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Exploring causal associations between alcohol and coronary heart disease risk factors: findings from a Mendelian randomization study in the Copenhagen General Population Study

Debbie A. Lawlor[1]*, Børge G. Nordestgaard[2,3,4], Marianne Benn[2,3,4], Luisa Zuccolo[1], Anne Tybjaerg-Hansen[2,3,5], and George Davey Smith[1]

[1]MRC Centre for Causal Analyses in Translational Epidemiology, School of Social and Community Medicine, University of Bristol, Oakfield House, Oakfield Grove, BS8 2BN Bristol, UK; [2]The Copenhagen General Population Study, Herlev Hospital, Copenhagen, Denmark; [3]Faculty of Health Sciences, Copenhagen University Hospital, University of Copenhagen, Copenhagen, Denmark; [4]Department of Clinical Biochemistry, Herlev Hospital, Copenhagen, Denmark; and [5]Department of Clinical Biochemistry, Rigshospitalet, Copenhagen, Denmark

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Aims To explore the causal effect of long-term alcohol consumption on coronary heart disease risk factors.

Methods and results We used variants in *ADH1B* and *ADH1C* genes as instrumental variables (IV) to estimate the causal effect of long-term alcohol consumption on body mass index (BMI), blood pressure (BP), lipids, fibrinogen, and glucose. Analyses were undertaken in 54 604 Danes (mean age 56 years). Both confounder-adjusted multivariable and IV analyses suggested that a greater alcohol consumption among those who drank any alcohol resulted in a higher BP [mean difference in SBP per doubling of alcohol consumption among drinkers: 0.76 mmHg (95% CI: 0.63, 0.90) from multivariable analyses and 0.94 mmHg (−3.03, 4.69) from IV analyses; *P*-value for difference in these results = 0.95]. The positive association of alcohol with HDLc in the multivariable analyses [4.9% (4.7, 5.1)] appeared stronger than in the IV analyses [1.5% (−4.5, 7.4)], and the weak inverse association with fibrinogen in the multivariable analysis [−2.0% (−2.1, −1.8)] was not present in the IV analyses [0.6% (−3.8, 5.0)], but statistically the results for both of these could not be reliably distinguished from each other (*P*-values 0.21 and 0.32, respectively). The weak inverse association of alcohol with BMI [−0.13 kg/m^2 (−0.16, −0.10)] and with triglycerides [−0.4% (−0.7, 0.4)] in multivariable analyses were in contrast to the strong positive association of alcohol with BMI [1.37 kg/m^2 (0.59, 2.15)] and the strong inverse association with triglycerides [−14.9% (−25.6, −4.3)] in IV analyses; $P = 0.006$ and 0.01, respectively, for difference between the two. Alcohol was not associated with non-HDLc or glucose.

Conclusion Our results show adverse effects of long-term alcohol consumption on BP and BMI. We also found novel evidence for a potentially beneficial effect on triglyceride levels, which needs further replication.

Keywords Alcohol • Lipids • Blood pressure • Glucose • Fibrinogen • ADH1B • ADH1C genes

Introduction

It has been suggested that the beneficial effect of modest alcohol consumption on coronary heart disease (CHD) is due to potential beneficial effects on high-density lipoprotein cholesterol (HDLc), insulin sensitivity, and fibrinogen levels.[1–9] Meta-analyses of short-term alcohol intervention studies suggest that it increases HDLc and adiponectin (a marker of insulin sensitivity) and decreases fibrinogen.[9] Many of the individual intervention studies are small, do not randomize to different amounts of alcohol and assess short-term effects; furthermore, there is marked heterogeneity between them.[9] There is also evidence that alcohol has detrimental effects on systolic and diastolic blood pressure (SBP and DBP).[10] Trials of effects on BP have also tended to be small, lack

* Corresponding author. Tel: +44 117 928726; Fax: +44 117 9287292, Email: d.a.lawlor@bristol.ac.uk

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randomization, assess short-term effects only, and there is heterogeneity between them.[10]

One method that is increasingly used to assess causal effects in observational data is Mendelian randomization.[11,12] This method uses genetic variants that are robustly associated with a risk factor as an instrumental variables (IV) to test the causal effect of the risk factor on an outcome of interest. Results from this method are less likely to be influenced by reverse causality or confounding than those obtained from conventional multivariable approaches.[11–13]

Alcohol is degraded to acetaldehyde in the liver by alcohol dehydrogenase (ADH) and then subsequently to acetate by acetaldehyde dehydrogenase.[14] Genetic variants in the *ADH* genes produce between-person variation in the speed with which people degrade alcohol and its metabolites.[14] This in turn influences alcohol reactions, with nausea and flushing associated with high acetaldehyde levels.[15] In studies of East Asian population variants in or near to the *ALDH2* gene, which encodes for ADH 2 and is common in East Asians, has been used as a proxy for alcohol consumption. In most studies, results suggest that greater alcohol consumption is associated with a higher body mass index (BMI), BP, HDLc, and lower low density lipoprotein (LDLc), but is not associated with triglycerides,[16–18] though in one study the association with lipids was in the opposite direction (variants related to greater alcohol consumption associated with lower HDLc and higher LDLc).[19] Mendelian randomization studies in East Asian populations also suggest that greater alcohol consumption is associated with reduced CHD.[17,18] However, since this variant is not polymorphic in European populations it cannot be used to examine the effects of alcohol on CHD in Europeans.

Among European origin populations, common functional polymorphisms in *ADH1B* and *ADH1C* are associated with flushing and levels of alcohol consumption; people who have fast degrading alleles consume less alcohol than those with slow degrading alleles.[15,20,21] One or both of these variants have been used to explore the causal effect of alcohol on CHD risk factors in European populations, but to date the studies have had small sample sizes (all fewer than 4000 participants) and have reported mixed results.[22–27]

The aim of this study was to use variants in *ADH1B* and *ADH1C* as IVs to estimate the causal effect of long-term alcohol consumption on BMI, SBP, DBP, HDLc, non-HDLc, triglycerides, fibrinogen, and glucose, in by far the largest Mendelian randomization study to date of this effect.

Methods

We used data from the Copenhagen General Population Study, a large general population cohort study that aims to eventually recruit 100 000 participants and collect genotypic and phenotypic data of relevance to a wide range of health-related problems. Individuals are randomly selected from the national Danish Civil Registration System and have to be aged 20 years or older and resident in greater Copenhagen; they also have to be white and of Danish decent. Recruitment began in 2003 and is still on-going. Additional study details have been previously published.[28,29]

At the time of genotyping for the present study 60 409 individuals had been recruited. Because a previous diagnosis of CHD may influence alcohol consumption and other lifestyle behaviours related to risk factors, and consequently bias the multivariable analyses and the comparison of those with the IV analyses, we excluded 3363 (5.5%) of the participants who had a previous diagnosis of CHD. Of the remaining 57 046, 57 023(99.9%) had valid data for both genetic variants and 54 604 (96%) had complete data on all variables included in any analyses presented here; these form our study sample. Missing data varied from 0 to 1% for any individual variable (Supplementary material online, *Table S1*).

All measurements were completed by trained staff at one clinic centre. Weight was measured without shoes and in light clothing to the nearest 0.1 kg on Soehnle Professional scales. Height was measured to the nearest 0.1 cm with a Seca stadiometer. Arterial BP was measured in the left arm, after 5 min of rest, with the participant seated; the appropriate cuff size was used. Non-fasting plasma total cholesterol, HDLc, triglycerides, fibrinogen, and glucose were measured using standard hospital assays (Konelab) and were subject to daily internal quality control assessing assay precision and monthly external quality control assessing assay accuracy. Non-HDLc was calculated as total cholesterol minus HDLc.

Amount of usual alcohol intake was reported as weekly consumption of beer in bottles and standard glasses of wine and spirits; each of these in Denmark contains the equivalent of ~12 g of pure alcohol. Information on being a lifetime abstainer or on giving up alcohol was not available and therefore those in the zero (no consumption) category are a mixture of these two. The *ADH1B* (rs1229984, Arg47His in exon 3) and *ADH1C* (rs698, Ile349Val in exon 8) genotypes were identified by TaqMan assays (see Supplementary material online). Genotype calls agreed with those using the Nanogen microelectronic chip technology.[20,30]

Details of how potential confounders were assessed have been reported in previous publications[20,28,29] and in the Supplementary material online.

Statistical analyses

All analyses were conducted in Stata version 11. HDLc, non-HDLc, triglycerides, fibrinogen, and glucose were natural log-transformed to improve normality. An exact test was used to examine Hardy–Weinberg Equilibrium (HWE) and linkage disequilibrium between the two variants was assessed with Lewontin's D' and r^2.[31,32]

Our a priori assumption was that the genetic variants would not be associated with potentially confounding factors of the alcohol-outcome associations, but we checked this using linear or logistic regression.

We used two approaches to examine the relationship of alcohol with outcomes—multivariable linear regression analyses and IV analyses, using *ADH1B* and *ADH1C* genotypes as instruments. Both methods estimate the same association; that of alcohol consumption with CHD risk factors.

Approximately 10% of participants reported no alcohol consumption and among those who reported some consumption, this was markedly right skewed. We examined associations in three different ways: (i) a 10-level categorical variable with all non-drinkers in one category and drinkers split into nine categories with approximately similar numbers in each; (ii) a logged continuous variable of alcohol intake per week among those reporting some consumption; and (iii) a binary variable comparing non-drinkers to drinkers. Method (i) allowed us to examine the shape of the association and deviation for all participants using multivariable analyses, but it was not possible to do this with IV analyses due to potential problems of weak instrument bias (the

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difference in consumption levels between genotype for some of these categories was very small). Method (ii) allowed us to examine a dose–response effect within those consuming some alcohol using both multivariable and IV analyses. To help with interpretation in these analyses, we transformed the right skewed alcohol variable to log base 2 so that results are the change in outcome per doubling of alcohol consumption among drinkers. Method (iii) allowed us to examine the association of any consumption vs. none using both analytical methods. For log-transformed outcome variables, the coefficients were back transformed and are presented as differences in means of the outcome by exposure on a percentage scale.

In the multivariable analyses, we used the category representing one to two drinks (12–24 g) per week as the reference. This allows the nature of the relationship between amount of alcohol consumed and each risk factor amongst those who drink any alcohol (i.e. the group in whom the main IV analyses) to be easily viewed.

In IV analyses, we used the control function estimator and used *ADH1B* and *ADH1C* genotypes jointly as multiple instruments (as categorical variables). We used a Sargan type test of over-identification to check the joint validity of using the two variants together. This tests whether they give consistent estimates when used individually. Further details of a range of additional IV analyses that were undertaken to test how robustour findings were, and of the methods used to statistically compare IV with multivariable analyses are provided in the Supplementary material online.

Additional analyses included examining: (i) by gender associations; (ii) by age associations; (iii) whether associations differed by type of alcohol; and (iv) whether there was an interaction between alcohol (none vs. some) and *ADH1B* in associations with outcomes. The rationale and assumptions for analyses (iv) are provided in the Supplementary material online.

Results

The distributions of all characteristics were the same in the 57 023 eligible participants and in the 54 604 participants with complete data (Supplementary material online, *Table S1*). Overall, 10% of the population reported drinking no alcohol and 9% reported drinking 25 or more drinks per week. Drinking differed by gender, with 13% of women reporting no consumption and 3% reporting 25 or more drinks per week, compared with 6% of men reporting no consumption and 17% reporting 25 or more drinks per week ($P < 0.0001$).

Both genotypes were in HWE ($P = 0.5$ and 0.7 for *ADH1B* and *ADH1C*, respectively). The linkage disequilibrium coefficient Lewontin's D' was 0.86 and r^2 was 0.006 between *ADH1B* and *ADH1C*.

All of the observed confounders were associated with alcohol consumption (Supplementary material online, *Table S2*), but neither genotype was associated with any of the observed confounding factors (*Table 1*).

Multivariable associations

Figure 1 shows the fully adjusted associations of alcohol category with outcomes. Detailed age and gender (Supplementary material online, *Table S3*) and fully adjusted (Supplementary material online, *Table S4*) associations with all outcomes by alcohol category are shown in Supplementary material online. In these analyses (that included all participants, including non-drinkers) alcohol consumption was positively associated with SBP, DBP, and HDLc and inversely associated with BMI and fibrinogen, with some evidence of weak inverse associations with non-HDLc, triglycerides, and glucose also.

Instrumental variable associations

Individuals with greater numbers of fast degrading alleles were more likely to be non-drinkers and on average drank less alcohol if they reported some consumption (*Figure 2* and *Table 2*). These associations are monotonic, but the difference between those with three or four alleles is greater than those between 0 and 1 or 1 and 2. Associations of *ADH1B*, *ADH1C* and the total allele score with alcohol consumption were similar in males and females (Supplementary material online, *Table S5A*; $P_{interaction}$ all ≥ 0.3). Genotypes appeared more strongly related to alcohol consumption at younger compared with older ages (Supplementary material online, *Table S5B*), but there was no strong statistical evidence that associations differed by age ($P_{interaction}$ all ≥ 0.1).

There was no strong statistical evidence that each variant alone differed as an IV for alcohol ($P_{overidentification} \geq 0.2$). Associations using different IV analysis methods (Supplementary material online, *Table S6*) were similar to the main analyses using the control function.

Table 3 shows the results of the main IV and fully confounder-adjusted multivariable analyses for the association of alcohol with CHD risk factors in those who drank some alcohol. Both confounder-adjusted multivariable and IV analyses suggested that greater alcohol consumption among those drinking some alcohol resulted in higher BP. The positive association of alcohol with HDLc in the multivariable analyses appeared stronger than in the IV analyses, and the weak inverse association with fibrinogen in the multivariable analysis was not present in the IV analyses, but statistically the results for both of these could not be reliably distinguished from each other. In contrast, the strong positive association of alcohol with the BMI and the strong inverse association with triglycerides in IV analyses were in marked contrast to the weak inverse associations from multivariable analyses for both outcomes ($P = 0.006$ and 0.01, for the difference between the results from two analytical methods for BMI and triglycerides, respectively). Amount of alcohol consumed was not associated with non-HDLc or glucose among those reporting some consumption.

Table 4 shows the multivariable and IV analyses results comparing non-drinkers to drinkers. For SBP, DBP, HDLc, and fibrinogen, the multivariable and IV analyses were consistent and suggested that not drinking any alcohol is associated with lower BP and HDLc and higher fibrinogen. In the IV analyses, non-drinking was associated with lower BMI and higher triglyceride levels, and there was statistical evidence that these associations differed from the multivariable associations ($P < 0.0001$ for the difference between the results from the two analytical approaches for both outcomes).

Additional analyses

None of the associations of alcohol with CHD risk factors in either multivariable or IV analyses differed by gender (all $P_{interaction} \geq 0.1$) or by age (all $P_{interaction} \geq 0.2$). When analyses were repeated with

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Table 1 Associations of observed confounders with *ADH1B* and *ADH1C* genotype ($n = 54\,604$)

	ADH1B			*ADH1C*			
	Mean (SD) or *n* (%) by genotype			Mean (SD) or *n* (%) by genotype			
	1/1 (slow) $n = 52\,326$	1/2 or 2/2 (fast) $n = 2278$	*P*-value[a]	2/2 (slow) $n = 9542$	1/2 (intermediate) $n = 26\,626$	1/1 (fast) $n = 18\,436$	*P*-value[a]
Age (years)	56.0 (13.2)	56.4 (13.1)	0.14	56.0 (13.1)	56.1 (13.2)	55.9 (13.3)	0.47
Women (*n* %)	29 648 (57)	1261 (55)	0.22	5496 (58)	15 061 (57)	10 352 (56)	0.11
Current smoker (*n* %)	11 110 (21)	477 (21)	0.74	2083 (22)	5610 (21)	3894 (21)	0.28
>4 h per week MVPA	25 410 (49)	1130 (50)	0.33	4649 (49)	12 934 (49)	8957 (49)	0.97
Prescribed antihypertensives	8934 (17)	388 (17)	0.96	1637 (17)	4578 (17)	3107 (17)	0.62
Prescribed statins	3906 (7)	148 (7)	0.12	714 (7)	1989 (7)	1351 (7)	0.83
Income >600 000 Kr	10 423 (20)	479 (21)	0.20	1885 (20)	5363 (20)	3654 (20)	0.60
Education >13 years	9079 (17)	399 (18)	0.84	1720 (18)	4571 (17)	3187 (17)	0.17
Height[b] (m)	1.71 (0.09)	1.71 (0.09)	0.62	1.71 (0.09)	1.71 (0.09)	1.71 (0.09)	0.65

MVPA, moderate or vigorous physical activity; Kr, Danish kroner.
[a]*F*-statistic for continuous variables and χ^2 for categorical variables testing the null hypothesis that distributions of the confounders do not differ by genotype (1 degree of freedom for *ADH1B* and 2 degrees of freedom for *ADH1C*).
[b]Height is included here as an association of the genetic variant with height might suggest a problem with population stratification.

the youngest age group (20–39 years) removed they were essentially the same as those presented in *Tables 3* and *4*.

The median (inter-quartile range) percentage contribution to total alcohol consumed was 69% (47, 92), 18% (0, 40), and 0% (0, 14) for wine, beer, and spirits, respectively. The Spearman correlation coefficients for amount consumed between total alcohol and each of wine, beer, and spirits were 0.83, 0.60, and 0.50, respectively (all *P*-values <0.00001). The pattern of association of *ADH1B* and *ADH1C* with wine and beer was similar to that with total alcohol consumption (Supplementary material online, *Figure S1A* and *B*). Variants did not appear to be associated with amount of spirits consumed (Supplementary material online, *Figure S1c*). Both the multivariable and IV analyses results were similar when either wine or beer was used instead of total alcohol (results available from authors on request). The multivariable analyses with amount of spirits consumed were similar to those with total alcohol (results available from authors), but since there was no association of variants with spirits we were not able to undertake IV analysis with this exposure.

Associations of *ADH1B* with observed confounders were similar among drinkers and non-drinkers, with the exception of smoking (Supplementary material online, *Table S7*). Among non-drinkers those with one or two fast-alleles were less likely to be smokers than those with none, whereas *ADH1B* was not associated with smoking amounts drinkers ($P_{interaction} = 0.02$). There was no strong statistical evidence of an interaction between *ADH1B* and alcohol consumption (none vs. some) in their associations with any outcomes, except for BMI (Supplementary material online, *Table S8*). Among non-drinkers those with one or two fast alleles, compared with none, had higher BMI, whereas among drinkers those with one or two fast alleles had lower BMI (by a similar magnitude to the opposite association in non-drinkers; $P_{interaction} = 0.03$).

Discussion

It has been suggested that HDLc is one of the main mechanism by which moderate alcohol consumption is cardioprotective.[4,5,9] Although our IV analyses were consistent with the positive multivariable association of alcohol with HDLc, they suggested that this association was possibly weak. Importantly, recent Mendelian randomization studies[33–37] and RCTs,[38] question whether HDLc is causally related to CHD, in stark contrast to findings supporting causal effects of cholesterol in remnants or triglyceride-rich lipoproteins and LDLc on CHD.[39–43] If HDLc is not causally related to CHD then it cannot explain any observed beneficial effect on CHD of moderate alcohol consumption.

Other mechanisms that have been proposed to explain reduced CHD risk in those drinking moderate amounts of alcohol are reduce fibrinogen and increased insulin sensitivity.[5,7–9] Consistent with evidence from intervention studies[9] we found greater levels of alcohol consumption to be associated with lower fibrinogen, though our IV analyses suggest that this might be very weak. Furthermore, the evidence that fibrinogen is causally related to CHD is not robust,[44,45] questioning the possibility that it could mediate a suggested beneficial effect of alcohol on CHD. Our analyses suggested no clear beneficial effect of alcohol on non-fasting glucose, but this is a weak proxy for insulin sensitivity. Without fasting insulin or a more direct measure of insulin resistance in our study we are unable to explore Mendelian randomization evidence for an effect of alcohol on insulin resistance.

We surprisingly found a potential beneficial effect of alcohol on triglyceride levels in our IV analysis. Meta-analysis of trials finds no association of alcohol with triglycerides apart from a possible detrimental effect at very high levels.[9] These intervention studies assess short-term effects and for the most part of modest levels vs. no consumption and are not directly comparable with our

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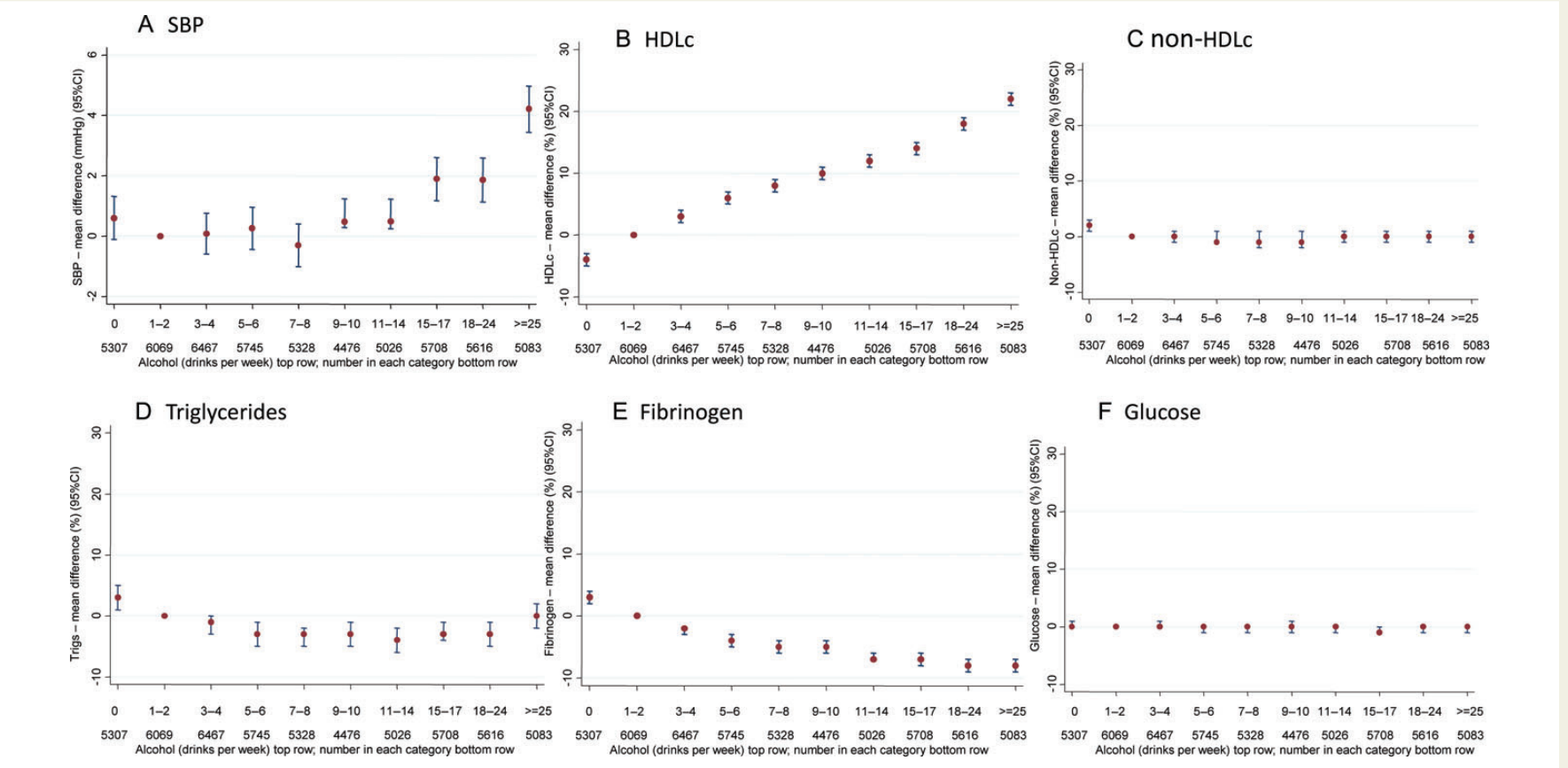

Figure 1 Multivariable associations of alcohol consumption with coronary heart disease risk factors; $n = 54\,604$. All associations are adjusted for age, gender, smoking, physical activity, antihypertensive and statin prescriptions, education, and income. The reference category in all analyses is one to two drinks per week; this takes the null value of 0 for all outcomes. Mean differences are indicated by dots and 95% confidence intervals by vertical lines.

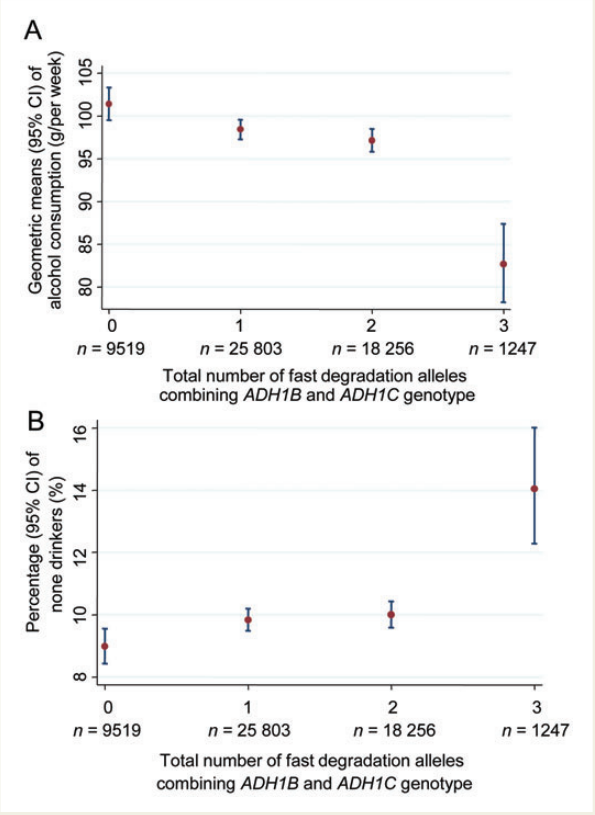

Figure 2 Association of combined *ADH1B* and *ADH1B* fast-allele score with average alcohol consumption levels in participants reporting drinking some alcohol and with not drinking alcohol; $n = 54\,604$. Shows geometric means (dots) and 95% confidence intervals of geometric means (vertical lines) of alcohol grams per week in those drinking some alcohol (*A*) and percentages (dots) and 95% confidence intervals of non-drinkers (*B*) by total number of fast-alleles.

findings. Because the influence of the genetic variant is present from conception for the whole of life it will begin to affect amount of consumption once someone starts to drink. Indeed we have shown that the variants are related to alcohol consumption across age groups from early adulthood to old age and that our associations do not differ by age. It is therefore possible that we have identified a novel potential beneficial effect of alcohol consumption on triglyceride levels. Recent evidence suggests that cholesterol in remnants, rather than triglyceride levels *per se* causes greater CHD, and our result regarding triglyceride levels may be a proxy for remnant cholesterol as the two are highly correlated.[39,40] Previous Mendelian randomization studies using the *ALDH2* variant in East Asian populations have suggested that alcohol consumption is not related to triglyceride levels in those populations,[17–19] and we accept that our finding related to this outcome should be treated with caution unless it is replicated.

For BP our IV and multivariable analyses, results were consistent with each other, with both confirming higher BP in those drinking more alcohol. These findings are consistent with a meta-analysis of randomized controlled trials of alcohol reduction,[9] and with previous Mendelian randomization studies in East Asian populations using the *ALDH2* variant.[16–19] Our IV analyses also suggest that greater alcohol consumption is associated with a higher BMI, which is consistent with previous East Asian population studies using the *ALDH2* variant.[16–19]

Study strengths and limitations

A strength of our study is the large sample size and the use of genetic variants that have been shown to be robustly associated with alcohol consumption[15,20,21] to examine the causal effect of long-term differences in alcohol consumption on a range of CHD risk factors. Assuming that the variants fulfil the assumptions of IV they will provide more valid estimates of causal associations than multivariable regression.[12] Although alcohol consumption was

Table 2 Association of *ADH1B* and *ADH1C* with alcohol consumption

	Mean difference (%) alcohol consumption in those consuming some alcohol (95% CI) $n = 49\,334$	OR of being a non-drinker in the whole cohort (95% CI) $n = 54\,604$
ADH1B one or two fast alleles vs. none	−15.0 (−19.2, −10.8)	1.49 (1.32, 1.69)
F-test	50	41[a]
P-value	<0.0001	<0.0001
ADH1C per fast allele	−2.1 (−3.3, −1.0)	1.06 (1.02, 1.10)
F-test	13	7[a]
P-value	<0.0001	<0.0001
Total *ADH1B* plus *ADH1C* allele score	−2.9 (−4.0, −1.8)	1.09 (1.05, 1.13)
F-test	27	17[a]
P-value	<0.0001	<0.0001

OR, odds ratio; CI, confidence intervals.
[a]From a model of the mean risk difference of not drinking.

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Table 3 Confounder adjusted multivariable and instrumental variable associations of alcohol with coronary heart disease risk factors in those who report some alcohol consumption (i.e. those reporting no consumption have been removed from these analyses)

	Mean difference of each outcome per doubling of alcohol consumption (95% CI). The null value for all results = 0							
	BMI (kg/m^2)	SBP (mmHg)	DBP (mmHg)	HDLc (%)[a]	Non-HDLc (%)[a]	Triglycerides (%)[a]	Fibrinogen (%)[a]	Glucose (%)[a]
Multivariable	−0.13 (−0.16, −0.10)	0.76 (0.63, 0.90)	0.58 (0.50, 0.66)	4.9 (4.7, 5.1)	0.0 (−0.2, 0.1)	−0.4 (−0.7, 0.0)	−2.0 (−2.1, −1.8)	−0.2 (−0.3, 0.0)
Instrumental variable	1.37 (0.59, 2.15)	0.94 (−3.03, 4.91)	0.23 (−1.95, 2.41)	1.5 (−4.5, 7.4)	4.2 (−1.2, 9.7)	−14.9 (−25.6, −4.3)	0.6 (−3.8, 5.0)	−2.1 (−5.6, 1.5)
$P_{IV\ vs.\ MV}$[b]	0.006	0.95	0.71	0.21	0.14	0.01	0.32	0.25

In the multivariable analysis results all results are adjusted for age, gender, smoking, physical activity, prescribed antihypertensives, prescribed statins, education, and income. In the instrumental variable analysis results the control function method was used with *ADH1B* and *ADH1C* used jointly as categorical (indicator) instrumental variables. The first stage *F*-statistic for all instrumental variable analyses = 29.

$n = 49\,334$.

CI, confidence interval.

[a]These results are differences in means on a percentage scale. This is because the distributions of these outcomes on their original scale (mmol/L) were strongly positively skewed, resulting in residuals in the regression models also being skewed. Consequently log transformations were used in the regression models and the resulting coefficients were transformed to give a difference on the percentage scale.

[b]Test of null hypothesis that there is no difference in association of alcohol with each outcome between the confounder-adjusted multivariable association (row 1) and the instrumental variable association using the control function (row 2); *P*-value obtained from the bootstrap distribution.

Table 4 Confounder adjusted multivariable and instrumental variable associations of drinking no vs. some alcohol with coronary heart disease risk factors

	Mean difference of each outcome comparing non-drinkers to those who drink any amount of alcohol (95% CI). The null value for all associations = 0							
	BMI (kg/m^2)	SBP (mmHg)	DBP (mmHg)	HDLc (%)[a]	Non-HDLc (%)[a]	Triglycerides (%)[a]	Fibrinogen (%)[a]	Glucose (%)[a]
Multivariable	0.88 (0.76, 0.99)	−0.20 (−0.75, 0.37)	−0.47 (−0.80, −0.14)	−13.1 (−13.9, −12.3)	2.0 (1.2, 2.8)	5.2 (3.7, 6.7)	7.2 (6.6, 7.9)	0.7 (0.2, 1.2)
Instrumental variable	−4.49 (−8.70, −0.29)	−9.40 (−30.16, 11.37)	−0.80 (−12.04, 10.44)	−16.6 (−47.3, 14.0)	−13.9 (−42.0, 14.3)	89.1 (34.2, 144.0)	5.4 (−17.5, 28.3)	8.3 (−10.2, 26.8)
$P_{IV\ vs.\ MV}$[b]	<0.0001	0.43	0.49	0.90	0.31	<0.0001	0.83	0.42

$n = 54\,604$.

CI, confidence interval.

In the multivariable analysis results all results are adjusted for age, gender, smoking, physical activity, prescribed antihypertensives, prescribed statins, education, and income. In the instrumental variable analysis results the control function method was used with *ADH1B* and *ADH1C* used jointly as categorical (indicator) instrumental variables. The first stage *F*-statistic for all instrumental variable analyses = 22.

[a]These results are differences in means on a percentage scale. This is because the distributions of these outcomes on their original scale (mmol/L) were strongly positively skewed, resulting in residuals in the regression models also being skewed. Consequently log transformations were used in the regression models and the resulting coefficients were transformed to give a difference on the percentage scale.

[b]Test of null hypothesis that there is no difference in association of alcohol with each outcome between the confounder adjusted multivariable association (row 1) and the instrumental variable association using the control function (row 2); *P*-value obtained from the bootstrap distribution.

associated with all of the measured confounders, the two genetic variants were not associated with any. Since this cohort consisted of white individuals of Danish descent, population stratification is unlikely, this is further supported by the lack of association of these variants with height (*Table 1*). It is conceivable that in social groups where there is pressure to consume alcohol any effect of the genetic variants will be over-ridden by this social pressure. However, we have shown that in the youngest age group (20–39 years) among whom this pressure might be greatest, the variants are if anything more strongly associated with consumption than at older ages. In our study the association of *ADH* fast alleles with alcohol when both variants were simply summed together appears to be largely driven by the difference in alcohol consumption between those with three or four alleles compared with all other individuals. However, our main results combined the two variants as separate categorical variables and these results were consistent with those from a weighted allele score IV (results in Supplementary material online). For all analyses the first-stage *F*-statistic did not suggest we had problems with weak instruments.

A key limitation is our inability to explore causal effects on insulin sensitivity due to the lack of appropriate measures of this, or on CHD outcomes due to the lack of numbers. Among the non-drinkers we cannot distinguish between lifetime abstainers and those who have stopped drinking because of ill-health. This limitation would potentially bias the multivariable analyses more so than the IV analyses, but could result in some underestimation of true associations in both. This will also affect the causal analyses we hoped to be able to undertake comparing the association of the variants with each outcome between lifetime abstainers and those who drink alcohol.

Since the genetic variants can only affect amount of alcohol consumed among those who have tried drinking some alcohol, we would not expect them to be related to CHD risk factors in those who have never consumed alcohol (see further discussion in Supplementary material online).[46] In our study, we found no evidence of statistical interaction between *ADH1B* and alcohol consumption with outcomes except for the BMI. The general lack of gene*alcohol consumption interaction could be because the group who (at the time of recruitment) report that they do not drink alcohol includes many who had been heavy drinkers and stopped drinking because of alcohol-related ill-health. Indeed the association of fast alleles with reduced odds of smoking in those reporting no alcohol could be because heavy drinkers who have quit alcohol are more likely to have no fast alleles and are more likely to smoke as a substitute for quitting alcohol (for additional discussion, see Supplementary material online). It could also be that even with this large sample size we lack statistical power to detect an interaction between *ADH1B* and alcohol consumption.

Other studies have examined the possible interaction of *ADH* variants with alcohol in their associations with CHD risk factors, primarily HDLc, but most of these have had very small sample sizes and report inconsistent results; like our study many were also unable to truly determine lifetime abstention.[26] Studies of East Asian populations using the *ALDH2* variant find interactions between alcohol and this variant supporting its causal role in increasing BP.[16,18,19] These findings fit with our main IV results regarding a detrimental effect of alcohol on BP. The interactions of *ADH1B* and alcohol consumption with BMI was driven both by an inverse association of fast alleles with BMI in drinkers (supportive of our assumption that greater consumption causes greater BMI), but also by a positive association in non-drinkers. The latter could be due to sick quitters, who are more likely to have no fast alleles being thinner, but as with the interaction for smoking, these analyses were not the main aim of our paper and could be chance findings given the number of interaction tests completed. They should be treated with caution unless replicated.

Whilst Mendelian randomization approaches tend to reduced bias they have considerably less statistical power than conventional multivariable approaches, and despite the very large sample size used here the IV analyses have wide confidence intervals. It is important, therefore, to replicate our findings, particularly for triglycerides, in other studies with greater statistical power.

To conclude, taking our results together with those of other studies, it seems unlikely that if moderate alcohol consumption is causally related to lower CHD risk this is mediated via HDLc or fibrinogen. Improved insulin sensitivity might mediate a potential beneficial effect, but we have been unable to test this. We have found evidence for a possible beneficial effect of alcohol on triglyceride levels, but these results require further validation. Finally, our results support a detrimental effect of alcohol consumption on BP and BMI.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

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Conflict of interest: none declared.

Authors' contributions

Study concept and design: Lawlor, Nordestgaard, Davey Smith; *Acquisition of data*: Nordestgaard, Tybjaerg-Hansen, Benn; *Batabase handling and updating*: Benn; *Statistical analysis*: Lawlor; *Drafting of the manuscript*: Lawlor; *Interpretation of results*: Lawlor, Nordestgaard, Benn, Zuccolo, Tybjaerg-Hansen, Davey Smith; *Critical revision of the manuscript for important intellectual content*: Lawlor, Nordestgaard, Benn, Zuccolo, Tybjaerg-Hansen, Davey Smith; *Obtained funding*: Nordestgaard, Tybjaerg-Hansen; *Administrative, technical, or material support*: Nordestgaard, Benn; Lawlor, Nordestgaard and Benn had full access to all of the data in the study

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and take responsibility for the integrity of the data (Nordestgaard and Benn) and the accuracy of the data analysis (Lawlor).

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