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# Alcohol consumption and telomere length: Mendelian randomization clarifies alcohol's effects

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Alcohol's impact on telomere length, a proposed marker of biological aging, is unclear. We performed the largest observational study to date (in  $n = 245,354$  UK Biobank participants) and compared findings with Mendelian randomization (MR) estimates. Two-sample MR used data from 472,174 participants in a recent genome-wide association study (GWAS) of telomere length. Genetic variants were selected on the basis of associations with alcohol consumption ( $n = 941,280$ ) and alcohol use disorder (AUD) ( $n = 57,564$  cases). Non-linear MR employed UK Biobank individual data. MR analyses suggested a causal relationship between alcohol traits, more strongly for AUD, and telomere length. Higher genetically-predicted AUD (inverse variance-weighted (IVW)  $\beta = -0.06$ , 95% confidence interval (CI):  $-0.10$  to  $-0.02$ ,  $p = 0.001$ ) was associated with shorter telomere length. There was a weaker association with genetically-predicted alcoholic drinks weekly (IVW  $\beta = -0.07$ , CI:  $-0.14$  to  $-0.01$ ,  $p = 0.03$ ). Results were consistent across methods and independent from smoking. Non-linear analyses indicated a potential threshold relationship between alcohol and telomere length. Our findings indicate that alcohol consumption may shorten telomere length. There are implications for age-related diseases.

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## INTRODUCTION

Telomere length is considered a potential biological marker of aging [1]. These repetitive nucleotide sequences, together with associated protein complexes, form a 'cap' at the ends of chromosomes, protecting them from damage. As a cell's replicative machinery cannot completely copy the ends of chromosomes, 50–100 base pairs are lost at each division. Telomere attrition therefore occurs with increasing cellular age. Critically short telomeres trigger cell death or replicative senescence, or occasionally continued division, mutation and genetic aberrations. Epidemiologically, shorter leucocyte telomere length (LTL) has been linked to several aging-related diseases including Alzheimer's disease, cancer and coronary artery disease [2, 3]. Telomere length is partly heritable and linked to sex [4], ethnicity and paternal age [5], but has also been linked to environmental and lifestyle factors, including exercise [6], smoking [7] and alcohol consumption [8].

Observational studies of the relationship between alcohol use and telomere length have produced conflicting results. The largest such study to date, of 4567 individuals, found no association between alcohol intake and either baseline or longitudinal change in telomere length [9]. Another analysis of two American cohorts ( $n = 2623$ ) also reported null findings [10]. On the other hand, a few small studies (sample size range: 255–1800) have observed associations with heavy drinking or AUD. Participants with AUD

have been reported to have shorter telomeres compared to healthy controls [11]. A longitudinal study of Helsinki businessmen observed that higher midlife alcohol consumption was associated with shorter telomere length in older age [8]. Drinking  $>30$  g/day of alcohol in older participants was associated with shorter telomeres in a Korean study [12]. Associations were stronger in those experiencing the alcohol flush reaction, raising the intriguing possibility that acetaldehyde, ethanol's toxic breakdown product, is mechanistically involved. In a recent review of 27 studies, 10 showed significant associations between alcohol use and telomere length [13]. The studies included cross-sectional and longitudinal designs. The majority comprised European participants with ages ranging from the third to seventh decade. Most studies observed positive associations between alcohol and LTL. However heterogeneity between studies in methods of quantifying telomere length and categorizing alcohol intake hindered meta-analysis and aggregation of the data.

MR seeks to identify potentially causal determinants of an outcome. It estimates the association between genetically predicted levels of an exposure and an outcome of interest. Residual confounding and reverse causation aim to be less of a concern than in most other methods of analyzing observational data [14]. With MR, genetic proxies can be used to study the effects of genetically-predicted variability in alcohol consumption

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or AUD risk. To our knowledge, no MR study of alcohol and telomere length has yet been attempted.

We conducted a large observational study of two alcohol phenotypes, alcohol consumption and AUD, and leucocyte. We then performed linear MR analyses to investigate the evidence for a causal effect between alcohol consumption/AUD and LTL. Estimates generated by our observational and genetic methods were compared. Genetic distinction between different alcohol use traits motivates their separate analysis. Quantity/frequency measures such as drinks per week and AUDIT-C (Alcohol Use Disorders Identification Test Consumption, a 3 item screening tool), while moderately genetically correlated with AUD, have distinct patterns of genetic correlation with other traits [13]. Furthermore, as there has been much speculation about potential J-shaped relationships between alcohol and health outcomes [15], we performed a non-linear MR analysis to examine the shape of the relationship between alcohol consumption and telomere length. Multiple robust methods were employed to test MR assumptions. These included use of non-drinkers as negative controls, testing one of the key assumptions that genetic proxies only impact an outcome via the exposure. Given the widespread exposure to alcohol across the world, clarification of any potential causal impact on telomere length is important.

## METHODS

All analyses were performed in R (version 3.6.0) unless otherwise specified.

### Study population

Participants were drawn from UK Biobank [16], a prospective cohort study which recruited ~500,000 volunteers aged 40–69 years in 2006–10. UK Biobank received ethical approval from the Research Ethics Committee (reference 11/NW/0382), and all participants provided written informed consent. Data used in this study included self-reported alcohol consumption, biological samples (blood) for genetic analysis, and long-term follow-up through hospital record linkage. UKB genetic data (single nucleotide polymorphisms, SNPs) were generated from the Affymetrix Axiom UK Biobank array (~450,000 individuals) and UK BiLEVE array (~50,000 individuals) following extensive quality control [17]. Ancestry principal components were generated using loadings from high-confidence SNPs in the 1000 Genomes Cohort. SNP dosages for instrumental variants were extracted from UKB v3 imputed genotype data using qctool (version 2.0.7). Participants with solely European ancestry (defined by self-report and ancestral PCs) were included to avoid population stratification.

### Alcohol traits

We used two different alcohol trait definitions, to correspond to alcohol quantity and AUD. 1) Alcohol consumption was self-reported at baseline. Participants were asked at study baseline whether they were current, never or previous drinkers. Answers to this question formed the basis of categorization. For current drinkers, the total estimated UK units (1 unit = 8 g ethanol; 1 US drink = 10 g ethanol) consumed weekly were calculated by summing across beverage types as previously described [18]. Total weekly units were categorized into quintiles for the whole sample to allow group comparisons. 2) AUD cases were defined by the presence of a relevant ICD-9 (303, 303.9, 303.01:3, 303.91:303.93) or ICD-10 code (F10.2, F10.21:F10.29) in linked NHS Hospital Episode Statistics.

### Telomere length measurements

LTL measurements were ascertained on DNA collected at the baseline assessment using a well-validated qPCR assay [19]. Measurements were reported as a ratio of the telomere repeat number to single-copy gene (T/S ratio), which were then log-transformed to approximate the normal distribution. Multiple quality checks to control and adjust for technical factors were undertaken as described elsewhere [19]. To aid comparison with other datasets, z-standardized LTL values were used. No exclusions, other than missing data, were made. A similar approach was taken in the original UKB telomere paper [19].

### Genetic variants

Genetically-predicted alcohol consumption was ascertained using an instrument composed of 93 variants associated with alcohol consumption

(log drinks per week) with genome-wide significance (GWS) in the largest published GWAS comprising 941,280 individuals [20]. This instrument has been evaluated in previous MR studies of known sequelae of alcohol [21]. Summary statistics were used that did not include data from UK Biobank participants ( $n = 226,223$ ) to avoid sample overlap, which can bias estimates towards observational associations [22]. All SNPs were associated with alcohol at GWS ( $p < 5 \times 10^{-8}$ ) and not in linkage disequilibrium (defined as  $r^2 > 0.1$ ). For AUD, 24 conditionally independent genetic variants were chosen from the largest published GWAS, comprised of European and European American individuals within the Million Veterans Program (MVP) and the Psychiatric Genomics Consortium (PGC) [23]. AUD cases were defined using ICD 9/10 codes within the MVP ( $n = 45,995$ ) and DSM-IV alcohol dependence within the PGC ( $n = 11,569$ ). Again all SNPs were associated with AUD at GWS ( $p < 5 \times 10^{-8}$ ). Genetic associations with LTL were obtained from the largest GWAS to date of telomere length, in 472,174 UKB participants [24].

### Statistical analysis

**Observational analysis.** Participants of European ancestry (to mirror the genetic analyses) with complete alcohol quantity, telomere, and covariate data were included. Separate multiple linear regression models were used to assess the relationship between LTL (dependent variable) and (1) alcohol consumption and (2) AUD. Factors previously described to associate with LTL were included as covariates in analysis: age at baseline, sex, educational qualifications, smoking, leucocyte count, and exercise. On the basis of previously observed sex differences in LTL [19], analyses were additionally stratified by sex. Alcohol consumption was fitted with linear and non-linear models to examine the shape of any alcohol-telomere relationship. The latter comprised two approaches. First, alcohol consumption was categorized into quintiles; second restricted cubic splines (RCS – 5 knots) were applied to alcohol intake. Non-linearity was formally tested with an F-test for equality of coefficients. To test the hypothesis that acetaldehyde is mechanistically involved in damage to telomeres, an interaction term between alcohol intake and *ADH1B* genotype (rs1229984) was included to test the hypothesis that higher levels of acetaldehyde are associated with shorter telomeres.

**Genetic analyses.** MR was used to obtain estimates for the association between genetically-predicted alcohol consumption/AUD and telomere length. Both linear and non-linear MR analyses were undertaken. Linear MR can be performed using summary statistics and so we were able to harness the power from large consortia GWAS in two-sample designs. Non-linear MR necessitates individual (participant-level) data, and thus was undertaken within the UKB in participants of European ancestry.

Two-sample MR analyses were conducted using MendelianRandomization package (version 0.5.1) and TwoSampleMR (version 0.5.6). Variants were harmonized between datasets, ensuring the association between SNPs and exposure and that between SNPs and the outcome reflected the same allele. Several MR methods were performed as broadly consistent results across methods strengthen the causal inference. Inverse variance weighted analysis regresses the effect sizes of variant-telomere associations against effect sizes of the variant-alcohol associations. A random effects model was implemented. Scatter plots and leave-one-out analysis were used to evaluate influential outliers. The MR-Egger method uses a weighted regression with an unconstrained intercept to remove the assumption (in IVW) that all genetic variants are valid instrumental variables (IVs). A non-zero intercept term in MR-Egger can be used as evidence of directional pleiotropy. The median MR method is also more resistant to pleiotropy. It takes the median IV estimate from all included variants, and therefore is robust when up to 50% genetic variants are invalid. MR-PRESSO attempts to reduce outlier bias by performing estimation of causal estimates after removal of outliers [25].

We performed additional sensitivity analyses to assess robustness of the findings. Given the prominence of *ADH1B* (rs1229984), we repeated the analyses excluding this variant. Checking for reverse causality in the alcohol/AUD-telomere relationship was done using the MR Steiger directionality test [26], as well as repeating MR analyses inverting the exposure and outcomes. Eighty-five SNPs (excluding  $n = 5$  palindromic SNPs) that are genome-wide significant for telomere length, had available associations with alcohol consumption, and 67 SNPs (excluding  $n = 1$  palindromic SNP) that had available associations with alcohol use disorder were used as instrumental variables to assess for a causal effect on alcohol. Differing availability of SNP associations reflects array

**Table 1.** Baseline characteristics of UK Biobank participants ( $n = 245,354$ ) included in observational analysis, according to alcohol status.

	Never drinkers <i>N</i> = 8240	Previous drinkers <i>N</i> = 9393	Current drinkers <i>N</i> = 227,721
Age <sup>1</sup> , years	59.1 (7.8)	57.4 (7.8)	56.6 (8.0)
Sex, females <i>n</i> (%)	6040 (73.3)	5106 (54.4)	109,430 (48.1)
Qualifications <i>none</i>	2235 (27.1)	2399 (25.5)	30,465 (13.4)
Degree <sup>3</sup>	1897 (23.0)	2443 (26.0)	80,773 (35.5)
Smoking never, <i>n</i> (%)	6618 (80.3)	4166 (44.4)	120,563 (52.9)
Previous, <i>n</i> (%)	1138 (13.8)	3828 (40.8)	85,512 (37.6)
Current, <i>n</i> (%)	484 (5.9)	1399 (14.9)	21,646 (9.5)
Systolic blood pressure <sup>1</sup> , mmHg	140.1 (20.3)	137.6 (19.6)	140.3 (19.5)
ADH1B dose <sup>1,4</sup>	1.9 (0.2)	2.0 (0.2)	2.0 (0.2)
Exercise, <sup>1</sup> MET minutes <sup>5</sup>	128.0 (107.4)	124.2 (109.1)	129.3 (101.0)

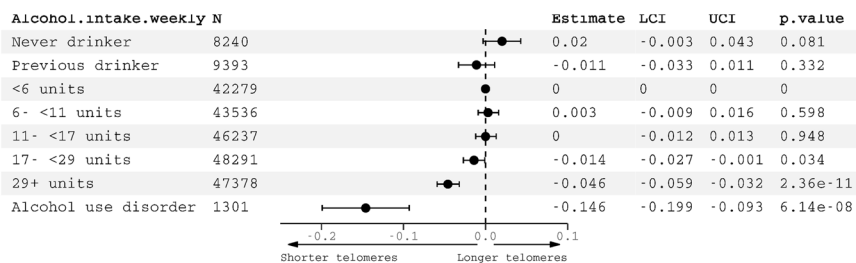
<sup>1</sup>Mean (standard deviation).

<sup>2</sup>Townsend Deprivation Index—calculated on census data. Higher score indicates greater material deprivation.

<sup>3</sup>Selected educational qualification categories shown for brevity. Degree indicates any college or university degree.

<sup>4</sup>SNP dosage of C allele of rs129984 (minor allele frequency = 0.03), associated with increased alcohol consumption compared to the T allele on a population level.

<sup>5</sup>Mean[exercise]T[ime] in minutes.



**Fig. 1** Observational associations with leucocyte telomere length in  $n = 245,354$  UK Biobank participants. Estimates generated from two regression models: (1) alcohol intake (estimates represent SD change in LTL) and (2) ICD diagnosis of alcohol use disorder, plotted together for comparison. Reference category for alcohol intake is <6 units weekly. Models adjusted for: age, sex, educational qualifications, leucocyte count, smoking, exercise.

differences between studies. We also conducted two-sample multi-variable MR (MVMR) to test whether the causal effect of alcohol on telomere length was confounded or mediated by smoking or physical activity. Genetic variant associations with smoking (cigarettes per day) were obtained from a large GWAS for the 92 SNPs used as instrumental variants for alcohol [20] and 18 SNPs used as instrumental variants for alcohol use disorder ( $n = 6$  had no available association statistics). Genetic associations for physical activity were obtained from a GWAS of device-measured physical activity [27]. Both IVW and MR Egger were conducted using MVMR in the MendelianRandomization package.

Nonlinear MR was undertaken by stratifying UKB subjects with complete alcohol, telomere and genetic data into quintiles based on residual alcohol consumption. This was defined as alcohol consumption minus the genetic contribution to alcohol intake (IV-free exposure). A genetic risk score for alcohol consumption was created by weighting each SNP dosage by the effect size (beta coefficient) obtained from the primary GWAS, and then summing across all SNPs. Stratifying directly on alcohol consumption could induce an association between the IV and outcome where there was none, invalidating MR assumptions. In each stratum a linear MR estimate of alcohol-telomere length was calculated using the ratio of coefficients method. To examine for the presence of a trend in the estimates a meta-regression of the stratum-specific estimates on the median value of alcohol intake in each stratum was performed. A conservative sensitivity analysis was performed excluding related participants ( $>0.088$  kinship coefficient). Sex-stratified analyses were performed.

To test the MR assumption that genetic variants only act upon telomere length through alcohol or AUD, we used never and previous drinkers in UKB as negative controls. MR analyses were performed separately in current, never and previous drinkers. If the assumption holds, there should be no association in non-drinkers. In all individual data MR analyses, age, sex and

the top 10 ancestral principal components were included as covariates. Again, a sensitivity analysis was performed excluding related participants ( $>0.088$  on the kinship matrix).

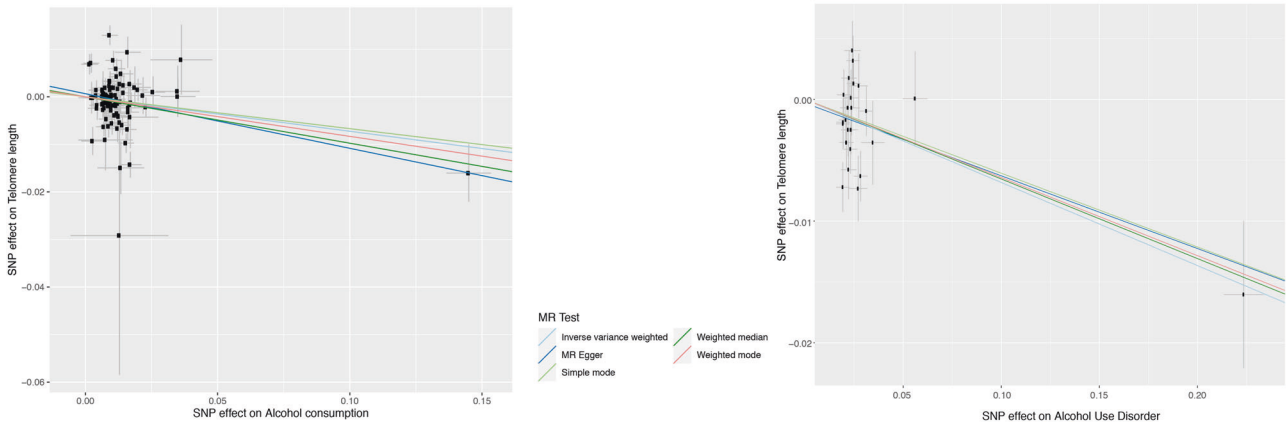
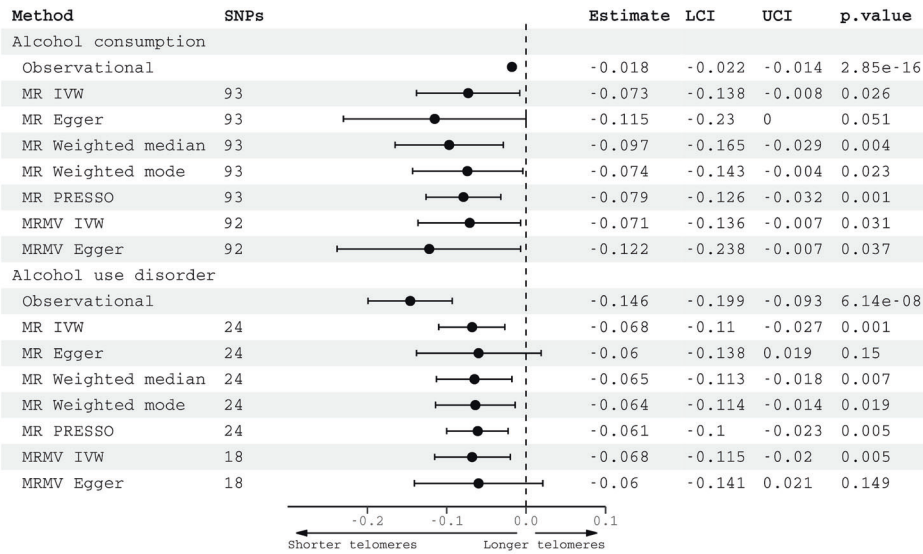
## RESULTS

### Participant characteristics UK Biobank

245,354 UKB participants were of European ancestry and had complete data on alcohol intake, telomere length and covariates and were included in the observational analysis (SFig. 1). The majority of participants were current drinkers, with only 3% ( $n = 8240$ ) reported as never drinkers and 4% ( $n = 9393$ ) previous drinkers. Never drinkers comprised a higher proportion of females, had fewer educational qualifications, lower smoking rates and lower levels of exercise than current drinkers (Table 1). Older age, male sex, smoking, lower educational qualifications and leucocyte count were independently associated with shorter LTL in both females and males (STable 1). Weekly exercise did not associate with LTL.

### Observational analysis

There was a significant observational association between high alcohol intake and shorter LTL (Fig. 1). Compared to lowest quintile drinkers (<6 units (48 g) weekly), highest quintile drinkers (>29 units weekly (232 g)) had significantly shorter telomeres ( $\beta = -0.05$ , 95% CI:  $-0.06$  to  $-0.03$ ,  $p = 2.36 \times 10^{-11}$ ). In the sex stratified analysis (STable 1 and SFig. 2) patterns of association with LTL were similar. Interaction terms between *ADH1B* genotype and alcohol quintile had large *p* values (STable 1). Individuals with



**Fig. 2 Top**—Multivariable-adjusted observational estimates (in 245,354 UKB participants) and two-sample Mendelian randomization estimates (two-sample design) for the association of genetically predicted alcohol consumption and alcohol use disorder with telomere length. Estimates for alcohol consumption observational associations represent SD change in telomere length for 1 SD increase in alcohol units weekly. MR estimates for alcohol consumption are per SD increase in genetically predicted log-transformed alcoholic drinks per week, and for AUD having a diagnosis of AUD. Bottom—SNP effects are plotted. A non-zero gradient to the lines indicates evidence for causality of alcohol on telomere length. Abbreviations: MR Mendelian randomization, SNPs single nucleotide polymorphisms, IVW inverse variance weighted, PRESSO Mendelian Randomization Pleiotropy RESidual Sum and Outlier, MRMV multivariable Mendelian randomization.

an ICD diagnosis of alcohol dependence ( $n = 1301$ ) in their linked clinical records had significantly shorter LTL compared with controls ( $\beta = -0.15$ , 95% CI:  $-0.20$  to  $-0.09$ ,  $p = 6.14 \times 10^{-8}$ ).

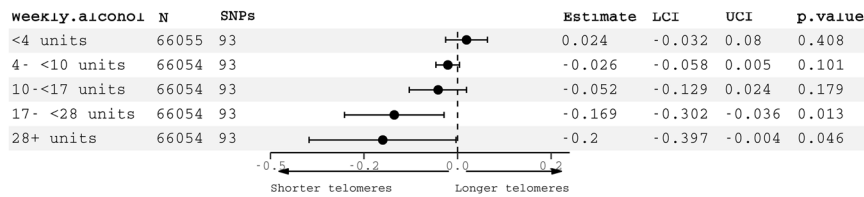
### Genetic analysis

Using 93 SNPs robustly and independently associated with alcohol consumption, univariable linear MR found an association between genetically-predicted alcohol consumption and telomere length (IVW  $\beta = -0.07$ , 95% CI:  $-0.14$  to  $-0.01$ ,  $p = 0.03$ ) (Fig. 2). Amongst drinkers in UK Biobank, the standard error of log-transformed weekly alcohol consumption is 1.17. This means that a 1 standard deviation increase in log-transformed alcohol consumption would represent an increase from 2 units of alcohol per week to 6.4 units, or from 10 units of alcohol per week to 32.2 units. Alternative methods gave consistent estimates, although there was some heterogeneity in estimates from different genetic variants (Fig. 2). Rs1229984, in *AD1HB*, an alcohol metabolism gene, was the most influential SNP in the analysis (Fig. 2). This SNP explained the most variance in alcohol consumption (and AUD) of all variants. Excluding rs1229984 from the analyses attenuated the MR IVW estimate, which became insignificant ( $\beta = -0.06$ , 95% CI =  $-0.13$  to  $0.01$ ,  $p = 0.09$ ).

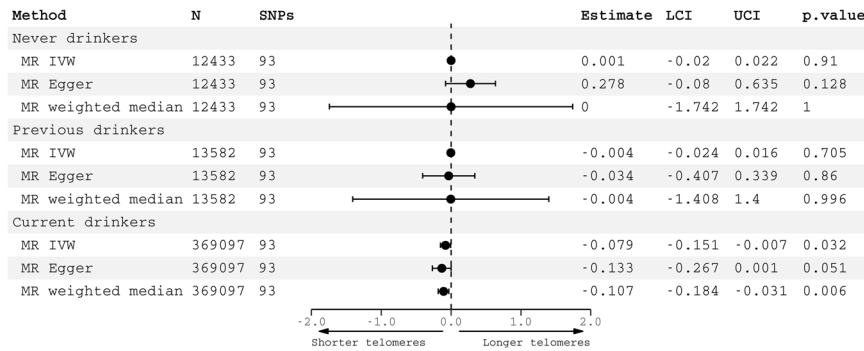
In the AUD MR, using as instrumental variables 24 SNPs robustly and independently associated with AUD, there was a significant association with telomere length (IVW  $\beta = -0.07$ , 95% CI:  $-0.11$  to  $-0.03$   $p = 0.001$ ). Again, the estimates were consistent across all MR methods (Fig. 2). Associations between genetically predicted alcohol intake and telomere length persisted in the multivariable IVW analysis ( $\beta = -0.07$ ,  $-0.14$  to  $-0.01$ ,  $p = 0.03$ ), controlling for smoking and physical activity. Similarly, associations between genetically-predicted AUD and telomere length remained significant in the IVW multivariable MR ( $\beta = -0.07$ , 95% CI:  $-0.12$  to  $-0.02$ ,  $p = 0.005$ ).

We found no evidence of reverse causation. MR Steiger tests for both alcohol consumption ( $p = 9.0 \times 10^{-86}$ ) and alcohol use disorder ( $p = 2.9 \times 10^{-131}$ ) indicated true causal effect directionality. Furthermore, neither associations between genetically-predicted telomere length and alcohol consumption (IVW  $\beta = 0.001$ ,  $-0.02$  to  $0.04$ ,  $p = 0.6$ ), nor AUD (IVW  $\beta = -0.02$ ,  $-0.06$  to  $0.02$ ,  $p = 0.3$ ) were significant (SFigs. 10–13).

In non-linear MR analyses, associations between genetically-predicted alcohol consumption and telomere length were only significant in the highest two quintiles of (IV-free) alcohol consumption, equating to >17 units weekly (17–28 units IVW



**Fig. 3 Non-linear Mendelian randomization estimates of associations between genetically predicted alcohol consumption and telomere length, stratified by weekly alcohol intake (IV-free exposure).** SNPs single nucleotide polymorphisms, LCI lower confidence interval, UCI upper confidence interval.



**Fig. 4 Negative controls for alcohol consumption.** Causal estimates for alcohol consumption on telomere length generated by Mendelian randomization analyses according to alcohol status. Effect estimates are per standard deviation increase in genetically predicted log-transformed alcoholic drinks per week. Abbreviations: SNPs single nucleotide polymorphisms, LCI lower confidence interval, UCI upper confidence interval.

$\beta = -0.17$ , 95% CI  $-0.30$  to  $-0.04$ ,  $p = 0.013$ ; 28+ units IVW  $\beta = -0.20$ , 95% CI:  $-0.40$  to  $-0.004$ ,  $p = 0.046$ ) (Fig. 3). In participants drinking smaller amounts, there was no association. The trend in estimates was significant, as deemed by a meta-regression of the stratum estimates on median alcohol consumption in each stratum ( $p = 0.0016$ ). These results provide a higher degree of confidence in a potentially causal effect in moderate to heavy drinkers than light drinkers. In a sensitivity analysis excluding all related individuals, estimates were similar, albeit with wider confidence intervals for quintile 5 (SFig. 14). Given the prominence of rs1229984 in the linear analyses, we repeated the non-linear analyses excluding this variant. Results were broadly similar, albeit with wider confidence intervals for quintile 5 (SFig. 15). Sex-stratified analyses yielded broadly similar patterns of associations (SFig. 16), although associations with shorter LTL were observed in males in quintiles 2 and 4, and females solely quintile 5. Confidence intervals were larger, reflecting differing alcohol intake group sizes between sexes.

The association between genetically-predicted alcohol consumption and telomere length was only observed in current drinkers (IVW  $\beta = -0.08$ ,  $-0.15$  to  $-0.007$ ,  $p = 0.03$ ) but not in never drinkers ( $\beta = 0.001$ ,  $-0.02$  to  $0.02$ ,  $p = 0.91$ ) nor previous drinkers ( $\beta = -0.004$ ,  $-0.024$  to  $0.02$ ,  $p = 0.71$ ) (Fig. 4). In a sensitivity analysis excluding all related individuals, patterns of association were similar (SFigure 17). Similarly, associations between genetically predicted AUD and telomere length were observed in current drinkers (IVW  $\beta = -0.10$ ,  $-0.15$  to  $-0.05$ ,  $p < 0.0001$ ) but not in never drinkers (IVW  $\beta = 0.18$ ,  $-0.01$  to  $0.36$ ,  $p = 0.06$ ) nor previous drinkers ( $\beta = -0.10$ ,  $-0.31$  to  $0.10$ ,  $p = 0.31$ ) (Fig. 5). Again, patterns of association were comparable in the smaller sample of unrelated participants (SFig. 18).

## DISCUSSION

### Key findings

Using observational and MR approaches we observed consistent associations between two alcohol phenotypes, alcohol consumption

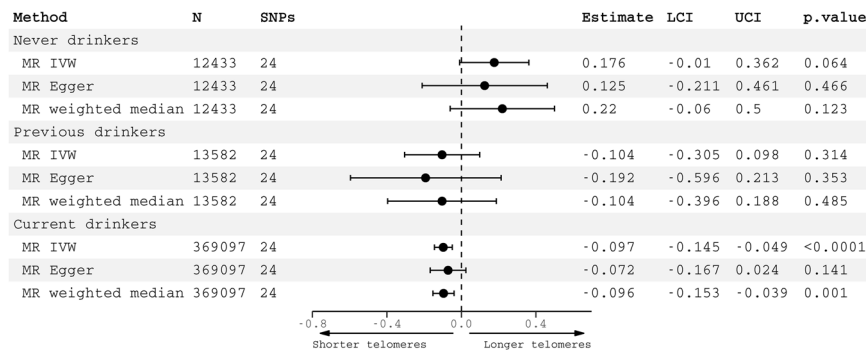
and AUD, and shorter telomere length. Non-linear analyses were suggestive of a threshold relationship between alcohol intake and telomere length.

### Discussion of findings

Estimates for associations between genetically-predicted alcohol consumption and telomere length were broadly consistent across the four MR methods employed. Whilst IV assumptions can never be tested empirically for each SNP, each method allows for different violations of the MR assumptions. Therefore consistent results across methods give greater confidence about the plausibility of the assumptions. The strongest association between genetically predicted alcohol consumption/AUD and telomere length was for rs1229984. The finding is biologically plausible, as this SNP is within an alcohol metabolism gene, *ADH1B*. It could result from greater power to detect a causal effect, as rs1229984 had the strongest associations with a broad range of alcohol use traits of any instrument used. The evidence in support of a causal effect of alcohol consumption on telomere length was weaker than that for AUD, given the relatively large  $p$  value (0.03), and the shift in estimate when the most influential SNP (rs1229984) was removed from the analysis.

Again, for AUD all MR methods gave consistent causal estimates. Alcohol consumption and alcohol use disorder are distinct phenotypes, with only partial overlap in their genetic associations. The reasons for this are unclear [28]. But unlike the quantity-frequency measure AUDIT-C, AUD shows strong genetic correlation with a range of psychiatric disorders and negative medical outcomes [29]. AUD heritability could be partially explained by inherited personality traits, such as impulsivity [30] or sensation-seeking [31] which are less relevant for lower intakes. Overlap with genetic risk to psychiatric disorders such as depression [32] could also be a factor, or even propensity to physiological side effects following large quantities of alcohol.

To contextualize the effect size, in the observational analysis, drinking >29 units (>232 g ethanol, ~ten 250 ml glasses of 14% alcohol by volume (ABV) wine) of alcohol weekly compared to <6



**Fig. 5 Negative controls for alcohol use disorder.** Causal estimates for alcohol use disorder on telomere length generated by Mendelian randomization analyses according to alcohol status. Effect estimates represent associations of a genetically-predicted diagnosis of AUD vs. no diagnosis. Abbreviations: SNPs single nucleotide polymorphisms, LCI lower confidence interval, UCI upper confidence interval.

units (~ two 250 ml glass of wine) was equivalent to 1–2 years of age-related change on telomere length. The MR effect sizes were greater – 1 SD higher genetically-predicted log-transformed alcoholic drinks weekly was equivalent to 3 years of aging. Possible explanations for greater associations in MR analyses are that these may capture the cumulative effects of lifetime propensity to drinking, and be subject to less confounding than observational estimates.

Significant associations between genetically-predicted alcohol and telomere length were only found in current drinkers, providing support that the only path from the genetic variants to LTL is through alcohol. Furthermore, the strength of evidence for a causal effect of alcohol on telomere length was greater in heavier drinkers. This finding suggests a threshold effect, in that a necessary minimum amount of alcohol consumption is required to damage telomeres. Similar relationships with alcohol have been described for other health outcomes [33]. Additionally, multi-variable MR analysis suggested that alcohol's effects are direct and not mediated or confounded by smoking or physical activity.

We are unable to temporally pinpoint alcohol's impact on LTL, especially as both alcohol consumption and LTL are heritable [19, 30]. We hypothesize three (not mutually exclusive) potential pathways: (1) direct effects of adult alcohol consumption on adult LTL, (2) parental alcohol consumption preconception influencing gamete and therefore inherited LTL, (3) maternal alcohol consumption leading to LTL shortening in utero. One mechanism by which alcohol could exert an influence on telomere length could be via oxidative stress and inflammation. Oxidative stress has been demonstrated, in vitro and in vivo, to affect telomere length [34]. Ethanol metabolism can produce reactive oxygen species and reactive nitrogen species [35, 36]. In addition, ethanol can reduce levels of critical cellular antioxidants, such as glutathione [37, 38], compounding the oxidative stress.

### Strengths

Strengths of this study include the triangulation of observational and MR approaches. The observational analysis is the largest to date. No previous MR analysis has been undertaken. Two alcohol traits, alcohol consumption and AUD were examined. Univariable and multivariable two-sample MR analyses were performed, as well as individual-level data interrogated in a non-linear MR analysis. Genetic associations were extracted from the largest GWAS available for both exposures and outcomes published. Multiple sensitivity analyses, including use of negative controls and multivariable MR were undertaken to explore the robustness of the findings.

### Limitations

Some limitations need to be acknowledged. Genetic variants explained a low variance of alcohol traits. Despite this, our analysis

had 85% statistical power to detect a 0.08 standard deviation change in telomere length for a 1 standard deviation increase in alcohol consumption. Due to size differences between groups, we had greater power to detect genetic associations in current drinkers compared to never drinkers. Estimates were similar when excluding related individuals, although less precise and no longer attained nominal statistical significance in quintile 5 in the nonlinear analyses. The observational analysis, use of negative controls, and non-linear MR analysis used self-report to determine alcohol intake. Although this is the only feasible method at scale, it may be subject to misclassification bias. MR estimates the causal effect of lifetime exposure to alcohol. Hence estimates do not necessarily equate to effect sizes if alcohol intake were modified following an intervention during adult life. Genetic associations with alcohol and telomere length were calculated in those with European ancestry and therefore may not apply to other populations with different ancestry groups. Two sample MR assumes that the two populations are broadly similar. UK Biobank is likely subject to a healthy volunteer bias. Prevalence of alcohol dependence in UKB was much lower than general population estimates [38]. This likely reflects cases being defined on the basis of ICD codes in linked health records, which would capture only the most severe cases. MR techniques rely on a number of assumptions which we have tried to test where possible, but residual uncertainty inevitably remains. Finally, telomere length was measured in leucocytes, but the extent to which this reflects other organ tissues is not clear [39].

### CONCLUSIONS

In conclusion, associations between alcohol traits, and genetically-predicted alcohol traits, and telomere length were found. Non-linear analyses suggested that a threshold alcohol intake might be necessary to impact telomere length. These findings lend support to alcohol, particularly at dependent levels, being a causal determinant of telomere length. Multiple sensitivity analyses to assess assumptions of the estimation methods offer a degree of confidence to their plausibility. These findings provide another piece of information in the arsenal of clinicians seeking to persuade patients of the harmful effects of alcohol. Shortened telomeres are proposed as causal risk factors for a number of age-related diseases like Alzheimer's disease. Furthermore, the dose of alcohol is important –even reducing drinking could have benefits.

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## AUTHOR CONTRIBUTIONS

AT conceived and designed the study. AT performed data analysis. HZ, DL and JG performed the AUD genetic association study. SB, BT, JG, TN, SS, KPE, VC and NS helped interpret the data and contributed intellectually to the interpretation of the results. AT drafted the manuscript and SB revised it. All authors reviewed and approved the final version.

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## ADDITIONAL INFORMATION

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