



Mendelian Randomization Causal Analysis

Increased alcohol consumption as a cause of alcoholism, without similar evidence for depression: a Mendelian randomization study

Marie Kim Wium-Andersen,^{1,2,3} David Dynnes Ørsted,^{1,2,3}
Janne Schurmann Tolstrup⁴ and Børge Grønne Nordestgaard^{1,2,3,5*}

¹Department of Clinical Biochemistry, and ²Copenhagen General Population Study, Copenhagen University Hospital, Herlev, Denmark, ³Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark, ⁴National Institute of Public Health, University of Southern Denmark, Copenhagen, Denmark and ⁵Copenhagen City Heart Study, Copenhagen University Hospital, Frederiksberg, Denmark

*Corresponding author. Department of Clinical Biochemistry, Herlev Hospital, Copenhagen University Hospital, Herlev Ringvej 75, 2730 Herlev, Denmark. E-mail: Boerge.Nordestgaard@regionh.dk

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Abstract

Background: Increased alcohol consumption has been associated with depression and alcoholism, but whether these associations are causal remains unclear. We tested whether alcohol consumption is causally associated with depression and alcoholism.

Methods: We included 78 154 men and women aged 20–100 years randomly selected in 1991–2010 from the general population of Copenhagen, Denmark, and genotyped 68 486 participants for two genetic variants in two *alcohol dehydrogenase (ADH)* genes, *ADH-1B* (rs1229984) and *ADH-1C* (rs698). We performed observational and causal analyses using a Mendelian randomization design with antidepressant medication use and hospitalization/death, with depression and alcoholism as outcomes.

Results: In prospective analyses, the multifactorially adjusted hazard ratio for participants reporting >6 drinks/day vs participants reporting 0.1–1 drinks/day was 1.28 (95% confidence interval, 1.00–1.65) for prescription antidepressant use, with a corresponding hazard ratio of 0.80 (0.45–1.45) for hospitalization/death with depression and of 11.7 (8.77–15.6) for hospitalization/death with alcoholism. For hospitalization/death with alcoholism, instrumental variable analysis yielded a causal odds ratio of 28.6 (95% confidence interval 6.47–126) for an increase of 1 drink/day estimated from the combined genotype combination, whereas the corresponding multifactorially adjusted observational odds ratio was 1.28 (1.25–1.31). Corresponding odds ratios were 1.11 (0.67–1.83) causal and 1.04 (1.03–1.06) observational for prescription antidepressant use, and 4.52 (0.99–20.5) causal and 0.98 (0.94–1.03) observational for hospitalization/death with depression.

Conclusions: These data indicate that the association between increased alcohol consumption and alcoholism is causal, without similar strong evidence for depression.

Key words: Alcohol, depression, antidepressant medication, alcoholism, Mendelian randomization, general population, *alcohol dehydrogenase* gene

Key Messages

- Previous studies have reported an association between alcohol consumption and depression and alcoholism, but whether these represent causal associations is unknown.
- The results from this study suggest that increased alcohol consumption is causally associated with alcoholism.
- This study did not find strong evidence of a causal association between alcohol consumption and depression or psychological distress.

Introduction

Heavy alcohol consumption is associated with increased risk of depression and alcoholism.^{1–11} However, heavy alcohol consumption is also associated with smoking, physical activity and socioeconomic status^{3,4,12} and a range of other factors, which may confound the associations. Also, because a person may increase alcohol consumption during the course of developing depression, it is difficult to determine whether heavy alcohol consumption precedes or rather is a consequence of depression. Thus, it is still unclear whether increased alcohol consumption is in fact causally associated with depression. For alcoholism, alcohol consumption is an obvious necessary factor, but whether heavy alcohol consumption per se is a direct cause of alcoholism is unknown.

An approach to examine the relationship between alcohol consumption and depression and alcoholism is the Mendelian randomization design.^{13,14} Here, genetic variants which influence an individual's alcohol consumption are used as proxies for adulthood lifelong alcohol consumption. The rationale is that these genetic variants are determined at conception by the random allocation of chromosomes during gamete formation, which means that they are typically not associated with any known or unknown confounders (e.g. smoking, physical activity, or socioeconomic status). As such, Mendelian randomization is analogous to a randomized controlled trial where participants are randomized to either 'intervention' or 'placebo', where the randomization ensures equal distribution of confounders and that the relationship is not prone to reverse causation. Consequently, if increased alcohol consumption per se is in fact a causal risk factor for depression and alcoholism, we would expect participants with genetic variants resulting in adulthood lifelong increased alcohol consumption to have a higher risk of depression and alcoholism.¹⁵ On the other hand, if alcohol consumption per se

is not causal, we would expect to find no increase in risk of depression and alcoholism by genotype.

We tested the hypothesis that increased alcohol consumption is causally associated with depression, psychological distress and alcoholism. For this purpose, we studied 78 154 men and women from the Danish general population and used a Mendelian randomization design. First, we tested whether increased alcohol consumption was associated with increased depression, psychological distress and alcoholism in an observational study. Second, we tested whether two genetic variants in two *alcohol dehydrogenase* (*ADH*) genes [*ADH-1B* (rs1229984) and *ADH-1C* (rs698)] were associated with increased alcohol consumption. *ADH* is an enzyme responsible for degradation of alcohol to acetaldehyde in the liver. The *ADH-1B**2 allele of *ADH-1B* increases *ADH* activity by 32% compared with the most frequent allele (*ADH-1B**1), resulting in greater accumulation of acetaldehyde.¹⁶ Conversely, the *ADH-1C**2 allele of *ADH-1C* decreases *ADH* activity by 2% compared with the most frequent allele (*ADH-1C**1), resulting in reduced accumulation of acetaldehyde. Acetaldehyde accumulation leads to nausea, flushing and tachycardia (the same effects as after ingestion of disulfiram), which means that participants with slow alcohol degradation alleles (*ADH-1B**1 and *ADH-1C**2) will accumulate acetaldehyde more slowly and thus be able to consume more alcohol than participants with fast degradation alleles.^{17,18} This association between both *ADH-1B* and *ADH-1C* and alcohol consumption has been observed in multiple studies, including studies of individuals of European ancestry.^{19–23} Third, we tested whether the two *ADH* genetic variants were directly associated with risk of depression, psychological distress and alcoholism. Finally, we used instrumental variable analysis to test whether alcohol consumption is causally associated with depression, psychological distress and alcoholism, and

compared the effects sizes with corresponding observational estimates.

Methods

The study was approved by Danish ethical committees and by Herlev Hospital. All participants gave written informed consent.

Participants

We used two large independent Danish general population studies, the Copenhagen General Population Study (CGPS) 2003–10 examination ($n = 67\,650$) and the Copenhagen City Heart Study (CCHS) 1991–94 and/or 2001–03 examinations ($n = 10\,504$).^{24,25} Participants from both studies were 20–100 years old and were randomly selected from the national Danish Civil Registration System,²⁶ to represent the Danish general population. All 78 154 participants were White and of Danish descent (i.e. the national Danish Civil Registration System showed that each participant and both parents were born in Denmark and were Danish citizens); participants without data on alcohol consumption were excluded ($n = 2\,068$). Participants filled in a questionnaire, which was reviewed together with an investigator on the day of attendance, had a physical examination performed and had blood samples drawn for biochemical measurements and for DNA extraction. If a participant appeared in more than one study, only data from the first examination were included. Because all individuals in Denmark have a unique identification number, we used the national Danish Civil Registration System to register emigration or death for all participants.

Alcohol consumption

Alcohol intake was reported by participants as weekly consumption of bottles of beer and standard glasses of wine and spirits. This information was used to calculate average alcohol consumption in drinks/day or drinks/week; one drink ~ 12 g alcohol. Because specific information on being a lifetime abstainer or on giving up alcohol was not available, participants reporting no consumption represent a combination of the two.

Alcohol dehydrogenase (ADH) genotypes

The *ADH-1B* genotype (rs1229984;Arg47His) and *ADH-1C* genotype (rs698;Ile349Val) were identified by Nanogen technology^{16,27–29} in the CCHS (9768 genotyped) and by TaqMan assays³⁰ in the CGPS (58 718 genotyped). Both genotypes are located on chromosome 4 in the *ADH-1B* gene and the *ADH-1C* gene, respectively. The linkage disequilibrium coefficient D' was 0.87 and r^2

was 0.01, and both genotypes were in Hardy–Weinberg equilibrium in both studies. The minor allele frequency of the *ADH-1B* and *ADH-1C* genotypes was 2% and 42%, respectively.

Depression

Depression was defined using three independent sources of information. First, we used information on self-reported antidepressant medication use ascertained as a affirmative answer to the question ‘Do you daily (or most days) use antidepressants, sedatives or relaxing pills?’ (Yes/no). These did not include sleeping pills or pain-relieving medication. Second, because antidepressant medication can only be obtained by prescription in Denmark, we obtained information about every prescription of antidepressant medication claimed by study participants from 1995 through 2010 from the national Danish Register of Medicinal Product Statistics. We used Anatomical Therapeutic Chemical (ATC) codes for: Selective Serotonin Reuptake Inhibitors (SSRI), N06AB; tricyclic antidepressants (TCA), N06AA; Noradrenaline Reuptake Inhibitor (NARI), Serotonin and Noradrenaline Reuptake Inhibitor (SNRI) and Noradrenergic and Specific Serotonergic Antidepressants (NaSSA), N06AX. We only included participants who at some point in their life had purchased antidepressant medication prescribed to them by a doctor for a period of at least 6 continuous months with an average daily dose of at least 0.75 of a standard WHO-defined daily dose.³¹ Third, diagnoses of depression among all participants were obtained: from the national Danish Patient Registry with information on all hospital discharge diagnoses of depression from psychiatric and somatic hospitals since 1977 and on diagnoses from emergency rooms and outpatient clinics since 1995;³² and from the national Danish Causes of Death Registry with information on causes of death on all individuals in Denmark since 1970.³³ Depression was categorized in ICD8 codes 296.0, 296.2, 298.0 and 300.4 until 1994, and ICD10 codes F32 and F33 from 1994 onwards.

Psychological distress

Psychological distress was ascertained by two self-reported questions: ‘Do you have the feeling that you have not accomplished very much recently?’ (Yes/no). Also ‘Do you feel like giving up?’ (Yes/no). The responses to each question were analysed separately.

Alcoholism

Diagnoses of alcoholism were obtained from the national Danish Patient Registry and the national Danish Causes of Death Registry. Alcoholism was categorized in ICD8 codes 291 and 303, and ICD10 code F10.

Covariates

Participants reported on: smoking status (never; former; current), number of cigarettes/day where other tobacco consumption was converted into cigarettes/day equivalents), leisure time physical activity (0–2 h of moderate activity/week; 2–4 h of moderate activity/week; >4 h of moderate activity or 2–4 h of vigorous activity/week; >4 h of vigorous activity/week), level of education after primary and lower secondary school [no education; shorter education (less than 3 years); basic vocational training (1–3 years); higher education (≥ 3 years); university education], level of income (lowest; middle; highest) and civil status (married; unmarried; separated; widow/widower). Body mass index (BMI) was measured as weight in kilograms divided by measured height in metres squared. Plasma levels of C-reactive protein (CRP) were measured with a high-sensitivity assay using latex-enhanced turbidimetry (Dako, Glostrup, Denmark) or nephelometry (Dade Behring, Deerfield, IL).²⁴

Statistical analyses

Stata version 12.1 (StataCorp, College Station, TX) was used. To achieve maximal statistical power, data from the CGPS and the CCHS were combined; however, results were similar within each study separately. In accordance with a Mendelian randomization study, we conducted four analyses as described below.

First, we tested whether alcohol consumption was associated with increased risk of depression, psychological distress and alcoholism. To include examination of very high alcohol intake in observational analyses, participants were divided into eight categories of drinks/day: 0, 0.1–1 (reference group), 1.1–2, 2.1–3, 3.1–4, 4.1–5, 5.1–6 and >6. We used logistic regression models to calculate odds ratios (ORs) with 95% confidence intervals (CIs) for cross-sectional endpoints. For prospective endpoints we used a Cox proportional hazards regression model with age as the underlying time scale (this means that age is automatically adjusted for), and left truncation (= delayed entry) in 1991–94, 2001–03 or 2003–10 as appropriate, to calculate hazard ratios (HRs) with 95% CI; participants with previous prescription antidepressant medication ($n = 5812$) / hospitalization with depression ($n = 422$) / hospitalization with alcoholism ($n = 1116$) were excluded from the relevant analyses. Follow-up began at examination and participants were censored at first event (prescription of antidepressant medication ($n = 3321$) / hospitalization/death with depression ($n = 777$) / alcoholism ($n = 736$)), death from other causes ($n = 6886$), emigration ($n = 380$) or end of follow-up June 2011, whichever came first. We tested the proportional hazards assumption by using Schoenfeld residuals; no important violations were

detected. For cross-sectional and prospective analyses we used two different models adjusted for (i) age and gender, and (ii) age, gender, smoking status, cigarettes/day, physical activity, education, income, civil status, BMI and plasma CRP. We had $\geq 99.9\%$ complete data. Missing values were imputed based on age and gender before multifactorial adjustment.³⁴

Second, we tested whether each of the *ADH* genotypes was associated with level of alcohol consumption. Furthermore, we combined *ADH-1B* and *ADH-1C* genotypes into one *ADH* genotype score with four combinations ranked according to alcohol consumption.

Third, we tested whether the *ADH* genotype combinations were directly associated with depression, psychological distress and alcoholism using unadjusted logistic regression models.

Fourth, to test the causal association between alcohol consumption and each of the endpoints, we performed instrumental variable analysis with a two-stage (sequential) regression model using each genotype and the genotype combinations as instruments to estimate the causal effect of an increase of 1 drink/day on risk of depression, psychological distress and alcoholism.³⁵ Participants reporting 0 drinks/day (a combination of lifetime abstainers and individuals who gave up drinking later in life) were excluded in this analysis to ensure that our results would show the effect of drinking more or less alcohol, and not the effect of drinking vs not drinking. The first stage was a linear regression of each of the *ADH* genotypes or genotype combination on level of alcohol consumption. F-statistics >10 indicate sufficient statistical strength to carry out valid instrumental variable analysis.¹⁴ The second stage was a logistic regression of alcohol consumption determined by genotypes (generated in the first stage) on depression, psychological distress or alcoholism to calculate causal ORs.³⁵ For comparison, we calculated observational ORs for the association between an increase of 1 drink/day and each of the endpoints using logistic regression.

Finally, we combined the endpoints of depression and psychological distress into four groups with participants having: none of the endpoints, one of the endpoints, two of the endpoints, and more than two endpoints. We performed age- and gender- and multifactorially-adjusted logistic regression models and instrumental variable analysis for an increase of 1 drink/day on risk of one endpoint, two endpoints and more than two endpoints versus none of the endpoints, respectively.

Results

Baseline characteristics of the 78 154 participants by alcohol consumption are listed in Table 1, by endpoints in

Table 1. Baseline characteristics of 78 154 individuals from the general population by alcohol consumption in drinks/day

	Alcoholic drinks/day							P-trend alcohol ^c	P-value depression	P-value alcoholism	P-trend genotype
	0	0.1–1	1.1–2	2.1–3	3.1–4	4.1–5	5.1–6				
No. (%)	9943 (13)	28 328 (36)	19 486 (25)	10 522 (13)	5131 (7)	2428 (3)	1130 (1)	1186 (2)			
Age, years, median (IQR)	59 (46–70)	54 (44–65)	58 (48–67)	60 (51–68)	61 (52–68)	60 (53–67)	60 (53–67)	59 (51–65)	5 × 10 ^{−91}	0.22	0.67
Women, No. (%)	7302 (73)	18 745 (66)	10 487 (54)	4342 (41)	1444 (28)	446 (18)	169 (15)	146 (12)	1 × 10 ^{−300}	2 × 10 ^{−64}	0.02 ^{NS}
Smoking, current smokers, No. (%)	3035 (31)	5637 (20)	4005 (21)	2480 (24)	1388 (27)	868 (36)	436 (39)	563 (47)	1 × 10 ^{−198}	3 × 10 ^{−34}	0.14
Cigarettes/day, median (IQR) ^a	15 (9–20)	13 (7–20)	13 (7–20)	15 (7–20)	15 (8–20)	20 (10–24)	20 (11–25)	20 (15–30)	5 × 10 ^{−142}	2 × 10 ^{−3} NS	0.80
Low leisure time physical activity, inactive or less than 2–4 h moderate/day, No. (%)	6389 (64)	15 093 (53)	9574 (49)	5105 (49)	2494 (49)	1226 (50)	587 (52)	692 (58)	7 × 10 ^{−5}	1 × 10 ^{−31}	0.74
Less than 3 years of education, No. (%) ^b	7884 (76)	16 939 (60)	11 015 (57)	5846 (56)	2761 (54)	1439 (59)	657 (58)	772 (65)	2 × 10 ^{−4}	3 × 10 ^{−46}	0.12
Lowest income, No. (%)	3473 (35)	4977 (18)	2926 (15)	1387 (13)	624 (12)	313 (13)	155 (14)	252 (21)	9 × 10 ^{−18}	5 × 10 ^{−114}	0.37
Married, No. (%)	5422 (55)	19 844 (70)	14 717 (76)	8197 (78)	4051 (79)	1893 (78)	839 (74)	770 (65)	3 × 10 ^{−31}	3 × 10 ^{−91}	0.02 ^{NS}
Body mass index, kg/m ² , median (IQR)	26 (23–30)	25 (23–28)	25 (23–28)	26 (23–28)	26 (24–28)	26 (24–29)	27 (24–29)	27 (24–29)	1 × 10 ^{−100}	0.69	0.04 ^{NS}
C-reactive protein, mg/l, median (IQR)	1.8 (1.2–3.4)	1.5 (1.1–2.5)	1.5 (1.1–2.4)	1.5 (1.1–2.4)	1.5 (1.1–2.4)	1.7 (1.2–2.7)	1.7 (1.2–3.0)	1.9 (1.2–3.4)	1 × 10 ^{−29}	8 × 10 ^{−40}	0.27

Baseline characteristics for participants in the Copenhagen General Population Study and the Copenhagen City Heart Study combined. IQR = interquartile range.

^a Based on current smokers. ^b After primary and lower secondary school. ^c P-trend when non-drinkers were excluded.^{NS} Not significant after correction for multiple comparisons using the Bonferroni correction (that is, P-values were multiplied with 40 multiple tests).

Supplementary Table 1A and B and by *ADH* genotype combinations in Supplementary Table 2 (available as Supplementary data at *IJE* online). All potential confounders were associated with increased alcohol consumption and/or with endpoints, but not with the genotype combination. Thus, genotype combination can be used as a largely unconfounded instrument to assess the causal association between adulthood lifelong increased alcohol consumption on psychological distress, depression and alcoholism. Mean follow-up time was 5.4 years (range: 0.0–19.7 years).

Alcohol consumption, depression, psychological distress and alcoholism

In the prospective analyses, for prescription antidepressant use the multifactorially-adjusted HR was 1.28 (95% confidence interval, 1.00–1.65) for participants reporting >6 vs 0.1–1 drinks/day, with a corresponding HR of 0.80 (0.45–1.45) for hospitalization/death with depression (Figure 1). In contrast, multifactorially-adjusted HRs for hospitalization/death with alcoholism were 1.67 (1.29–2.15) for 1.1–2 drinks/day, 2.95 (2.27–3.83) for 2.1–3 drinks/day, 3.54 (2.62–4.77) for 3.1–4 drinks/day, 5.97 (4.41–8.07) for 4.1–5 drinks/day, 7.34 (5.23–10.3) for 5.1–6 drinks/day and 11.7 (8.77–15.6) for participants reporting >6 drinks/day vs participants reporting 0.1–1 drinks/day.

In cross-sectional analyses, for self-reported antidepressant use the multifactorially-adjusted ORs were 1.36 (1.07–1.73) and 1.43 (1.15–1.78) for participants reporting 5.1–6 and >6 vs 0.1–1 drinks/day (Figure 2). Corresponding ORs were 1.18 (95% CI, 1.02–1.36) and 1.47 (1.29–1.69) for not accomplishing much, and 1.34 (1.06–1.69) and 1.79 (1.47–2.17) for wanting to give up. Participants reporting 0 drinks/day also had increased risk of self-reported antidepressant use, not having accomplished much and wanting to give up, but this group represents a combination of lifetime abstainers and individuals who gave up drinking later in life.

ADH genotypes and alcohol consumption

Compared with participants with the *ADH-1B**2/2 genotype (fast metabolizers), participants with the *ADH-1B**1/1 genotype (slow metabolizers) had 63% higher alcohol consumption (4.1 drinks more per week) (*P*-trend = 5×10^{-29}) (Supplementary Figure 1, available as Supplementary data at *IJE* online). Likewise, compared with participants with the *ADH-1C**1/1 genotype (fast metabolizers), participants with the *ADH-1C**2/2 genotype (slow metabolizers) had 5% higher alcohol consumption (0.5 drinks more per week) (*P*-trend = 1×10^{-6}). When genotypes were combined, participants with the slowest

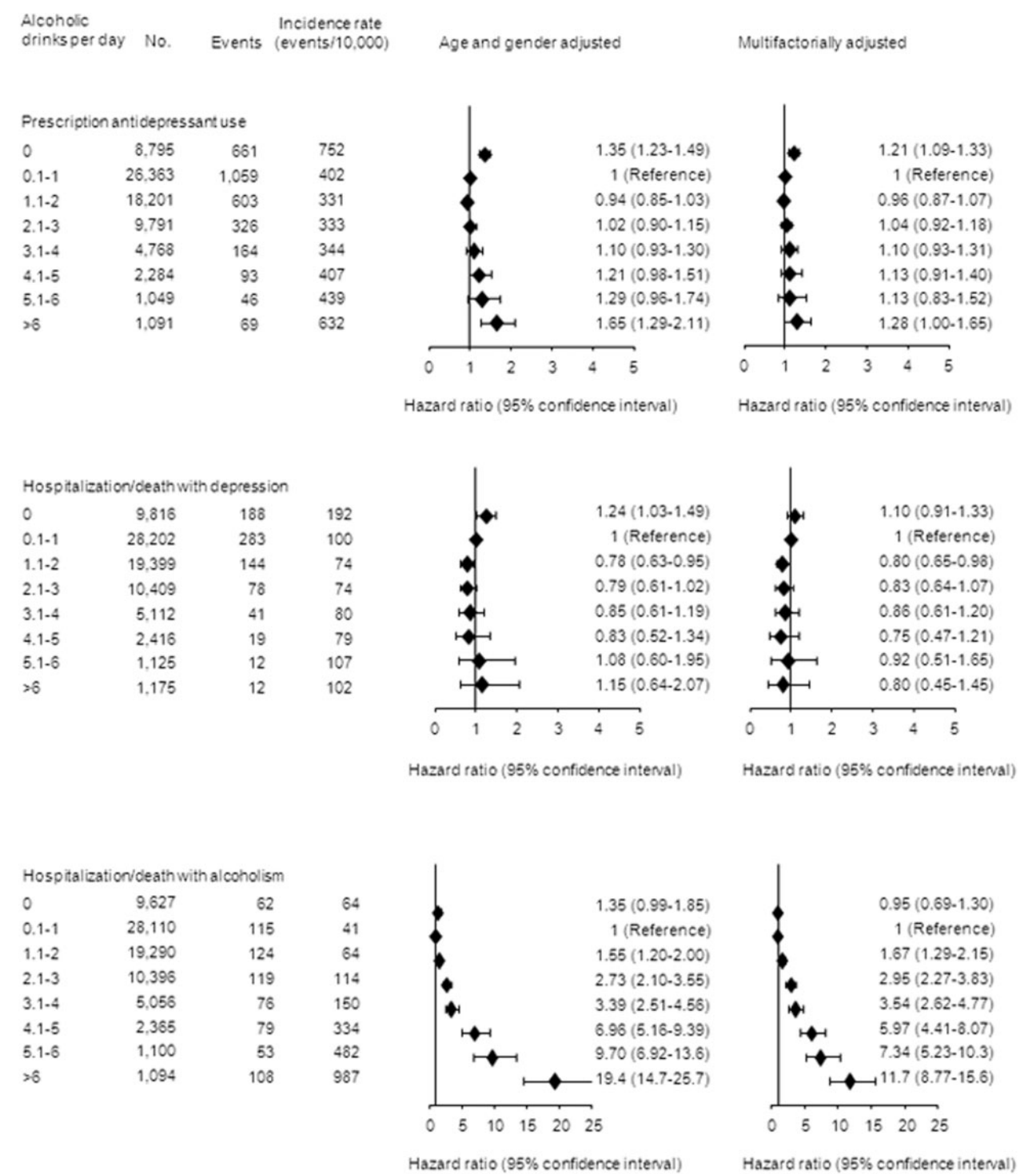


Figure 1. Prospective associations between alcohol consumption, prescription antidepressant medication and hospitalization/death with depression and alcoholism in the general population. Based on 78 154 participants from the Copenhagen General Population Study and Copenhagen City Heart Study, combined. Multifactorially-adjusted was for age, gender, smoking status, cigarettes/day, physical activity, education, income, civil status, body mass index and plasma C-reactive protein.

metabolizing genotype combination had a 27% increase in alcohol consumption (2.3 drinks more per week) compared with participants with the fastest metabolizing genotype combination (P -trend = 6×10^{-17}).

ADH genotypes, depression, psychological distress and alcoholism
Genotype combinations associated with increased alcohol consumption were not associated with increased

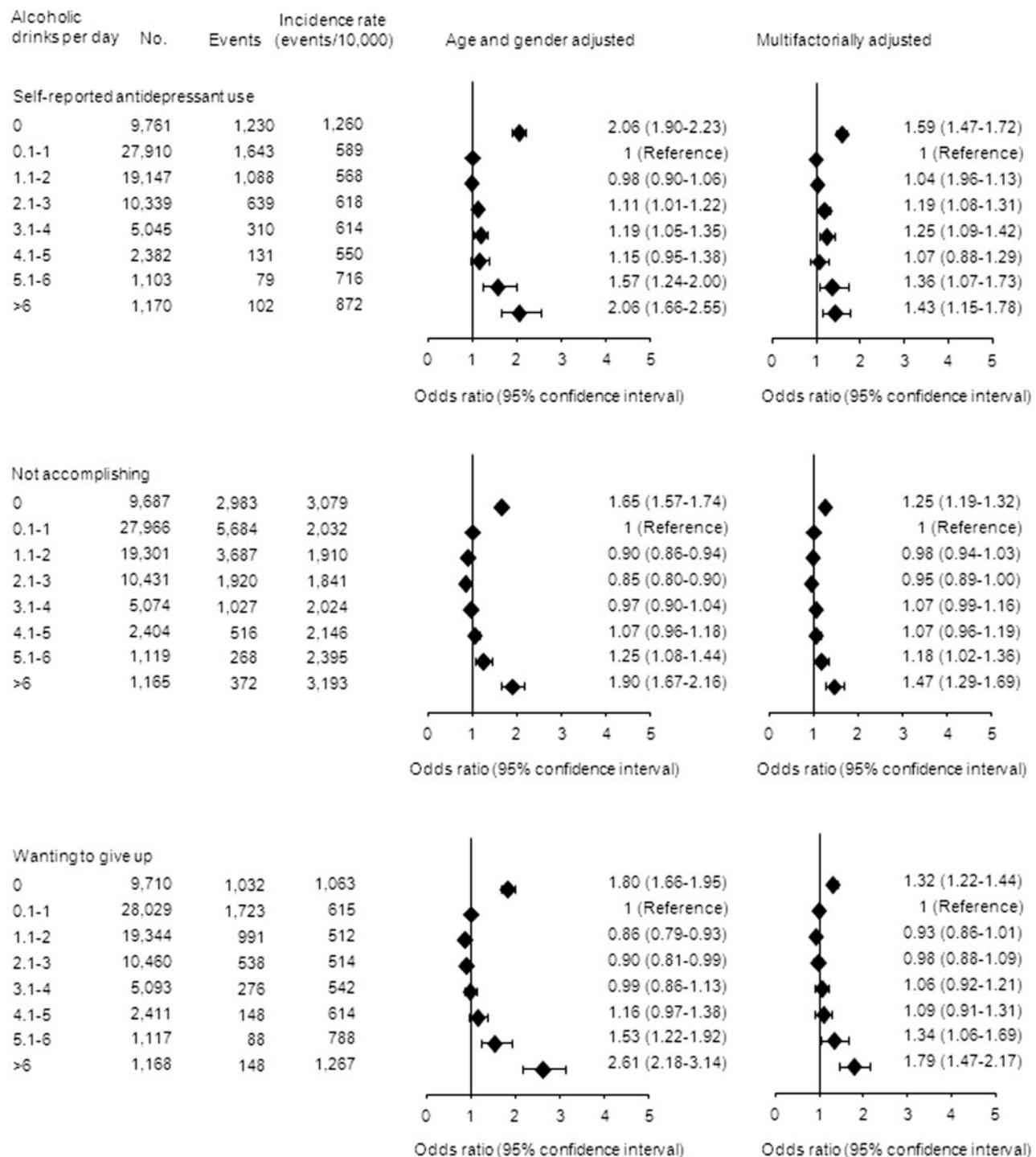


Figure 2. Cross-sectional associations between alcohol consumption, self-reported antidepressant medication, and psychological distress in the general population. Based on 78 154 participants from the Copenhagen General Population Study and Copenhagen City Heart Study, combined. Not all participants answered questions concerning use of antidepressant medication or psychological distress, therefore numbers vary slightly. Multifactorially-adjusted was for age, gender, smoking status, cigarettes/day, physical activity, education, income, civil status, body mass index and plasma C-reactive protein.

risk of self-reported antidepressant use (P -trend = 0.86), prescription antidepressant use (P -trend = 0.75), hospitalization/death with depression (P -trend = 0.06), not having accomplished much (P -trend = 0.43) or wanting to

give up (P -trend = 0.45) (Figure 3). In contrast, genotype combinations were associated with increased risk of hospitalization/death with alcoholism (P -trend = 1×10^{-6}).

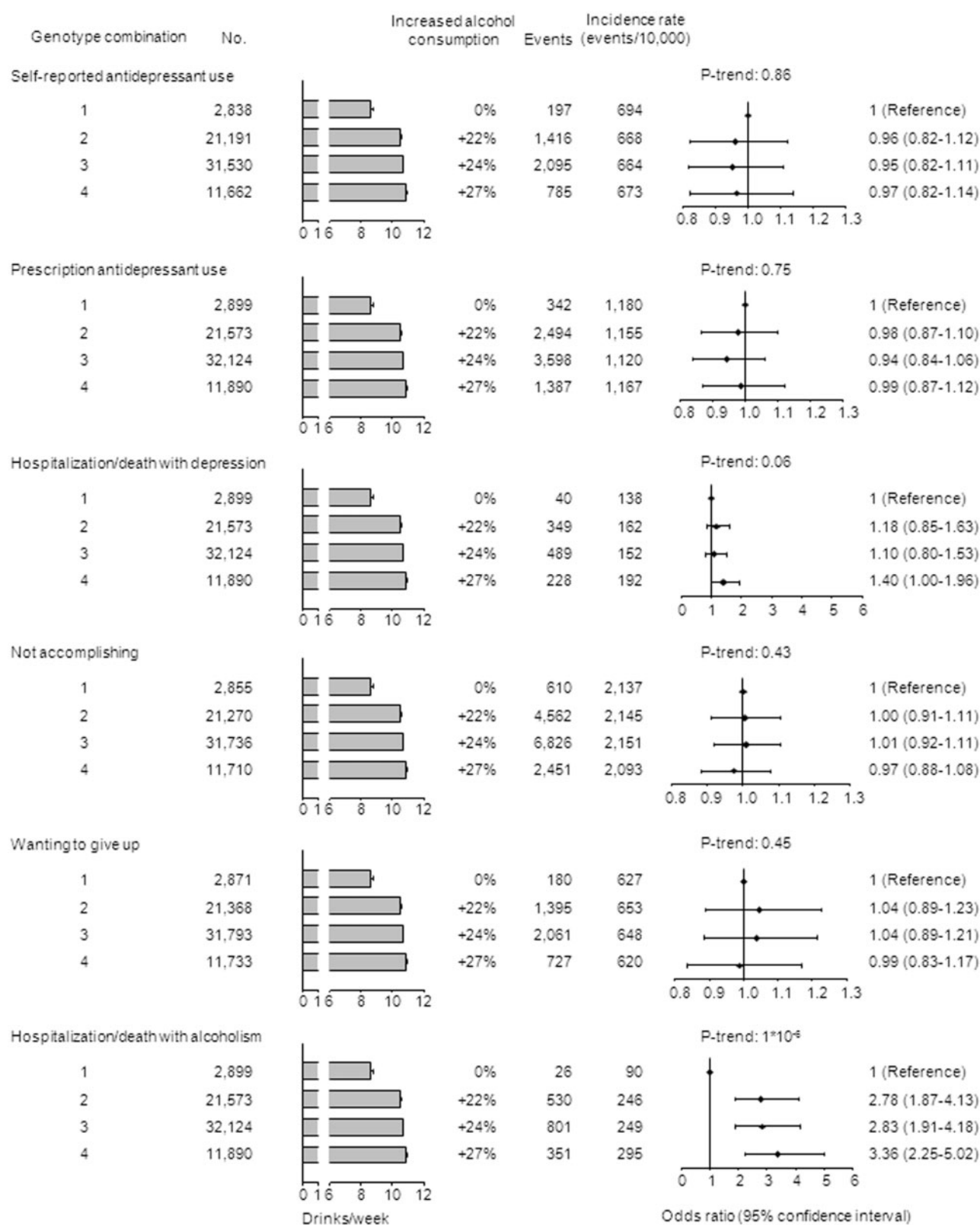


Figure 3. Associations between *ADH* genotypes, depression, psychological distress and alcoholism. Based on 68 486 participants from the Copenhagen General Population Study and Copenhagen City Heart Study, combined. Not all participants answered questions concerning use of antidepressant medication or psychological distress, therefore numbers vary slightly. Odds ratios were unadjusted, because genotypes do not associate with potential confounders (see [Supplementary Table 2](#), available as [Supplementary data](#) at [IJE](#) online). Genotype combinations were for *ADH-1B* genotype and *ADH-1C* genotype with 1 being the fastest metabolizing genotype combination and 4 the slowest. *ADH*, alcohol dehydrogenase.

Alcohol consumption, depression, psychological distress and alcoholism: causal vs observational estimates

For self-reported antidepressant use, instrumental variable analysis yielded a causal OR of 0.86 (0.45–1.64) for an increase of 1 drink/day estimated from the combined genotype combinations, whereas the corresponding multifactorially-adjusted observational OR was 1.07 (1.05–1.09) (Figure 4). For prescription antidepressant use, corresponding ORs were 1.11 (0.67–1.83) causal and 1.04 (1.03–1.06) observational; for hospitalization/death with depression, 4.52 (0.99–20.5) causal and 0.98 (0.94–1.03) observational; for not accomplishing much 1.01 (0.69–1.48) causal and 1.03 (1.02–1.04) observational; and for wanting to give up 1.03 (0.54–1.96) causal and 1.07 (1.05–1.09) observational. In contrast, for hospitalization/death with alcoholism ORs were 28.6 (6.47–126) causal and 1.28 (1.25–1.31) observational.

Combined endpoint of depression and psychological distress

For having one of the endpoints (three for depression and two for psychological distress), the multifactorially-adjusted OR was 1.01 (0.99–1.02) for an increase of 1 drink/day vs having none of the endpoints (Figure 5). Corresponding ORs were 1.05 (1.03–1.07) for having two of the endpoints and 1.10 (1.07–1.13) for having more than two endpoints. When adjusted only for age and gender, corresponding ORs were even higher. Corresponding causal ORs were 0.99 (0.66–1.48), 1.10 (0.59–2.05) and 1.03 (0.44–2.41).

Sensitivity analysis

When we observationally examined men and women separately, results were similar to those in Figure 1 and 2 (Supplementary Figures 2–5, available as Supplementary data at *IJE* online). When we excluded participants with previous self-reported antidepressant use or hospitalization/death with depression from the prospective analysis of risk of prescription antidepressant use, as well as when we excluded participants with previous self-reported antidepressant use or prescription antidepressant use from the prospective analysis of risk of hospitalization/death depression, results were also similar (see Supplementary Figure 6, available as Supplementary data at *IJE* online). Furthermore, when we excluded participants reporting >3 drinks/day from the instrumental variable analysis (and thus only examined moderate drinkers), causal risk estimates were similar, albeit with wider confidence intervals (see Supplementary Table 3, available as Supplementary data at *IJE* online).

Discussion

The principal findings of this study of 78 154 men and women from the Danish general population are that

increased alcohol consumption was associated causally with alcoholism, without similar strong evidence for depression or psychological distress. Whereas there seemed to be no effect for alcohol consumption on antidepressant medication use or psychological distress, we cannot exclude that there could be an effect on hospitalization/death with depression, as the genotypes associated with increased alcohol consumption had nominally increased risk of this endpoint, and as the causal OR for hospitalization/death with depression for the genotype combinations was 4.52 (95% CI 0.99–20.5). The *P*-values of greater than 0.05 could be due to low power due to small numbers in this group rather than to no effect, or to the small effect of the genotypes on alcohol consumption. There seemed to be no effect of alcohol consumption on the other measures of depression, but these more loosely define depression and are not clinical measures.

It should be mentioned, however, that our effect sizes of genetically increased alcohol consumption cannot be used to evaluate the magnitude of the effect but only the presence of a causal association. This is because self-reported alcohol consumption likely does not fully capture the lifetime alcohol consumption, as participants may report alcohol consumption differently (e.g. variation in the size of one drink or drinking beer/wine/spirits with higher or lower alcohol percentage). Our causal effect estimates could therefore be inflated and should be interpreted with caution.

In both the cross-sectional and the prospective analyses of the observational association between alcohol consumption and the depression phenotypes, there is evidence of a U- or J-shaped association which has also been shown in previous studies.^{1,7} There could be different explanations for this:

- i. abstinence itself may be a predictor of depressive symptoms;
- ii. alcohol consumption leads to depressive symptoms and as a result patients with depressive symptoms quit drinking; and
- iii. confounding (e.g. non-drinking is associated with risk factors of depressive symptoms). Indeed, our data showed a clear possibility of confounding, as the relationship between alcohol consumption and most of our covariates were J-shaped as well (Table 1).

Unfortunately, we did not have information on lifetime abstainers or on giving up alcohol later in life.

Mechanistically, a causal association between increased alcohol intake and increased risk of alcoholism may be explained in a simple straightforward manner. Increased alcohol intake may cause compulsion to drink even more.³⁶ Likewise, increased alcohol intake may cause minor withdrawal symptoms by affecting the mesolimbic dopamine

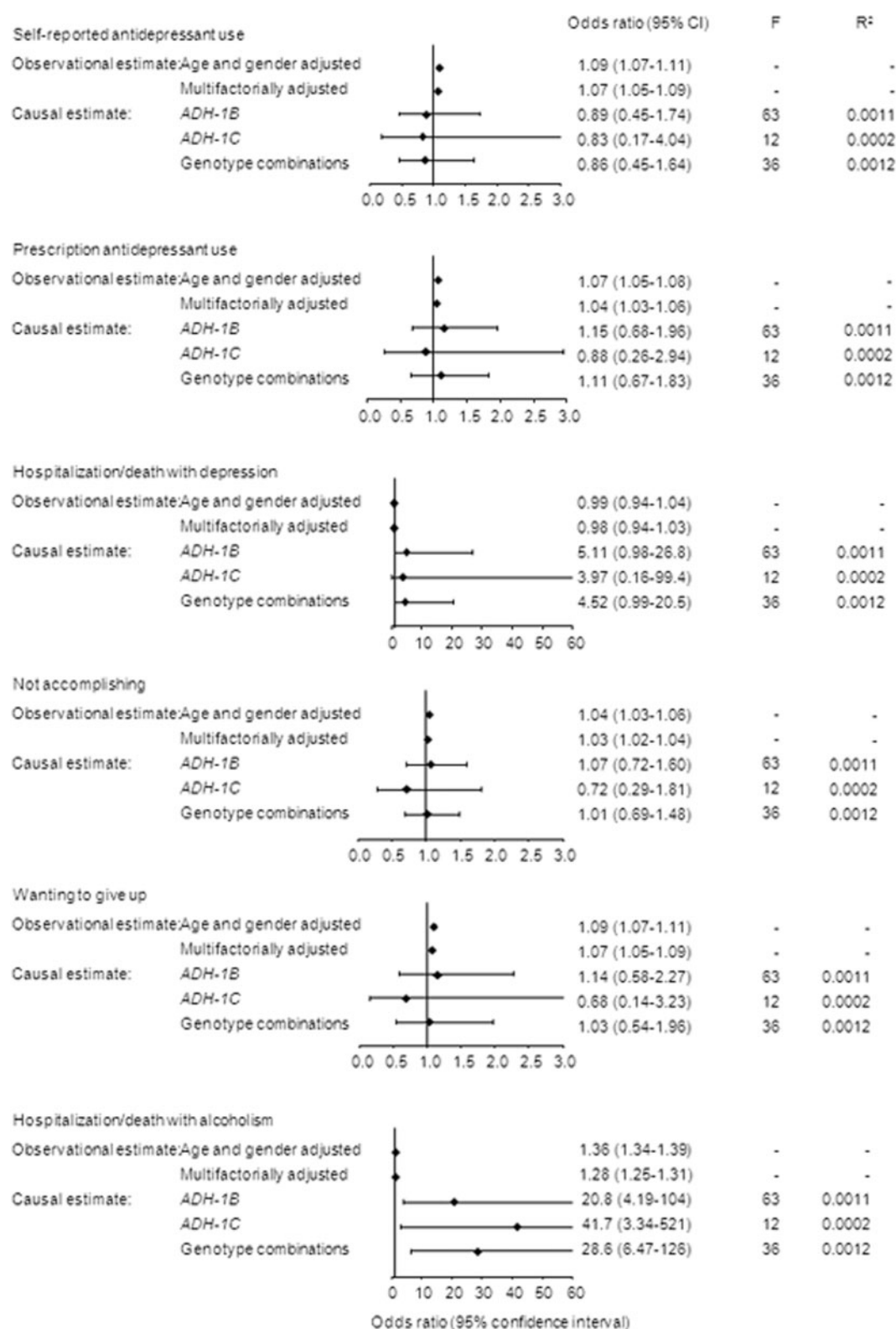


Figure 4. Observational and causal risk estimates for depression, psychological distress and alcoholism for an increase in alcohol consumption of 1 drink/day. Based on 58 543 participants from the Copenhagen General Population Study and Copenhagen City Heart Study, combined. Participants reporting 0 drinks/day were excluded. CI, confidence interval. R² expresses the alcohol consumption explained by the genotypes and F-statistics evaluate the strength of the instrument with F > 10 indicating sufficient statistical strength to carry out instrumental variable analysis.

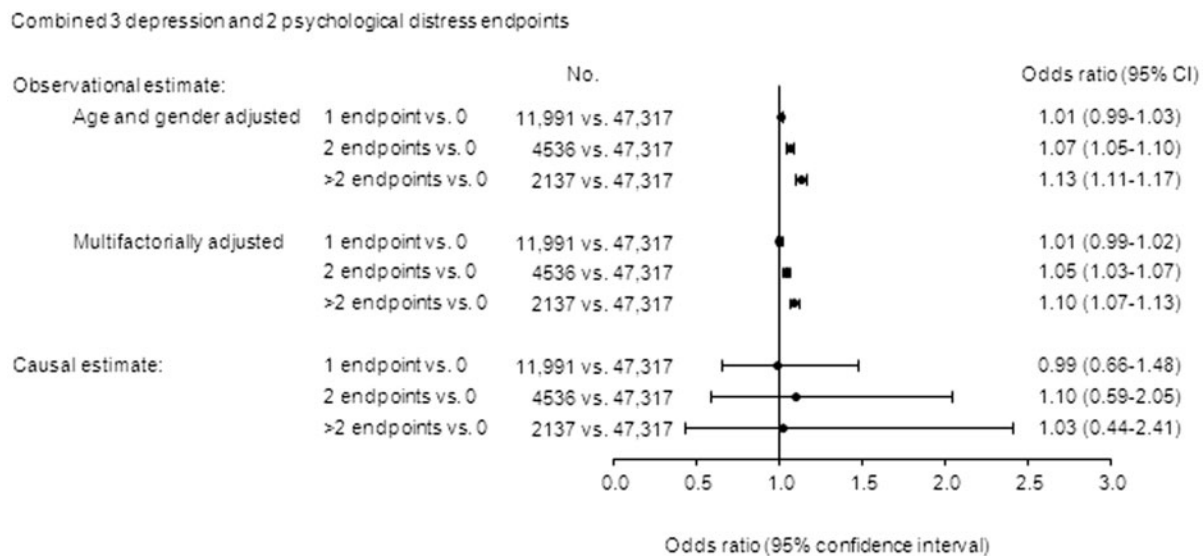


Figure 5. Observational and causal risk estimates for one of the depression/psychological distress endpoints, two of the depression/psychological distress endpoints and more than two of the depression/psychological distress endpoints, vs no depression/psychological distress endpoints, for an increase in alcohol consumption of 1 drink/day. Based on 58 543 participants from the Copenhagen General Population Study and Copenhagen City Heart Study, combined. In total, 47 317 participants did not have any of the endpoints. For each analysis, participants with a different number of endpoints were excluded and participants reporting 0 drinks/day were excluded from all analyses. CI, confidence interval.

system and γ -aminobutyric acid systems,³⁶ which may prevent alcohol drinkers from stopping after an initial period with daily alcohol intake. The present genetic data also suggest that less accumulation of acetaldehyde may be an important condition for development of alcoholism, which may explain the large causal risk estimate for a 1 drink/day increase in alcohol intake. In contrast, although alcohol intake clearly affects mood,³⁷⁻³⁹ a plausible biological mechanism relating increased alcohol intake to depression is more difficult to envisage, in accordance with the present finding of no evidence of a strong causal relationship between alcohol intake and depression. Our results suggest that the association is more likely caused by confounding or reverse causation, that is depression or psychological distress causing individuals to increase drinking to relieve depressive symptoms (self-medication hypothesis). However, if alcohol is used to self-medicate symptoms of depression, then one would expect the Mendelian randomization analyses to reveal a negative causal association. In our study, the limited power available for this Mendelian randomization analysis means it is difficult to draw firm conclusions, but if there is no support for alcohol consumption increasing depression, there is similarly no evidence for alcohol consumption decreasing depression. Depression may increase alcohol consumption (and future Mendelian randomization studies might investigate this) but even if it does so, alcohol consumption does not seem to be effective in relieving depressive symptoms.

An important strength of our large study of the general population is that we had information on alcohol

consumption, *ADH* genotypes, depression, psychological distress and alcoholism in a single study on all participants, where previous studies have examined: either the association between alcohol consumption and depression^{1,4-7,9,11,40,41} or alcoholism;³ or the association between *ADH* genotypes and depression^{42,43} or alcoholism.^{17,44-46} Only one recent study, including 3900 men aged 65-83 years, examined the *ADH1B* genotype, alcohol consumption and risk of depression⁴⁷ and the results also suggested that alcohol consumption does not cause depression. This finding is further supported by genetic studies of genotypes associated with increased alcohol consumption which have failed to find an association between the genotypes and depression.^{42,43} Further strengths include the completeness of the Danish registers, which meant we were able to perform prospective analyses of prescription antidepressant medication use and hospitalization/death with depression and alcoholism with no losses to follow-up during a period of up to 20 years.

A weakness of this paper is the measure of depression as we, contrary to many previous studies,^{1,5,7-9,41} did not have any diagnostic scoring scales on depression; instead we had to ascertain depression as use of antidepressants and hospitalization/death with depression. Prescription of antidepressants has considerable limitations, as this relies on presentation to a doctor or to another social/healthcare service, which may be influenced by alcohol consumption. Furthermore, the willingness of the doctor to prescribe antidepressants for depression may also be influenced by alcohol consumption. Finally, antidepressants are also

prescribed for other reasons such as analgesia; however, to exclude some of the participants receiving antidepressants for other reasons than depression, we chose to include only participants who had received antidepressant medication for at least 6 months with a daily defined dose above 0.75. Using register information on hospitalizations or death with depression as a measure of depression will result in a highly selected group for the following two reasons. First, because most people with depression in Denmark are treated in general practice or by private psychiatrists, these participants will only be registered with a hospital diagnosis of depression if their depression is severe enough to be hospitalized or if they are hospitalized for a different condition, and using hospital discharge diagnoses might thus underestimate the number of participants with depression. Second, hospitalization may be reduced in those who drink heavily: in this case the exposure is affecting the likelihood of detecting the outcome, thus potentially underestimating the effect. Finally, we also included psychological distress as responses to two questions regarding 'not accomplishing much' and 'wanting to give up', but neither is a core feature of depression although they are both likely to be associated with it. Importantly, however, unlike previous studies we used information from three independent depression endpoints as well as from two psychological distress endpoints, and found similar results.

Another potential limitation to our study is that all participants were White, and therefore our results may not necessarily apply to other races; however, previous studies have reported associations between *ADH* genotypes and alcoholism in European-Americans, European-Australians, African-Americans, Mexican-Americans and Asians,^{44–46,48} and we are not aware of data to suggest that our results should not be applicable to other races. Also, two fundamental assumptions in a Mendelian randomization analysis are that the genotypes used should be independent of the confounding factors of the association between the exposure of interest and the outcome and that the genotypes should influence the outcome only through the exposure of interest (i.e. alcohol consumption with consequent acetaldehyde production).¹³ In our data, genotypes were not associated with any of our measured confounders but we cannot exclude that the genotypes were associated with unmeasured confounders.

Another possible limitation of this study is the assumption that the genotypes should influence the outcomes only through alcohol consumption and consequent production of acetaldehyde. To investigate the possibility of pleiotropic effects, other than the enzyme alcohol dehydrogenase's ability to metabolize retinol to retinoic acid,⁴⁹ we searched expression quantitative trait loci databases (the SCAN.org database) to see if the genotypes regulate

expression of mRNAs or proteins but we did not find evidence of pleiotropic effects. Thus, although none are known at present, we cannot totally exclude that the genotypes could affect the outcome by alternative pathways (other than through alcohol consumption)⁵⁰ or that these genotypes are in linkage disequilibrium with other variants which may influence risk of the endpoints. Unfortunately, because we do not have information on lifetime abstainers, it was not possible for us to test whether genotypes were also associated with the outcome in lifetime abstainers which would suggest pleiotropic effects. Pleiotropic effects of the genotypes could influence susceptibility to alcoholism as those who are fast metabolisers will be able to tolerate more alcohol. This could account for the much greater effect seen in the IV estimate compared with the observed effect.

Also, we cannot exclude that some of the covariates lie on the causal pathway between alcohol consumption and depression; it is possible that increased alcohol consumption will result in a different lifestyle (i.e. smoking, physical activity or obesity) which in turn will affect the risk of depression, meaning that a possible depression association is not caused by alcohol alone but through another covariate.

In conclusion, we found that increased alcohol consumption was associated causally with alcoholism, without similar strong evidence for depression or psychological distress. However, as this is one of the first studies to examine the causal associations between alcohol consumption and risk of both depression and alcoholism in the general population, further studies are needed in order to confirm our results.

Supplementary Data

Supplementary data are available at *IJE* online.

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