drinking household, making confounding by parental *ALDH2* unlikely (42).

This Mendelian randomization analysis suggests low to moderate alcohol use does not have a beneficial effect on some potential mediators linking alcohol use with lower CVD, but might be harmful for QT interval and HR. Previous studies in the West showing an inverse association of moderate alcohol use with CVD may be due to confounding by unmeasured healthier attributes of moderate users. However, the cardioprotective effect of alcohol could also be mediated via other pathways not examined here. Future Mendelian randomization analyses, with credible genetic instruments, examining the mechanism by which alcohol affects cardiovascular events, are needed to elucidate the overall effect of alcohol use on cardiovascular health across the globe.

REFERENCES


Table 1: Alcohol Consumption and Socio-demographic Characteristics by *ALDH2* Genotype Among 4,588 Men From The Guangzhou Biobank Cohort Study (2003-2008)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Two inactive alleles (AA) n=394 (%)</th>
<th>One inactive allele (GA) n=1,917 (%)</th>
<th>No inactive alleles (GG) n=2,277 (%)</th>
<th>(^a P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol units (10g ethanol per day)</td>
<td>0.07 (0.70)</td>
<td>0.24 (1.16)</td>
<td>0.92 (2.52)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age group (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50-54</td>
<td>11.2</td>
<td>10.1</td>
<td>9.2</td>
<td></td>
</tr>
<tr>
<td>55-59</td>
<td>20.3</td>
<td>21.2</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>60-64</td>
<td>25.1</td>
<td>23.7</td>
<td>26.5</td>
<td></td>
</tr>
<tr>
<td>65-69</td>
<td>20.1</td>
<td>23.3</td>
<td>23.2</td>
<td>0.23</td>
</tr>
<tr>
<td>70-74</td>
<td>17</td>
<td>15.8</td>
<td>14.9</td>
<td></td>
</tr>
<tr>
<td>75-79</td>
<td>5.8</td>
<td>4.5</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>80+</td>
<td>0.5</td>
<td>1.5</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than primary</td>
<td>2.8</td>
<td>2.4</td>
<td>2.3</td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>25.4</td>
<td>27.2</td>
<td>25.8</td>
<td></td>
</tr>
<tr>
<td>Junior middle</td>
<td>30</td>
<td>30.4</td>
<td>30.8</td>
<td></td>
</tr>
<tr>
<td>Senior middle</td>
<td>26.4</td>
<td>25.3</td>
<td>24</td>
<td>0.68</td>
</tr>
<tr>
<td>Junior college</td>
<td>9.4</td>
<td>8.5</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>College</td>
<td>6.1</td>
<td>6.3</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>41.4</td>
<td>40.4</td>
<td>40.1</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>29.7</td>
<td>27.3</td>
<td>27.4</td>
<td>0.69</td>
</tr>
<tr>
<td>Current</td>
<td>28.9</td>
<td>32.2</td>
<td>32.5</td>
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<tr>
<td>Physical activity (IPAQ)</td>
<td></td>
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<tr>
<td>Inactive</td>
<td>8.6</td>
<td>7.6</td>
<td>7.4</td>
<td></td>
</tr>
<tr>
<td>Minimally active</td>
<td>35.8</td>
<td>38.6</td>
<td>41.8</td>
<td>0.1</td>
</tr>
<tr>
<td>HEPA active</td>
<td>55.6</td>
<td>53.8</td>
<td>50.8</td>
<td></td>
</tr>
<tr>
<td>Tea use</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(^c)Never</td>
<td>23.6</td>
<td>26.8</td>
<td>28.9</td>
<td>0.06</td>
</tr>
<tr>
<td>Current</td>
<td>76.4</td>
<td>73.2</td>
<td>71.2</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) P-value from Analysis of variance for continuous variables and from a \(\chi^2\) test for categorical variables, 2 sided  
\(^b\) Presented as mean (standard deviation)  
\(^c\) Never use includes never, occasional and former users  
ALDH2: Aldehyde dehydrogenase 2; HEPA: health-enhancing physical activity (i.e., vigorous activity at least 3 days a week achieving at least 1,500 metabolic equivalent (MET) minutes per week or activity on 7 days of the week, achieving at least 3,000 MET minutes per week; IPAQ: International Physical Activity Questionnaire
Table 2: Adjusted association of *ALDH2* Genotype with Corrected QT Interval, Uncorrected QT Interval, and Heart Rate Among 2,326 Men Never Alcohol Users From the Guangzhou Biobank Cohort Study (2003-2008)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>ALDH2 genotype</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA (n=305)</td>
<td>GA (n=1,113)</td>
<td>GG (n=908)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected QT interval (ms)</td>
<td>β</td>
<td>95% CI</td>
<td>β</td>
<td>95% CI</td>
<td>β</td>
<td>95% CI</td>
</tr>
<tr>
<td>Ref</td>
<td>-</td>
<td>-0.71, 1.91</td>
<td>-0.49, 2.21</td>
<td>0.49, 3.18</td>
<td>0.49, 3.18</td>
<td>0.85</td>
</tr>
<tr>
<td>Uncorrected QT interval (ms)</td>
<td>Ref</td>
<td>-0.59, 3.09</td>
<td>-1.14, 2.66</td>
<td>1.14, 4.93</td>
<td>1.14, 4.93</td>
<td>0.53</td>
</tr>
<tr>
<td>Heart rate (beats per minute)</td>
<td>Ref</td>
<td>0.15, 1.63</td>
<td>0.65, 2.18</td>
<td>0.65, 2.18</td>
<td>0.65, 2.18</td>
<td>0.31</td>
</tr>
</tbody>
</table>

*a*Adjusted for age, education, physical activity, smoking, and tea consumption

*ALDH2*: Aldehyde dehydrogenase 2; CI: Confidence Interval
Table 3: Associations of One Alcohol Unit (10g Ethanol) per Day With Corrected QT Interval, uncorrected QT interval and Heart rate Using a Mendelian Randomization Design and an Observational Multivariable Linear Regression Analysis Among 4,588 Men From the Guangzhou Biobank Cohort Study (2003-2008)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Mendelian randomization</th>
<th>Instrumental variable analysis</th>
<th>Observational</th>
<th>Multivariable regression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>95% CI</td>
<td>β</td>
<td>95% CI</td>
</tr>
<tr>
<td>Corrected QT interval (ms)</td>
<td>1.04</td>
<td>-0.61, 2.70</td>
<td>0.27</td>
<td>-0.03, 0.57</td>
</tr>
<tr>
<td>Uncorrected QT interval (ms)</td>
<td>-0.71</td>
<td>-3.00, 1.59</td>
<td>-0.33</td>
<td>-0.75, 0.09</td>
</tr>
<tr>
<td>Heart rate (beats per minute)</td>
<td>0.98</td>
<td>0.04, 1.92</td>
<td>0.33</td>
<td>0.16, 0.50</td>
</tr>
</tbody>
</table>

aF statistic was 77.4
bAdjusted for age, education, physical activity, smoking, and tea consumption
CI: Confidence Interval
Table 4: Associations of Alcohol With Corrected QT Interval, Uncorrected QT interval, and Heart rate Using a Mendelian Randomization Design and an Observational Multivariable Linear Regression Analysis Among Men From the Guangzhou Biobank Cohort Study (2003-2008), Excluding QRS≥120ms, Excluding Heavy Alcohol Users or Excluding Former Alcohol Users

<table>
<thead>
<tr>
<th>Selection and outcome</th>
<th>n</th>
<th>F statistic</th>
<th>β</th>
<th>95% CI</th>
<th>β</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>4,401</td>
<td>78.3</td>
<td>1.67</td>
<td>0.06, 3.28</td>
<td>0.13</td>
<td>-0.17, 0.43</td>
</tr>
<tr>
<td>Corrected QT interval (ms), QRS&lt;120ms</td>
<td>4,303</td>
<td>67.6</td>
<td>3.14</td>
<td>-4.10, 10.4</td>
<td>-0.14</td>
<td>-1.40, 1.13</td>
</tr>
<tr>
<td>Uncorrected QT interval (ms)</td>
<td>4,303</td>
<td>67.6</td>
<td>-2.35</td>
<td>-12.4, 7.71</td>
<td>-0.05</td>
<td>-1.61, 1.71</td>
</tr>
<tr>
<td>Corrected QT interval (ms), QRS&lt;120ms</td>
<td>4,125</td>
<td>64.7</td>
<td>5.19</td>
<td>-1.90, 12.3</td>
<td>-0.27</td>
<td>-1.50, 0.96</td>
</tr>
<tr>
<td>Heart rate (beats per minute)</td>
<td>4,303</td>
<td>67.6</td>
<td>3.27</td>
<td>-0.84, 7.39</td>
<td>-0.05</td>
<td>-0.76, 0.66</td>
</tr>
</tbody>
</table>

Excluding heavy drinkers

<table>
<thead>
<tr>
<th>Selection and outcome</th>
<th>n</th>
<th>F statistic</th>
<th>β</th>
<th>95% CI</th>
<th>β</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected QT interval (ms)</td>
<td>4,314</td>
<td>81.8</td>
<td>0.81</td>
<td>-0.75, 2.38</td>
<td>0.29</td>
<td>-0.02, 0.59</td>
</tr>
<tr>
<td>Uncorrected QT interval (ms)</td>
<td>4,314</td>
<td>81.8</td>
<td>-0.55</td>
<td>-2.72, 1.62</td>
<td>-0.35</td>
<td>-0.77, 0.07</td>
</tr>
<tr>
<td>Corrected QT interval (ms), QRS&lt;120ms</td>
<td>4,145</td>
<td>82.4</td>
<td>1.46</td>
<td>-0.06, 2.99</td>
<td>0.14</td>
<td>-0.16, 0.44</td>
</tr>
<tr>
<td>Heart rate (beats per minute)</td>
<td>4,314</td>
<td>81.8</td>
<td>0.76</td>
<td>-0.12, 1.65</td>
<td>0.35</td>
<td>0.18, 0.52</td>
</tr>
</tbody>
</table>

Excluding former drinkers

<table>
<thead>
<tr>
<th>Selection and outcome</th>
<th>n</th>
<th>F statistic</th>
<th>β</th>
<th>95% CI</th>
<th>β</th>
<th>95% CI</th>
</tr>
</thead>
</table>

aAdjusted for age, education, physical activity, smoking, and tea consumption
bBased on 10g ethanol per day
CI: Confidence Interval; QRS: QRS complex