Clinical Epigenetics

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Dissecting the causal effects of smoking, alcohol consumption, and related DNA methylation markers on electrocardiographic indices

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Abstract

Background Tobacco and alcohol are recognized risk factors for heart disease, yet their causal effects on electrocardiogram (ECG) signaling and mechanisms remain unclear. Previous studies may be susceptible to confounding or bias, and this study dissected the genetic architecture linking tobacco and alcohol consumption with P-wave duration, PR interval, and QT interval.

Methods Utilizing genetic instruments for tobacco and alcohol consumption, associated methylation quantitative trait locus (mQTL), and summary-level GWAS data for ECG indices, we assessed heritability and genetic causal associations using linkage disequilibrium score regression and Mendelian randomization (MR) analysis. Fine mapping was performed via colocalization analysis and summary-data-based MR (SMR) to identify potential shared genetic variants.

Results A positive causal relationship was found between drinks per week (DrnkWk) and QT interval [β (95%CI): 1.06 (0.91, 5.05), $P=0.005$], with causality substantiated through multiple robust MR models. Multivariable MR confirmed independence from smoking phenotypes. In epigenetic MR analyses, two alcohol-related CpG loci (cg03345232 and cg04605617) were causally associated with QT interval changes, with cg04605617 mapping to *PLA2G2C* gene significantly prolonging QT. The mQTL rs10916683 at cg04605617 is a strong eQTL for *PLA2G2C*. Additionally, cg03345232 shared a causal variant (rs12881206) with QT interval predisposition through colocalization analysis. SMR analysis did not identify shared putative functional genes passing the HEIDI test between DrnkWk and the QT interval.

Conclusions There is a causal relationship between DrnkWk and QT interval prolongation, and targeting specific DNA methylation sites like cg04605617 mapped to *PLA2G2C* may provide novel targets for preventing QT interval prolongation.

Keywords Alcohol consumption, QT interval, Genetic architecture, DNA methylation, Mendelian randomization

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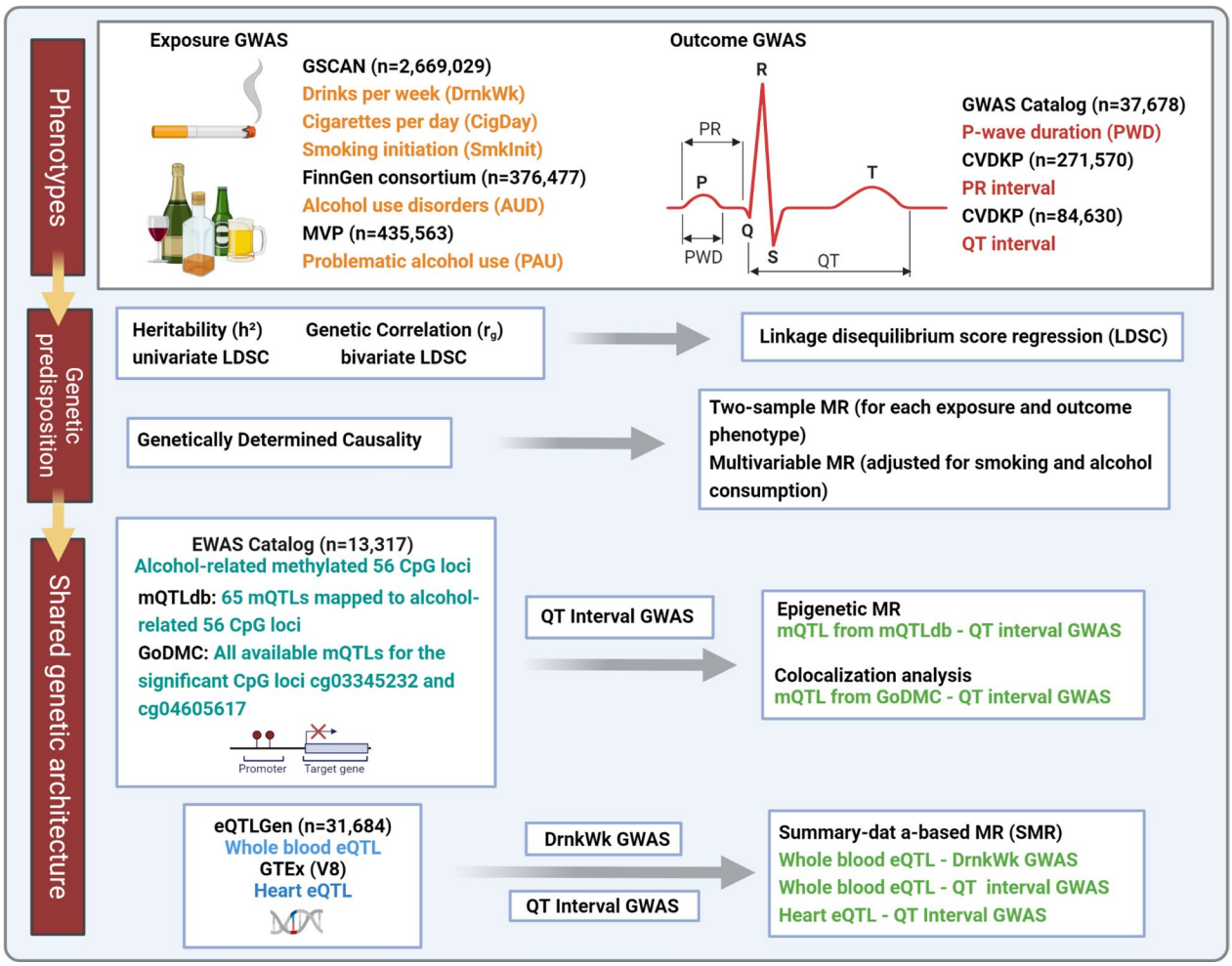
Graphic abstract



Introduction

Non-invasive indices derived from the electrocardiogram (ECG), such as P-wave duration, PR interval, and QT interval, play a pivotal role in providing insights into cardiac structural and electrical conduction abnormalities [1–3]. These indices are closely associated with the risk of atrial and ventricular arrhythmias, with prolonged QT intervals linked to ventricular arrhythmias, particularly torsade de pointes (TdP) [4–9]. The clinical significance of these ECG parameters lies in their causal impact on conditions such as atrial fibrillation and sudden cardiac death (SCD) [10, 11].

Observational studies reveal that ECG signals are influenced by various pathophysiological factors, such as age, gender, genetics, medications, electrolytes, hormones, and the autonomic nervous system [12, 13]. Underlying genetic and environmental determinants, such as poor lifestyle choices, possess the potential to disturb normal cardiac rhythms by modifying these pivotal factors that contribute to alterations in ECG indices [14]. Large cohort studies suggest that the amalgamation of genetic composition and collective health behaviors and factors exhibits a log-additive impact on the susceptibility to cardiovascular disease [15, 16]. The investigation of the causal association between these underlying factors and ECG signals prompts exploration into the impact of two prevalent adverse lifestyles, smoking and alcohol consumption, on ECG indices.

Smoking and alcohol use, global modifiable risk factors, exhibit intricate connections with cardiovascular disease. Both tobacco use and alcohol consumption, whether acute or chronic, are associated with arrhythmias

[17–22]. Alcohol overdose is frequently associated with "holiday heart syndrome," characterized by a heart block PR and a prolonged QT interval [23–25]. Notably, epigenome-wide association studies (EWAS) show the effects of smoking and alcohol on DNA methylation, a regulatory mechanism influencing gene expression and potentially regulating cardiac ion channel activity [26–28]. However, the extent to which such epigenetic changes mediate the response of electrocardiographic signaling molecules to smoking and alcohol consumption, thereby affecting the normal regulation of cardiac rhythm, remains unclear. Additionally, establishing causal associations in observational studies is inherently challenging due to confounding bias, necessitating causal inference, and genetic architecture investigation.

Here, using large-scale genome-wide association studies (GWAS) and EWAS summary statistics, we sought to explore genetic correlations, causality, and shared risk loci with putative functions between tobacco and alcohol consumption and ECG indices, unraveling the underlying genetic association.

Method

Study design

This study aimed to investigate the genetic architecture between smoking and drinking exposure and ECG parameters. Five different smoking and drinking phenotypes were used as exposure factors, encompassing three distinct types of alcohol consumption behavior: drinks per week (DrnkWk, indicating the average number of drinks reported by each participant per week), alcohol use disorders (AUD, a binary phenotype representing a clinical diagnosis of chronic alcohol dependence), and problematic alcohol use (PAU, a broader phenotype incorporating both clinical and subclinical patterns of alcohol misuse), and two different stages of tobacco use: cigarettes per day (CigDay, a continuous measure reflecting the average number of cigarettes smoked daily), smoking initiation (SmkInit, a binary phenotype denoting whether an individual has ever smoked, serving as a proxy for early-life smoking behavior and lifetime exposure risk). The ECG markers, specifically P-wave duration (PWD), PR, and QT interval durations, were utilized as outcome measures. PWD, PR, and QT intervals were chosen due to their established roles in arrhythmic risk stratification and their potential modulation by genetic and environmental factors. Prolonged QT intervals, in particular, are linked to ventricular arrhythmias and SCD. Other ECG parameters, such as QRS duration, were not included due to the limited availability of GWAS data with sufficient statistical power, specifically within European populations. We used linkage disequilibrium score regression (LDSC) to estimate the heritability and genetic correlations between exposures and outcomes [29]. LDSC provides an initial assessment of shared genetic background between smoking, alcohol consumption, and ECG traits. A genetic correlation would suggest common genetic influences but does not establish causality. Two-sample MR (TSMR) was used to provide genetically causal evidence in two independent, non-overlapping populations, and multivariable MR (MVMR) was applied to address crosstalk genetic effects between smoking and drinking phenotypes, given genetic correction between the two ($|rg|=0.16–0.27$) [30–32]. To identify whether DNA methylation (mQTL) mediates the observed causal relationships, we conducted epigenetic methylation MR. Colocalization analysis was then used to determine whether the same genetic variant drives both methylation changes and ECG alterations, strengthening the mechanistic interpretation of the findings.[33]. Finally, we adopted summary-data-based MR (SMR) to investigate shared gene expression pathways using eQTL from whole blood and cardiac tissue [34].

GWAS summary statistics sources

We obtained GWAS summary statistics of smoking (CigDay and SmkInit) and drinking (DrnkWk) from the GWAS & Sequencing Consortium of Alcohol and Nicotine Use (GSCAN) database [35]. GSCAN Phase 2 integrated data from 60 GWAS involving up to 3.4 million participants from four major ancestries, focusing on nicotine and substance use [30]. To ensure genetic homogeneity, we extracted stratified GWAS data for European populations ($n=2{,}669{,}029$) stratified without United Kingdom Biobank (UKB). Additionally, GWAS in the FinnGen consortium for AUD (diagnostic codes: ICD-10 F10.2 or F10.3, 376,477 individuals) and the Million Veteran Program (MVP) GWAS meta-analysis for PAU (435,563 individuals) were used [36]. Summary statistics for PR and QT intervals were obtained from the Cardiovascular Disease Knowledge Portal (CVDKP). The CVDKP database curates a multi-ancestry GWAS dataset of 40 studies for the PR interval, with 271,570 Europeans accounting for 92.6% of the total participants [37]. The QT interval GWAS is a large-scale meta-analysis of 84,630 participants from the UKB [38]. PWD GWAS was derived from a recently published GWAS study (GCST004826) involving 37,678 Europeans, available in the NHGRI-EBI GWAS Catalog [39] (Table 1). Measurements of these parameters in milliseconds (ms) were obtained from resting supine 12-lead or 3-lead ECGs taken in the first 15 s before exercise testing on a bicycle. Heart rate-corrected QT (QTc) intervals were calculated using the Bazett formula, defined as $\mathrm{QTc} = \frac{\mathrm{QT(ms)}}{\sqrt{\mathrm{RR}\ (s)}}$[38]. More detailed recordings of ECGs and covariate adjustment for reliable results analysis can be found in the

Table 1 Information for GWAS summary statistics

Exposure	Samples	Source	Ancestry
Drinks per week (DrnkWk)	2,669,029	GSCAN	European
Cigarettes per day (CigDay)	2,669,029	GSCAN	European
Smoking initiation (SmkInit)	2,669,029	GSCAN	European
Alcohol use disorders (AUD)	376,477	FinnGen consortium	European
Problematic alcohol use (PAU)	435,563	MVP	European
Outcome			
P-wave duration (PWD)	37,678	NHGRI-EBI GWAS Catalog	European
PR interval	271,570	CVDKP	European
QT interval	84,630	CVDKP	European

GSCAN, GWAS & Sequencing Consortium of Alcohol and Nicotine Use; *MVP* Million Veteran Program, *CVDKP* Cardiovascular Disease Knowledge Portal

original studies [37–39]. All studies, including the FinnGen consortium, have their study protocols and have been approved by local ethics committees.

Genetic proxies for alcohol and tobacco use

Genetic variants used as instrumental variables (IVs) meet the core assumptions of MR (Fig. 1). To serve as genetic proxies for smoking and alcohol use, single nucleotide polymorphisms (SNPs) needed to exhibit genome-wide significance (GWS) of $P<5\times10^{-8}$ from each exposure summary-level dataset. Extracted SNP data were subsequently subjected to linkage disequilibrium (LD) clumping ($r^2=0.001$, kb = 10,000) to ensure genetic independence. Palindromic variants were excluded if they had intermediate allele frequencies that hindered the inference of positive-strand alleles. The F-statistic greater than 10 was used to exclude weak instrumental bias [40].

Genetic instruments for alcohol-related DNA methylation

We obtained whole-blood DNA methylation patterns associated with alcohol consumption from an

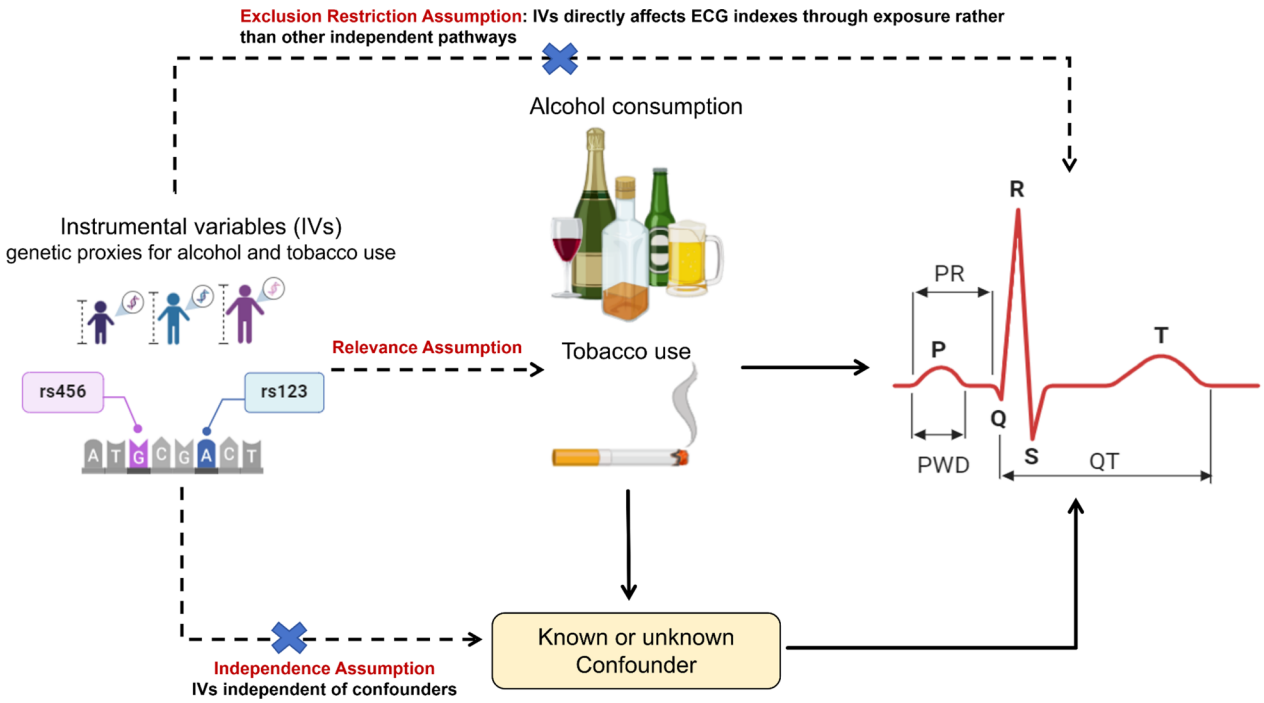


Fig. 1 Core assumptions of MR using genetic proxies for alcohol and tobacco use. The relevance assumption ensures IVs are strongly associated with the exposures. The independence assumption indicates IVs are independent of confounders. The exclusion restriction assumption ensures IVs affect ECG indices only through alcohol and tobacco use, not other pathways

epigenome-wide association study (EWAS) with a meta-analysis of 13 populations (13,317 individuals) in the MRC-IEU EWAS Catalog [41]. The Infinium HumanMethylation450 BeadChip was employed to measure DNA methylation levels in blood samples, and an EWAS model was constructed using DNA methylation β value (the ratio of methylated probe intensities divided by the sum of methylated and non-methylated probe intensities) as the outcome variable and for multiple covariates (e.g., age, sex, BMI, smoking, batch effect, and white cell composition). A total of 363 CpG loci in 9643 Europeans were identified as being associated with alcohol consumption within the Bonferroni-adjusted threshold of epigenomic significance at $P<1\times10^{-7}$ (0.05/440,000). We then derived mQTL robustly associated with these CpG loci based on the Accessible Resource for Integrated Epigenomic Studies (ARIES) project database (mQTLdb). The ARIES initiative utilized the Illumina Infinium HumanMethylation450 (HM450) BeadChip for epigenetic data and the Illumina Infinium Human Hap550/660-w quad SNP genotyping platform for genetic data in 1018 mother–offspring pairs from the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort. Matrix eQTL software conducted preliminary SNP-CpG association analysis, followed by exact linear regression and genome-wide complex trait analysis (GCTA) for significantly independent mQTLs related to each CpG site. Identified mQTLs at middle-age time points were further analyzed for subsequent methylation MR.

Statistical analyses

Heritability and genetic correlation

LDSC methods have proven effective in estimating genetic correlations across multiple traits or diseases [32]. We used the ldscr package to assess single-trait SNP heritability (h^2) and cross-trait genetic correlations (r_g), including all exposures and outcomes. The analysis began with preparing and cleaning GWAS summary statistics for each trait. LD scores were computed using reference panel data to quantify the extent of LD across the genome. SNP-based heritability was then calculated using a univariate LDSC model to estimate the proportion of phenotypic variance explained by the SNPs. For cross-trait genetic correlations, a bivariate LDSC model was employed, leaving the intercept unconstrained to account for potential confounders such as population stratification. The genetic correlations (r_g) between different phenotypes were estimated by fitting the bivariate LDSC model to the GWAS summary statistics of the exposures and outcomes. The estimated r_g values were interpreted to understand the genetic overlap between different traits.

Mendelian randomization

TwoSampleMR package (version 0.5.7) and MR pleiotropy residual sum and outlier (MR-PRESSO) package for TSMR and MVMR, respectively, allowed us to assess causality through a variety of models and to perform sensitivity analyses on the results [31]. The analysis relies on the inverse variance weighted (IVW) method for unbiased and effective causal estimates. This method assumes the validity of all SNPs as IVs and estimates the causal effect by regressing the SNP-ECG measure on the SNP-alcohol and tobacco effect sizes, weighted by the inverse of the SNP-ECG marker standard error. Robust analyses were implemented using the MR-Egger regression, weighted median models, and the radial MR framework [42]. Radial MR, particularly leveraging a Cochran's Q-statistic-based weighted regression method, generates radial plots to transform data, providing immediate visual diagnostics and robust weighting that can detect subtle outlier effects often overlooked by traditional statistical methods [42]. Potential directional horizontal pleiotropy was addressed using the Egger intercept test and the MR-Robust Adjusted Profile Score (MR-RAPS) [43]. MR-RAPS generated a consistent and asymptotically normal estimate by adjusting for profile scores, with ANOVA results ($P>0.05$), confirming that IVs weights were independent of regression residuals. This suggests effective control of potential pleiotropy, thereby enhancing the reliability of causal effect estimation. Furthermore, additional sensitivity analyses, including heterogeneity assessments and leave-one-out analysis, were performed to ensure the robustness of the findings. When evaluating the causal effects of DNA methylation at alcohol-related CpG sites, the method varies based on the number of associated mQTLs. For CpG sites linked to a single mQTL, only the Wald ratio and its corresponding SE are computed. In contrast, if a CpG site is associated with multiple independent mQTLs, the aforementioned IVW method is applied. To address multiple tests, we employed Bonferroni correction.

Fine mapping under a single causal variant assumption

For those individual CpG loci convincingly associated with ECG marker changes (Bonferroni corrected $P<0.05$/number of CpG loci), we performed an approximate Bayes factor colocalization analysis to finely map each feature under a single causal variant assumption. All available mQTLs for the CpG loci of interest were obtained from the Genetics of DNA Methylation Consortium (GoDMC) [44], which includes a diverse range of 36 cohorts from various studies and populations, such as ALSPAC, Brisbane Systems Genetics Study (BSGS), and GSK, providing a valuable resource for examining

genetic influences on DNA methylation. These mQTL data were then integrated with the GWAS summary data. Colocalization analyses using the "coloc" R package were designed to investigate whether the association of these CpG locus-associated mQTL with ECG marker change was driven by shared causal variation. This analysis tested the posterior probability of five hypotheses: H0 (no causally variable loci), H1 (only trait 1 has causally variable loci), H2 (only trait 2 has causally variable loci), H3 (each of the two traits has a different causally variable locus), and H4 (the two traits share a causally variable locus). The results provide the Bayes factor and posterior probability for each SNP, and we considered an H4 posterior probability of 95% or higher for a single mQTL as evidence of colocalization.

SMR

SMR is a robust methodology for investigating the shared genetic basis between traits using summary statistics from GWAS and QTL [34]. We used GWAS and eQTL data to detect associations between trait-associated SNPs and gene expression and applied the heterogeneity in dependent instrument (HEIDI) test to identify potential horizontal pleiotropy in causal associations. Whole-blood eQTL gene expression was obtained from cis-eQTL summary data from eQTLGen Consortium, while heart left ventricle eQTL data were from the GTEx project (v8, only SNPs with $P < 1 \times 10^{-5}$ are included). We utilized SMR software (version 1.3.1) with default parameters, configuring the cis window to 1 Mb, the MAF threshold to 0.01, and requiring at least one cis-eQTL at $P < 5 \times 10^{-8}$. The hg19 reference genome and Ensembl were used for gene annotation. Significant SMR probes were identified using Bonferroni-corrected thresholds (0.05/number of probes), and the HEIDI test P value thresholds > 0.05 were considered indicative of a lack of heterogeneity.

Result

Genetic risk effects of drinking, smoking, and ECG indices

We assessed the heritability and genetic associations of smoking, alcohol consumption, and ECG indices using univariate and bivariate LDSC. The phenotype of cigarette and alcohol consumption could be explained by 3.1–9.3% genetic background, whereas PWD, PR, and QT interval showed higher genetic effects from 10.5 to 19.3%. Regarding cross-trait genetic correlations, we observed significant genetic correlations between AUD and QT interval ($r_g = 0.126$, $P = 0.002$), SmkInit and QT interval ($r_g = 0.096$, $P = 0.002$), and CigDay and QT interval ($r_g = 0.184$, $P = 2.04\text{E-}6$) (Table S1). Before performing the TSMR analysis, we evaluated bias and Type I error rates related to sample overlap using the Burgess et al. tool (https://sb452.shinyapps.io/overlap/) under the null hypothesis. By inputting exposure and outcome sample sizes, we estimated overlap proportions (0.0–1.0) with default settings. The Type I error rate remained stable at 0.05, with no inflation, and the bias was consistently 0.000, confirming the reliability of our estimation method. When MR analyses were performed, we identified 10 (*F*-statistic: 42.17–286.69), 10 (*F*-statistic: 30.68–71.09), 29 (*F*-statistic: 39.88–484), 16 (*F*-statistic: 31.38–790.45), and 46 (*F*-statistic: 34.34–103.69) genetic instruments for DrnkWk, AUD, PAU, CigDay, and SmkInit, respectively (Table S2). IVW results showed that genetically predicted PAU was associated with longer PWD [β (95%CI): 11.64 (8.25, 15.03), $P = 1.76\text{E-}11$], and SmkInit was associated with shorter PR intervals [β (95%CI): −1.23 (−2.12, −0.33), $P = 0.007$]. Notably, the QT interval was more significantly affected by the genetic predisposition to tobacco and alcohol consumption, with three of the five exposures significantly prolonging the QT interval [AUD, β (95%CI): 1.35 (0.24, 2.46), $P = 0.017$; DrnkWk, β (95%CI): 1.06 (0.91, 5.05), $P = 0.005$; SmkInit, β (95%CI): 2.06 (0.32, 3.80), $P = 0.020$] (Fig. 2).

To gauge the stability of the TSMR results, multiple robust models were further used to provide causal estimates consistent with IVW. The casual correlation between PAU and longer PWD passed the test of weighted median models and radial IVW, but failed MR-Egger's assumptions [β (95%CI): −0.17 (−24.28, 23.93), $P = 0.990$]. In contrast, MR-Egger suggested a weak correlation between SmkInit and longer PR intervals, but weighted median models radial IVW did not provide significant causal inference (Fig. 3A and Table S3). The radial plot revealed that no influential outliers were affecting the causal relationship between PAU and PWD. However, 9 potential outlier points were detected in the association between SmkInit and longer PR intervals (Fig. 3B). The outlier-corrected analysis (radial Egger corrected) maintained the negative result consistent with other robust methods (Fig. 3A). For QT intervals, genetic predispositions to AUD and SmkInit were found to have potential outliers, with 1 and 5 outlier points, respectively. Although the causal relationship remained significant after outlier removal, this association between genetically determined AUD, SmkInit, and prolonged QT intervals was almost exclusively rejected in other sensitivity analyses due to inconsistent causal directions (Fig. 3A). Nevertheless, all robust analytical models, including radial MR outlier correction, supported a causal effect between DrnkWk and longer QT intervals [$\beta_{\text{MR-Egger}}$ (95% CI): 4.59 (0.85, 8.33), $P = 0.018$; $\beta_{\text{penalized weighted median}}$ (95% CI): 5.05 (2.00, 8.10), $P = 0.001$; $\beta_{\text{Radial IVW}}$ (95%CI): 2.98

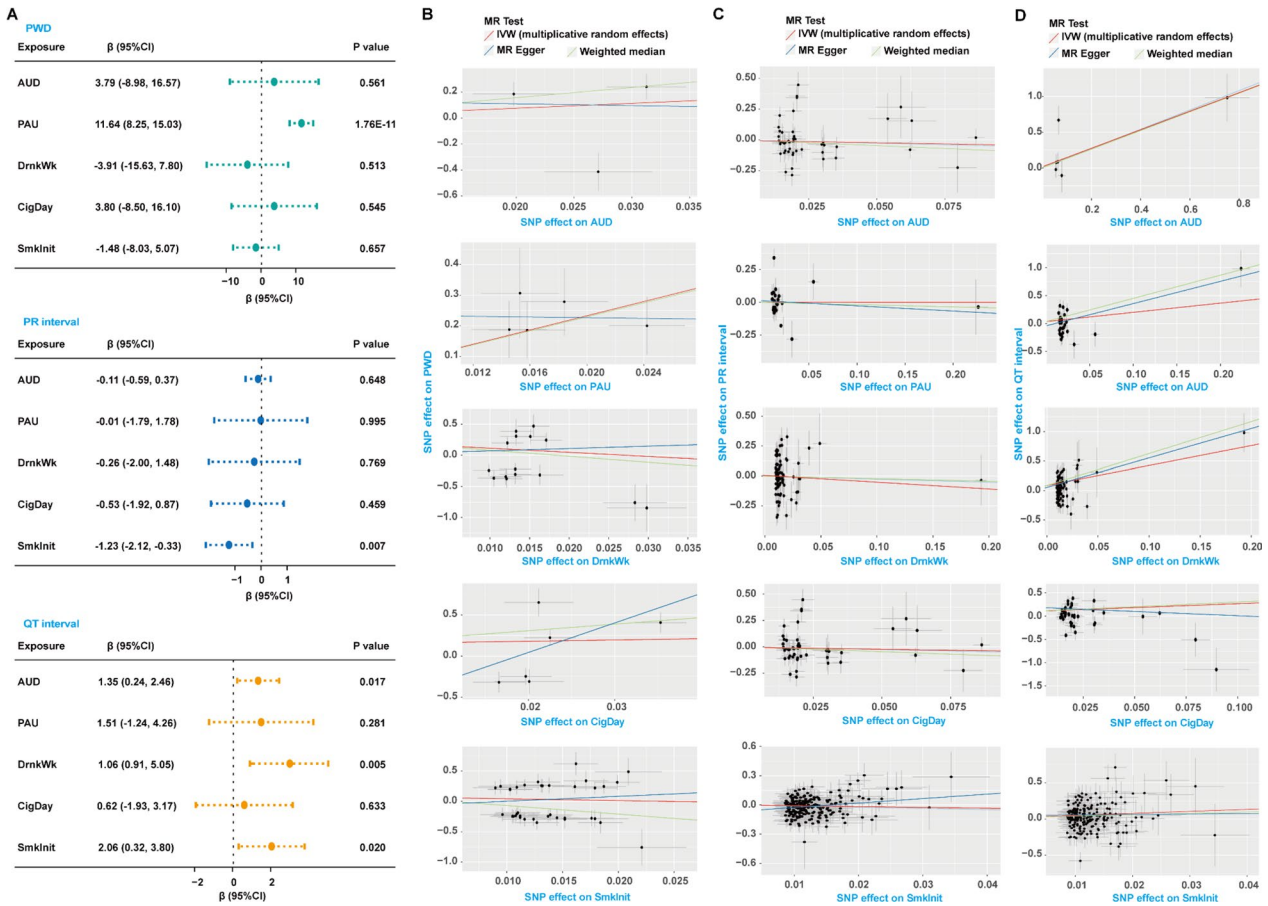


Fig. 2 Mendelian randomization (MR) estimates the causal effect between tobacco and alcohol consumption and cardiac ECG indexes. **A** MR estimates are evaluated by the inverse variance weighted (IVW) method. **B** Scatterplot for MR-estimated individual variant regression coefficients for tobacco and alcohol consumption on PWD. **C** Scatterplot for MR-estimated individual variant regression coefficients for tobacco and alcohol consumption on PR interval. **D** Scatterplot for MR-estimated individual variant regression coefficients for tobacco and alcohol consumption on QT interval. PWD, P-wave duration; AUD, alcohol use disorders; PAU, problematic alcohol use; DrnkWk, drinks per week; CigDay, cigarettes per day; SmkInit, smoking initiation; *β*, effect sizes; 95%CI, 95% confidence interval

(0.91, 5.05), $P=0.005$] (Fig. 3 and Table S3). Importantly, after adjusting for CigDay and SmkInit, the MVMR analysis substantiated that the DrnkWk is causally linked to prolonged QT intervals (Table 2). Additionally, examinations based on the MR-Egger intercept and MR-RAPS tests indicated the absence of directional horizontal pleiotropy ($P>0.05$) for DrnkWk and QT interval, further suggesting adherence to the MR exclusive restriction assumption in the aforementioned analyses (Table 3). Leave-one-out analysis showed no single SNP significantly impacted results and no heterogeneity was observed (IVW: $P=0.061$) (Fig. 4A and B). MR-RAPS diagnostics (ANOVA: $P=0.221$) indicated IV weights were independent of residuals, ensuring unbiased estimates. Residual and Q-Q plots validated normality assumptions, supporting reliable MR findings (Fig. 4C).

The causal effect of alcohol-related DNA methylation CpG loci on QT interval predisposition

Given the observed causal relationship between DrnkWk and prolonged QT intervals, and the fact that EWAS suggests that drinking influences DNA methylation in blood and tissues, and that aberrant epigenetic mechanisms (patterns of DNA methylation) potentially modulate a wide range of cardiovascular disorders, including cardiac arrhythmias [26–28], using GCTA results, we identified 65 SNPs associated with 56 CpG sites from the mQTLdb database, all of them belonging to cis-mQTL (Table S4). These mQTL were integrated with the QT interval GWAS to be included in the methylation MR analyses. Eight CpG loci (cg03345232, cg04605617, cg07091481, cg07104958, cg07512517, cg07567724, cg12807764, and cg20374917) were identified as causally associated with QT interval susceptibility, achieving nominal significance at the $P<0.05$

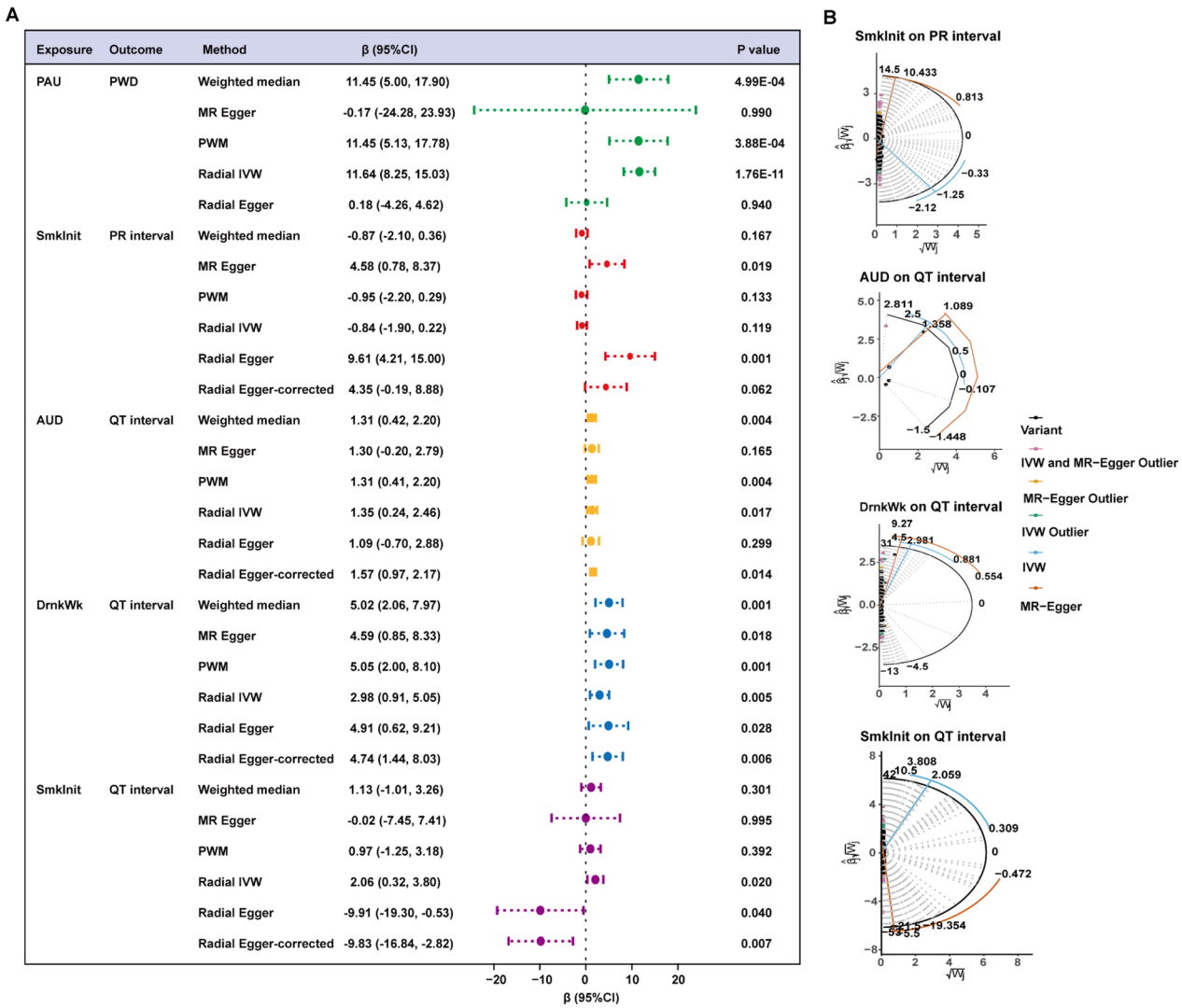


Fig. 3 Robust Mendelian randomization (MR) estimates the causal relationship between tobacco and alcohol consumption and ECG indices. **A** Forest plot of causal results assessed by applying robust MR methods. **B** Radial MR plots showing ratio estimates for each genetic variant as well as overall MR estimates, and variant outliers that affect causal estimates. PWD, P-wave duration; AUD, alcohol use disorders; PAU, problematic alcohol use; DrnkWk, drinks per week; SmkInit, smoking initiation; PWM, penalized weighted median; radial IVW, radial inverse variance weighted; *β*, effect sizes; 95%CI, 95% confidence interval. Radial Egger corrected represents outlier(s)-corrected analyses

Table 2 Multivariable Mendelian randomization analysis adjusted for smoking phenotypes

Exposure	Outcome	Adjusted phenotypes	Beta	Std. Error	*P* value
DrnkWk	QT interval	CigDay	5.05	2.25	0.025
		SmkInit	4.97	2.23	0.009

DrnkWk, drinks per week; CigDay, cigarettes per day; SmkInit, smoking initiation

level; two of these loci, cg03345226 and cg04605617, in particular, survived Bonferroni correction ($P < 0.05/56 = 8.93\text{E-}04$) (Fig. 5A, Tables 4 and S5). The CpG site cg04605617 was most significantly associated with QT interval prolongation [β (95%CI): 0.175 (0.13, 0.22), $P = 1.82\text{E-}13$] and mapped to the promoter region of the protein-coding gene Phospholipase A2 Group IIC (*PLA2G2C*) (chr1: 20,501,558) (Fig. 5B). Although the function of *PLA2G2C* is poorly understood, it is predicted to enable calcium ion binding activity. To investigate whether the two mQTLs rs10916683 and rs2872909 of cg04605617 affect the expression of the *PLA2G2C* gene in blood and or heart, we queried it on eQTLGen and GTEx Portal and found rs10916683 to be a strong eQTL for the *PLA2G2C* gene in whole

Table 3 Directional horizontal pleiotropy test

Exposure	Outcome	Method	Intercept value	Std. Error	F value	*P* value
AUD	PWD	MR-Egger intercept	0.14	1.19		0.926
		MR-RAPS			3.54	0.352
	PR interval	MR-Egger intercept	0.002	0.04		0.952
		MR-RAPS			0.12	0.988
	QT interval	MR-Egger intercept	0.01	0.12		0.904
		MR-RAPS			2.36	0.456
		Radial Egger	0.36	0.93		0.715
		Radial Egger corrected	−0.55	0.34		0.197
PAU	PWD	MR-Egger intercept	0.22	0.22		0.399
		MR-RAPS			14.96	0.191
	PR interval	MR-Egger intercept	0.01	0.04		0.727
		MR-RAPS			0.41	0.861
	QT interval	MR-Egger intercept	−0.08	0.06		0.191
		MR-RAPS			1.10	0.396
DrnkWk	PWD	MR-Egger intercept	−0.15	0.37		0.698
		MR-RAPS			2.24	0.133
	PR interval	MR-Egger intercept	0.006	0.03		0.831
		MR-RAPS			1.48	0.178
	QT interval	MR-Egger intercept	−0.031	0.03		0.315
		MR-RAPS			0.86	0.538
		Radial Egger	−0.27	0.27		0.317
		Radial Egger corrected	−0.22	0.21		0.303
CigDay	PWD	MR-Egger intercept	−0.82	0.52		0.187
		MR-RAPS			0.78	0.693
	PR interval	MR-Egger intercept	−0.005	0.03		0.891
		MR-RAPS			1.09	0.384
	QT interval	MR-Egger intercept	0.09	0.07		0.171
		MR-RAPS			0.21	0.969
SmkInit	PWD	MR-Egger intercept	−0.15	0.20		0.459
		MR-RAPS			0.93	0.490
	PR interval	MR-Egger intercept	−0.07	0.02		0.002
		MR-RAPS			2.83	0.046
		Radial Egger	−1.71	0.44		1.41E-04
		Radial Egger corrected	−0.92	0.37		0.014
	QT interval	MR-Egger intercept	0.03	0.05		0.573
		MR-RAPS			0.38	0.980
		Radial Egger	1.16	0.46		0.012
		Radial Egger corrected	1.17	0.34		0.001

PWD P-wave duration, *AUD* alcohol use disorders, *PAU* problematic alcohol use, *DrnkWk* drinks per week, *SmkInit* smoking initiation, *CigDay* cigarettes per day, *MR-RAPS* MR-Robust Adjusted Profile Score, radial Egger corrected represents outlier(s)-corrected analyses

blood ($P=9.36$E-16) (Table S6). Another CpG locus cg03345232 exhibited a significant causal relationship with a shortened QT interval [β (95%CI): −0.435 (−0.657, −0.213), $P=1.22$E-04], and it was mapped to the protein-coding gene Ras and Rab interactor 3 (*RIN3*). Nevertheless, we did not find evidence supporting the classification of either of the two mQTLs, rs77826962, or rs12884739 at this locus, as eQTLs influence gene expression (Table S6).

Genetic basis shared between alcohol consumption and QT interval susceptibility

For the convincing causal relationship between the CpG locus and QT interval alteration described above, we

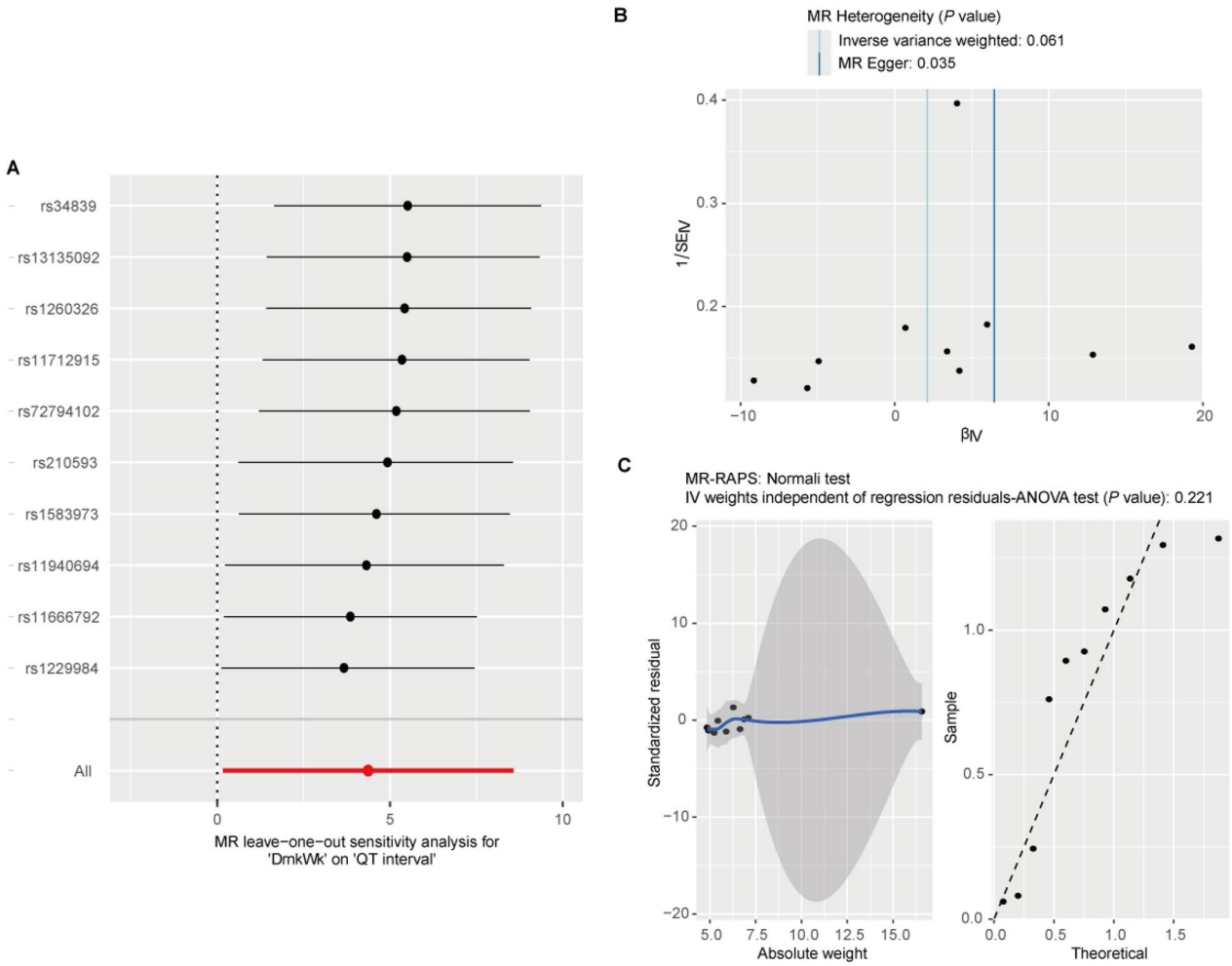


Fig. 4 MR sensitivity analysis of the causal relationship between DrnkWk and prolonged QT intervals. **A** Leave-one-out analysis identifying the effect of individual variables on the total effect. **B** Funnel plot showing the results of the heterogeneity analysis of the instrumental variable (IV) for causal estimation; **C** MR-RAPS diagnostic plots of the association between DrnkWk and QT interval prolongation, including residual plots showing the IV weights versus the standardized residuals and Q-Q plots assessing normality

performed an approximate Bayes factor colocalization analysis to replicate the MR findings and attempted to find individual causal variants mediating this relationship. We obtained a broader spectrum of mQTLs associated with cg03345232 and cg04605617 in the GoDMC dataset. Figure 5C shows that methylation at cg03345232 shared a genetic causal variant, rs12881206, with a 99.7% posterior probability of H4 (PPH4) with QT GWAS signaling. However, for the mQTLs at cg04605617, the colocalization analysis did not reveal a single shared variant site with a posterior probability exceeding 95.0%. Furthermore, we applied SMR integrating eQTL and GWAS summary data, to identify putative functional genes underlying the association between DrnkWk and QT interval. We identified 77 and 33 significant variants in whole-blood eQTL for DrnkWk and QT intervals, respectively ($P_{\mathrm{DrnkWk}} < 0.05/15629 = 3.20\text{E-}06$, $P_{\mathrm{QT}} < 0.05/15617 = 3.20\text{E-}06$), and 19 significant variants ($P < 0.05/4662 = 1.07\text{E-}05$) were found for QT intervals in heart left ventricle (Table S7). Among these variants, we observed that DrnkWk and QT intervals share a protein-coding gene, *NPIPB6*, and a long non-coding RNA gene, *NONHSAG018995.2* (ENSG00000251417). Unfortunately, both of these variants did not pass the horizontal pleiotropy test by HEIDI ($P < 0.05$) (Table S7).

Discussion

This study presents evidence of a genetic association and causality between tobacco and alcohol consumption and ECG indices. It sheds light on the molecular genetic architecture underlying alcohol consumption's impact on QT interval prolongation and provides valuable insights into their regulatory mechanisms.

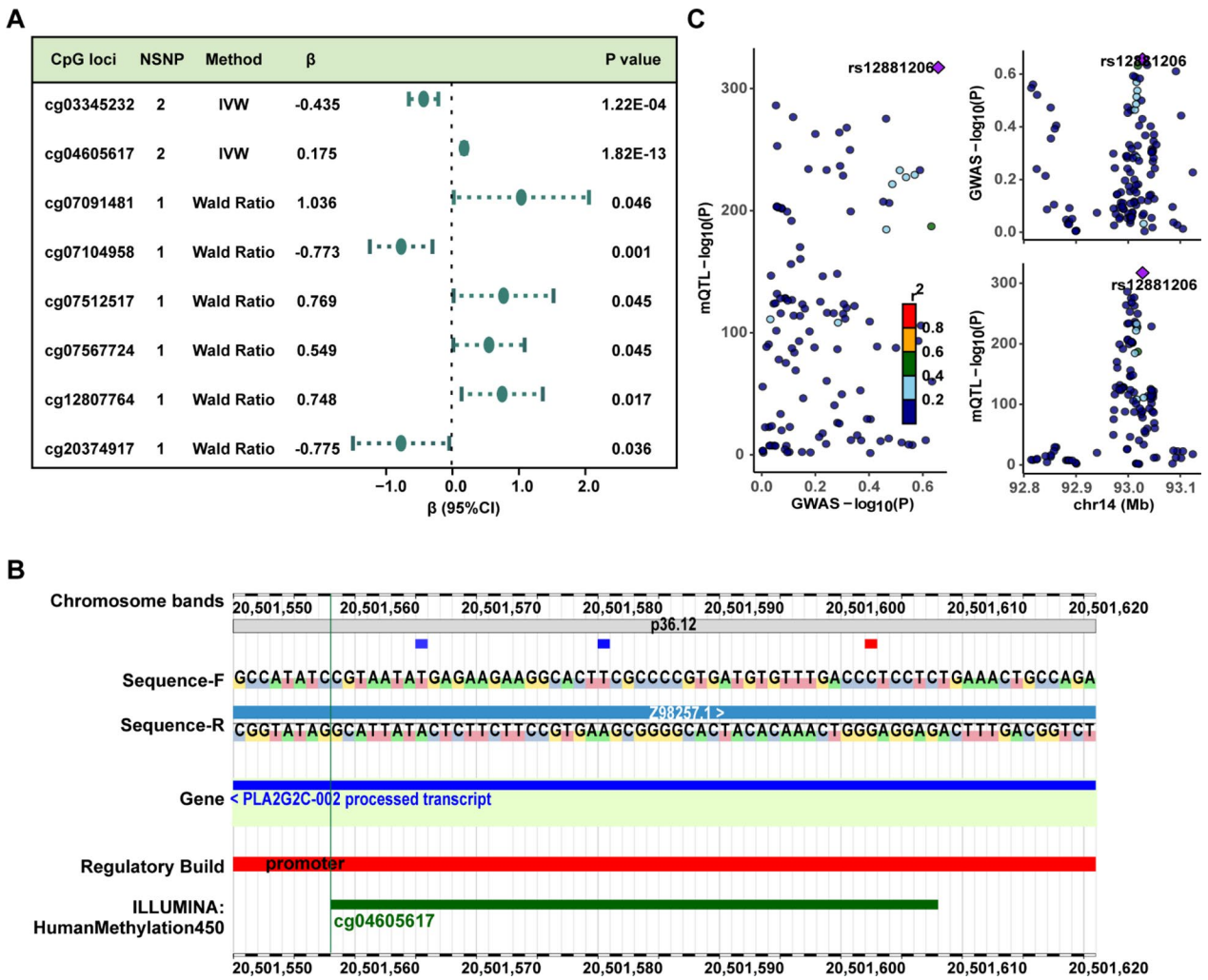


Fig. 5 Epigenetic MR estimates and colocalization analysis. **A** Epigenetic causal estimates of significant CpG loci for alcohol-related methylation on QT interval susceptibility. **B** Genomic localization of DNA methylation site: CpG island cg04605617.cg04605617 localizes to chromosome 1 at position 20,501,558 and maps to the promoter region of the gene *PLA2G2C*. **C** Regional map of evidence for colocalization of methylated CpG sites with QT interval susceptibility. 99.7% posterior probability supports that rs12881206 is a shared causal variant for cg03345232 methylation and QT interval susceptibility. IVW, radial inverse variance weighted; mQTL, methylation quantitative trait loci

Table 4 Causal estimates of significant CpG loci for alcohol-related methylation on QT interval susceptibility by epigenetic Mendelian randomization

CpG	CpG.Chr	CpG.Pos	Nearest gene(s)	No.SNP	Method	Beta	SE	LCI	UCI	*P* value
cg03345232	14	92,981,121	*RIN3*	2	IVW	−0.44	0.11	−0.66	−0.21	1.22E-04
cg04605617	1	20,501,558	*PLA2G2C*	2	IVW	0.18	0.02	0.13	0.22	1.82E-13
cg07091481	10	82,169,149	*C10orf58*	1	Wald ratio	1.04	0.52	0.02	2.06	0.046
cg07104958	10	46,168,551	*ANUBL1*	1	Wald ratio	−0.77	0.24	−1.24	−0.30	0.001
cg07512517	7	38,408,106	-	1	Wald ratio	0.77	0.38	0.02	1.52	0.045
cg07567724	1	153,777,721	*GATAD2B*	1	Wald ratio	0.55	0.27	0.01	1.09	0.045
cg12807764	5	146,864,669	-	1	Wald ratio	0.75	0.31	0.14	1.36	0.017
cg20374917	11	128,603,874	*FLI1*	1	Wald ratio	-0.78	0.37	-1.50	-0.05	0.036

IVW Inverse variance weighted, *SE* standard error, *LCI* lower 95% confidence interval, *UCI* upper 95% confidence interval

The evaluation of genetic associations across traits supports the hypothesis that genetic factors play a crucial role in the association between tobacco and alcohol consumption and ECG indices. Substantial genetic causal associations between tobacco and alcohol exposure and cardiac electrophysiological changes were highlighted by MR analysis. Using multiple robust MR hypothesis models, we provided compelling causal evidence in univariate MR analyses between weekly alcohol consumption and QT interval prolongation. To maintain the direction and significance of the causal effects, we employ stringent criteria, such as MR-Egger and weighted median models, to ensure the robustness of the MR estimates and to reduce the Type 1 error rate. MVMR further confirmed the independence of this genetic association from the smoking phenotype, eliminating the confounding effect of genetic correction between smoking and alcohol phenotypes. Epigenetic MR analyses revealed a significant causal relationship between alcohol-related DNA methylation and QT interval prolongation. Eight CpG loci were identified as potential mediators of this causality, with two surviving Bonferroni corrections, including cg04605617 mapping to the promoter region of *PLA2G2C*. The mQTL rs10916683 at cg04605617 is a robust eQTL for *PLA2G2C*. Despite SMR not identifying shared functional genes without pleiotropy for alcohol-induced QT interval prolongation, the colocalization analysis revealed an mQTL, rs12881206, shared with a high posterior probability between cg03345232 methylation and QT GWAS signals. They underscore the potential regulatory role of DNA methylation in modulating QT intervals.

These results align with observational studies indicating a positive correlation between alcohol consumption and QT interval prolongation, suggesting a potential increased arrhythmia risk by affecting ventricular repolarization [25, 38, 45, 46]. Importantly, the identification of the CpG locus cg04605617 in the promoter of the relatively obscure gene *PLA2G2C* provides an intriguing avenue for cardiovascular genetic exploration. *PLA2G2C* encodes a calcium-dependent phospholipase belonging to the phospholipase A_2 (PLA_2) family, contributing to signal transduction, membrane homeostasis, and immune regulation [47–49]. Although limited, polymorphisms in *PLA2G2C* sense changes in plasma triglycerides, and functional experiments have found that it produces a lysophospholipid antigen in mouse hepatocytes, leading to the propagation of an antiviral immune response via NKT cells [50–52]. Moreover, allergy and inflammation appear to induce the expression of PLA_2, including *PLA2G2C* [53]. Other members of the PLA_2 family, such as SPLA2-IIA, have also been associated with cardiovascular diseases, particularly atherosclerosis. Elevated levels of SPLA2-IIA are linked to an increased cardiovascular risk, likely due to its role in promoting inflammation within the arterial wall [54]. Given the structural similarities, PLA2G2C may also affect similar pathways; however, direct evidence supporting this hypothesis is currently lacking. Notably, EWAS reveals that methylation at the cg04605617 site within *PLA2G2C* is significantly linked to high arsenic exposure, hinting at a potential association with cardiovascular disease [55]. We also note that *PLA2G2C* is functionally annotated in Gene Ontology to enable calcium binding [56], and that the significant *PLA2G2C* mQTL and eQTL identified, rs10916683, are also notable polymorphic sites where its homolog, *PLA2G2A*, functions [57]. The observed causal link between cg04605617 methylation and QT interval prolongation suggests that alterations in DNA methylation at this locus may impact *PLA2G2C* expression and calcium ion kinetics in cardiac cells, which are integral to cardiac excitation–contraction coupling and have profound effects on cardiac electrophysiology [58, 59]. However, the precise mechanistic contributions remain incompletely understood. Determining how methylation at this specific locus regulates PLA2G2C expression could uncover novel epigenetic regulatory networks governing cardiac electrical activity. Functional validation studies addressing these gaps would significantly enhance the translational relevance of the current findings.

Furthermore, we identified rs12881206 between alcohol consumption and QT interval predisposition with a posteriori probability exceeding 0.95 to match the H4 hypothesis from colocalization analysis, implying that they share the same causal variant in this genomic region. Upon querying dbSNP, we identified this polymorphic locus as an intronic variant of RIN3 on chromosome 14, previously undocumented for clinical significance. However, RIN3 is a guanine nucleotide exchange factor that activates Ras and Rab5 proteins [60]. Given their roles in cell signaling and endocytosis regulation [60], further exploration of the potential mechanistic links between these findings has the potential to uncover new insights into cardiovascular risk factors. In contrast, this study did not identify a definitive genetic causal relationship between smoking and ECG alterations. Nevertheless, it is imperative to emphasize that smoking constitutes a well-established role as a cardiovascular risk factor through diverse mechanisms such as inflammation, oxidative stress, and endothelial dysfunction [61]. Consequently, the research findings should not be misconstrued as indicating that smoking is benign for cardiovascular health. Statistical significance should not be confused

with biological relevance, and caution should be exercised in attributing causality based solely on observational data and statistical associations. Previous studies have reported associations between smoking and ECG indices, particularly QT interval prolongation [17, 20]. While CigDay showed a strong genetic correlation with QT interval, MR did not confirm causality, underscoring the need to distinguish correlation from causation. LDSC quantifies the overall genetic overlap between traits by assessing genome-wide correlations, which may arise from shared genetic influences rather than direct causal pathways. In contrast, MR leverages genetic variants as IVs to estimate the direct effect of an exposure on an outcome, minimizing confounding and reverse causation. By relying on independent genetic instruments, MR helps distinguish true causal relationships from associations driven by pleiotropy or indirect genetic linkages, providing stronger evidence for causal inference. The strengths of our study lie in leveraging large-scale, high-quality GWAS data, employing multiple robust MR models to ensure stable causal inference, and using mQTL datasets for genetic co-localization and SMR analyses to explore potential sharing mechanisms. However, limitations include the use of blood DNA samples, which may not fully represent tissue-specific methylation profiles. The reliance on mQTL effects from a specific demographic and age group (middle-aged time point), along with the European pedigree in MR analyses, raises concerns about generalizability. Our MR analyses, primarily in European ancestry individuals, enhance population homogeneity but limit generalizability to other populations. Additionally, the CpG loci associated with alcohol consumption in our study were derived from the latest EWAS in the EWAS Catalog, which linked alcohol use to specific methylation sites. The mQTL data for these loci were obtained from ALSPAC. However, due to differences in alcohol consumption rates across cohorts, while the mQTL-CpG associations are robust, the extent of behavioral influence across cohorts is difficult to estimate [62]. These limitations underscore the need for diversified populations and tissues in future research to enhance the validity and applicability of the findings. In conclusion, our study reveals a potential genetic mechanism for alcohol-induced QT interval prolongation, identifying the CpG locus cg04605617 in the promoter region of the little-known *PLA2G2C* gene. This suggests that alcohol-related DNA methylation may influence the QT interval through a novel pathway. The presence of rs10916683 as a significant eQTL variant implies broader systemic effects of alcohol on the cardiovascular system. However, the limited understanding of the biological function of the CpG locus and its associated shared variant (rs12881206) raises questions about its direct impact on cardiac physiology. Despite these limitations, our findings provide valuable insights into the complex genetic architecture linking alcohol consumption to specific cardiac electrophysiologic changes. The identification of a causal relationship between alcohol consumption and QT interval prolongation underscores the importance of lifestyle factors in cardiovascular health. Specifically, the study highlights the significant role that genetic and epigenetic mechanisms play in mediating the effects of alcohol on cardiac electrical activity. Understanding these pathways can guide clinical interventions aimed at reducing alcohol-related cardiac risks. Identifying specific DNA methylation sites like cg04605617 mapped to *PLA2G2C* offers potential biomarkers for risk stratification. Clinically, these findings support routine ECG monitoring in individuals with heavy alcohol use and genetic predisposition to prolonged QT intervals. Moreover, epigenetic interventions, such as targeted methylation modulators, could be explored as a novel therapeutic approach for arrhythmia prevention. To further validate and expand these findings, future studies should replicate the associations in larger, multi-ethnic cohorts with broader age ranges. Functional experiments in vitro or animal models could elucidate the underlying mechanisms, and prospective clinical studies evaluating changes in alcohol consumption on methylation profiles and ECG parameters would strengthen the causal inference. Importantly, although blood-based mQTLs provide useful insights into systemic regulatory mechanisms, their relevance to cardiac-specific methylation remains uncertain. Tissue-specific differences in DNA methylation patterns could influence the functional interpretation of our findings. Future studies integrating cardiac tissue-derived mQTL and single-cell epigenomic data will be crucial to validating whether methylation changes at *PLA2G2C* directly impact QT interval regulation.

Conclusion

This study reveals a causal link between alcohol consumption and QT interval prolongation through specific genetic and epigenetic mechanisms. The identification of cg04605617 within *PLA2G2C* as a candidate locus offers new insights into the regulatory mechanisms underlying cardiac electrophysiology. However, given the tissue-specific nature of epigenetic modifications, further validation in cardiac tissues is warranted. These findings contribute to the broader understanding of alcohol's influence on cardiac function and may inform future research into epigenetic-based therapeutic strategies.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13148-025-01851-x.

Supplementary material 1.

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Author contributions
ZZ and YS helped in conceptualization, methodology, data curation, formal analysis, resources, software, visualization, writing—original draft, funding acquisition. XL and TL contributed to methodology, investigation, formal analysis, supervision. XT helped in conceptualization, writing—review & editing, project administration, supervision, funding acquisition.

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Availability of data and materials
No datasets were generated or analyzed during the current study.

Declarations

Ethics approval and consent to participate
The data used in this study were sourced from publicly available GWAS and DNA methylation datasets, all of which received ethical approval in their original studies with informed consent from participants. As the data were anonymized and de-identified, no additional ethical approval was required for our reanalysis. We adhered to the ethical guidelines set by the original studies.

Consent for publication
All authors approved the final version and agreed to be responsible for the study.

Competing interests
The authors declare no competing interests.

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