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Moderate alcohol use and cognitive function among men using a Mendelian randomization design in the Guangzhou Biobank Cohort Study

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Alphabetical list of all abbreviations used in the manuscript

ALDH2 - Aldehyde dehydrogenase 2

ANOVA – Analysis of variance

DNA - Deoxyribonucleic acid

HDL-cholesterol - High Density Lipoprotein- cholesterol

HEPA - Health-enhancing physical activity

GBCS - Guangzhou Biobank Cohort Study

GHHARE - The Guangzhou Health and Happiness Association for the Respectable Elders

IPAQ - International Physical Activity Questionnaire

MET - Metabolic equivalent

MMSE - Mini Mental State Examination

RCT - Randomized Controlled Trial

SNP - Single nucleotide polymorphism

2SLS - Two Stage Least Squares

Running head

Alcohol and cognition using Mendelian randomization

Abstract

Observational studies usually show moderate alcohol use is associated with better cognitive function. Such studies are vulnerable to residual confounding arising from systematic differences between moderate alcohol users and others. A Mendelian randomization study in a suitable population, such as Southern Chinese men, where alcohol use is low to moderate and determined by genotype, offers an alternative and superior approach to clarify the causal effect of moderate alcohol use on cognitive function. The authors used *ALDH2* genotypes (AA, AG/GA or GG), as an instrumental variable in two-stage least squares (2SLS), to obtain unbiased estimates of the effect of alcohol units (10 gram of ethanol) per day on cognitive function, assessed from delayed 10-word recall score (n=4,707) and Mini Mental State Examination (MMSE) (n=2,284) among men from the Guangzhou Biobank Cohort Study. *ALHD2* genotypes were strongly associated with alcohol consumption with an F-statistic of 71.0 in 2SLS. Alcohol was not associated with delayed 10-word recall score (-0.03 words per alcohol unit, 95% confidence interval (CI) -0.18, 0.13) or MMSE score (0.06, 95% CI -0.22, 0.34). Moderate alcohol use is unlikely to be cognitively protective.

Medical Subject Headings

Alcohol drinking; Cognition; Mendelian randomization analysis; developing countries

Several prospective western observational studies show that alcohol use generally has a U-shaped association with cognitive function such that moderate alcohol users usually have better cognitive function than never users (1-3) although there are exceptions, for example where the association was non-significant in a relatively small sample (4). However, observational study designs are susceptible to residual confounding (5, 6) and cannot disentangle the true effects of alcohol from apparent effects generated by the clustering of several unmeasured healthier attributes in moderate alcohol users (7). Replication of observational results in populations with a different confounding structure may strengthen the likelihood of a causal association between exposure and outcomes (6, 8). There are few studies of moderate alcohol use and cognition from non-Western settings. In two large cross-sectional studies from Southern China, with a different social patterning of moderate alcohol use from western settings, we found occasional, rather than moderate, alcohol use, compared to never use, associated with better cognitive function (9, 10), suggesting observed effects are not biologically driven by alcohol. Another study from China found moderate alcohol use impaired cognitive function (11). Large randomized controlled trials (RCTs) assessing the effect on cognitive function of increasing from negligible or low alcohol use to moderate alcohol use are unethical, given the carcinogenicity of alcohol and its association with accidents, and are also impractical, given prevalent alcohol use (12). A Mendelian randomization design, using gene(s) randomly allocated at conception as an

instrumental variable, provides an alternative approach which is less vulnerable to confounding than observational studies (13).

Mendelian randomization has previously been proposed as a way to verify the causal role of moderate alcohol use in a suitable population where a gene, such as aldehyde dehydrogenase 2 (*ALDH2*), affects alcohol use (13-15). People with inactive *ALDH2* alleles flush and feel discomfort following alcohol use because of acetaldehyde exposure. Southern Chinese men are particularly suitable for a Mendelian randomization study of alcohol use based on *ALDH2*, although polymorphisms of other genes, such as *ADH*, may be suitable in other populations. Inactive alleles of *ALDH2* are common in East Asia. Among Southern Chinese men alcohol use is generally low to moderate (16, 17), it is also socially acceptable for men to abstain from or only use alcohol on special occasions (16, 17) and *ALDH2* genotype influences alcohol use (14, 15). In contrast, among Southern Chinese women alcohol use is much more constrained by social convention (18). Considering these unique characteristics in Southern Chinese men, we used a Mendelian randomization study design in the Guangzhou Biobank Cohort Study, with *ALDH2* genotypes as an instrumental variable for alcohol use, to assess if moderate alcohol use was causally protective of cognitive function among men. We also assessed the same association using a traditional observational design for comparison.

MATERIALS AND METHODS

Participants

The Guangzhou Biobank Cohort Study (GBCS) is a collaboration between the Guangzhou No.12 Hospital and the Universities of Hong Kong and Birmingham (19). Recruitment of participants draws from "The Guangzhou Health and Happiness Association for the Respectable Elders (GHHARE)", a community social and welfare association unofficially aligned with the municipal government where membership is open to anyone aged 50 years or older for a monthly, nominal fee of 4 Yuan (50 US cents). Recruitment was in three phases; phase 1 from September 2003 to November 2004, phase 2 from April 2005 to May 2006, and phase 3 from September 2006 to January 2008. Follow- up of the participants started in 2008. Approximately 7% of permanent Guangzhou residents aged 50 years or more are members of GHHARE, of whom 33% enrolled for phase 1, 2 or 3 and were included if they were capable of consenting, ambulatory, and not receiving treatment modalities that, if omitted, may result in immediate life-threatening risk, such as chemotherapy or radiotherapy for cancer, or dialysis for renal failure.

Participants underwent a detailed interview and physical examination at baseline recruitment. The methods of measurement have previously been reported (19). Alcohol use

was recorded in terms of frequency, type of beverage and usual amount per occasion. Cognitive function was assessed using a test from a test battery developed for the Consortium to Establish a Registry for Alzheimer disease (20). The Consortium tests cover general cognitive function, confrontational naming, semantic fluency, constructional ability, and new learning ability. We used the test of new learning ability (10-word list learning task). Four words in this test were taken from the original English language test (21): “arm”, ”letter”, ”ticket” , and “grass”. “Pole”, “shore”, “cabin”, and “engine” were replaced with “corner”, “stone”, “book”, and “stick” as in the adapted Consortium 10-word list learning task (21), and “butter” and “queen” were replaced by “soy sauce” and “chairman” as these are more culturally appropriate. During the learning phase, the 10-word list was read out to the participant who was then asked to recall immediately the words they remembered; this was repeated 3 times. After a 5-minute period of distraction, during which the interview was continued, the participant was then asked to recall as many of the 10 words as he or she was able, giving the delayed recall score out of 10. The adapted Consortium 10-word list learning task has been validated as a culturally and educationally sensitive tool for identifying dementia in population-based research in developing countries (21).

In phase 3, we additionally used the Mini Mental State Examination (MMSE), a test of

general cognitive function covering orientation, attention, memory and recall and language (22). Three of the 11 tasks in the original MMSE were modified to be culturally appropriate and consistent with other adaptations for Chinese populations (23). Orientation in place was adapted according to geographical divisions of China and screening setting to: “country”, “province”, “city”, “hospital”, and “floor”. In the 3-word registration and recall “table” and “penny” were replaced by “newspaper” and “train”, while the third word remained as “apple”, to ensure all three words were frequently used two-character Chinese words. The modified MMSE has the same scale as the original MMSE (23), hence the psychometric properties of the measures should be similar. In addition, the modified MMSE has been used before (10, 24, 25). The Guangzhou Medical Ethics Committee of the Chinese Medical Association approved the study and all participants gave written, informed consent before participation.

DNA extraction and SNP analysis

Biological samples for DNA extraction used in the present study were obtained in GBCS phase 3 at baseline and in phases 1 and 2 at follow-up. DNA was extracted at Guangzhou No.12 Hospital either at baseline from fresh blood using a standard phenol-chloroform extraction procedure and stored at -80^{0}C or from blood or buffy coat previously stored at -80^{0}C using a standard magnetic bead extraction procedure. Genotyping was performed

using the MassARRAY system (Sequenom, San Diego, CA, USA) and the iPLEX assay at a commercial company.

Exposures

We used *ALDH2* genotypes (AA, AG/GA or GG), identified by SNP rs671, as an instrumental variable for alcohol use because the association of *ALDH2* genotypes with alcohol use was non linear. An initial analysis results were similar using an additive model for *ALDH2* genotypes.

Alcohol use

Alcohol use was considered as alcohol units (10 gram (g) ethanol) per day based on total consumption obtained from the frequency, quantity and type of beverage recorded at baseline; infeasible quantities (>30 alcohol units per day) were excluded. Former alcohol users were included as non-drinkers (0 alcohol unit per day) because former alcohol users may have abstained from alcohol because of poor health unrelated to former alcohol use; excluding them could create a bias. Moreover, many former users in this study reported having previously been very infrequently alcohol users, i.e. once or twice a year, unlike the sick quitters in western populations who are mainly former heavy users (26). Mean previous alcohol use among former drinkers was 1.0 alcohol units per day, with a few former

drinkers reporting heavy previous alcohol use (11%) and most former drinkers (75%) reporting occasional previous alcohol use. As previously, we also created an alcohol category defined among men as never user, occasional user (<1/week, often only once or twice per year), moderate user (weekly and ≤210 g ethanol per week), heavy user (weekly and >210g per week) and former user (10, 17).

Outcomes

The outcomes were delayed 10-word recall score (out of 10) for all 3 phases and MMSE score (out of 30) for phase 3 only.

Statistical analysis

We tested the Hardy-Weinberg equilibrium at the SNP locus on a contingency table of observed-versus-predicted frequencies with an exact test. We used ANOVA to assess the association of *ALDH2* genotypes with alcohol consumption. We used chi-square tests to assess if *ALDH*2 genotypes and alcohol categories were associated with potential confounders, such as socio-economic position, lifestyle, hypertension or diabetes. For the Mendelian randomization design we used two-stage least squares (2SLS), with *ALDH*2 genotypes (AA, AG/GA and GG) as an instrumental variable. The first step predicted alcohol units from *ALDH*2 genotypes and the second step assessed the association of

alcohol units with delayed 10-word recall and MMSE scores. Alleles of *ALDH2* randomly allocated at conception are unlikely to determine, and hence confound, subsequent socio-economic position or lifestyle, although alcohol use could affect smoking (27); we only adjusted the 2SLS regression for potential confounders associated with *ALDH2* genotype. We used the Hausman test to assess whether the 2SLS estimates differed from unadjusted ordinary least squares estimates (28). For comparison, we also present the adjusted associations of alcohol units with the outcomes under multivariable linear regression models in a traditional observational design. We adjusted these multivariable linear regression models for age (in 5-year groups), education, physical activity, longest held occupation and smoking status, as categorized in Table 1, but we did not adjust for household income, hypertension or diabetes as they did not alter the estimates (29). We also repeated the 2SLS and the multivariable linear regression analysis firstly excluding all heavy alcohol users and secondly excluding all former alcohol users. All statistical analyses were done using Stata 10.1 (StataCorp LP, College Station, TX).

RESULTS

Of the 8,450 men in GBCS phases 1, 2 and 3, 5,606 had bio-materials available for DNA extraction. We only have bio-materials for phases 1-2 participants who returned for follow up by the end of 2010. Of these 5,606 men, 4,986 had viable DNA, of whom 4,707 (56% of

all men in GBCS) had complete information on *ALDH2* genotype, alcohol use and delayed 10-word recall score. Of the 2,569 men in GBCS phase 3, 2,284 (89% of all men in GBCS phase 3) had complete information on *ALDH2* genotype, alcohol use and MMSE score. For the observational multivariable linear regression, 7,934 (94%) men had complete information on alcohol category, confounders and 10-word recall score while 2,458 (96%) men in phase 3 had complete information on alcohol category, confounders and MMSE score. *ALDH2* genotypes had distributions consistent with Hardy-Weinberg equilibrium. Genotype frequencies were similar in samples from recruitment phases 1 and 2 follow-up (AA 8.0%, AG/GA 41.0% and GG 51.1%) as in samples from phase 3 baseline (AA 9.0%, AG/GA 42.4% and GG 48.5%).

Table 1 shows that among men *ALDH2* genotype was not associated with socioeconomic position, lifestyle, hypertension or diabetes. *ALDH2* genotypes were strongly associated with alcohol consumption, such that men with two active *ALDH2* alleles consumed ten times as much alcohol per day as men with 2 inactive alleles. The variance in alcohol use explained by *ALDH2* was 3%, which is typical for Mendelian randomization studies (30).

Table 2 shows that the main alcoholic beverage was rice wine. Table 2 also shows that alcohol category was associated with socio-economic position, lifestyle and hypertension

among men. Compared to never users, occasional alcohol users were younger, more educated, more likely to have manual job, more likely to smoke, more physically active and less likely to have hypertension. Moderate alcohol users were less educated and more likely to smoke but similar in other aspects to never users.

Among men *ALDH2* genotype was a valid instrumental variable for alcohol consumption with an F-statistic from 2SLS of 71.0. An F-statistic greater than 10 or 11 suggests an adequate instrumental variable with a low risk of bias due to a weak instrument, because the F-statistic is inversely related to the relative bias (bias of instrumental variable analysis: bias of observational analysis) (31). Table 3 shows that in the Mendelian randomization design alcohol was not associated with delayed 10-word recall score or MMSE score. Estimates from 2SLS were not different from those using unadjusted ordinary least squares, as indicated by the Hausman p-value. Using multivariable linear regression in an available case analysis, alcohol was not associated with higher delayed 10-word recall score or MMSE score. Exclusion of former alcohol users did not change the estimates. However, when heavy users were excluded, alcohol was associated with higher delayed 10-word recall score, but not with MMSE score.

DISCUSSION

Using a Mendelian randomization study design in Southern Chinese men, where alcohol use is generally low to moderate, we found that low to moderate alcohol use was not associated with cognitive function. Our novel analytic approach adds to the literature by suggesting that the positive association of moderate alcohol use with cognitive function previously observed may be socially driven, perhaps by residual confounding, rather than biologically driven by alcohol (1, 2, 32).

Although we used a Mendelian randomization design which has similar properties to an RCT (13), there were some limitations. First, in our population, the pattern of alcohol use is different from in western settings. The range of alcohol use was mainly low to moderate. Hence, we cannot investigate the effect of heavy alcohol use on cognitive function. However, the harms of heavy alcohol use are already known (12). Nevertheless, alcohol use among men in this study has the expected positive associations with blood pressure and HDL-cholesterol (17). Second, this is not a representative sample although the disease prevalence is similar to that of a population representative sample in China (19). In addition, we only had samples from men recruited in phases 1 and 2 if they returned for follow-up. However, *ALDH2* genotype frequencies were similar for those available from phases 1 and 2 as for phase 3. Furthermore *ALDH2* genotypes were not associated with socio-economic position or lifestyle. Nevertheless, participants returning for follow-up might have been

healthier. Mendelian randomization, similar to RCTs, is less susceptible to confounding because the genes used as instrumental variables are unlikely to be systematically related to socio-demographic characteristics or lifestyle (13), and hence the estimates remain relatively unbiased within this study. Third, we only have measurement at one time of cognitive function making reverse causality a possibility in the observational analysis but not for Mendelian randomization analysis (33). Forth, although it is possible that *ALDH2* genotypes could directly affect cognitive function irrespective of alcohol use due to pleiotrophy or linkage disequilibrium, there was no association of *ALDH2* genotype and cognitive function in never/occasional users in both sexes (data not shown), consistent with a Korean longitudinal study (34). Fifth, results from the Mendelian randomization and observational analysis were similar. However, that does not negate the value of a Mendelian randomization design, as an observational analysis is inevitably open to residual confounding that could bias estimates towards as well as away from the null. A similar pattern of associations was also observed when alcohol use was considered in categories in an observational design in this study (10)**.** Lastly, given 3% of the variance in alcohol use explained by *ALDH2*, our study only has sufficient power to detect an effect size of about 0.3 (35), which is 0.56 words for delayed 10-word recall and 0.79 score for MMSE, so we cannot rule out the possibility of small benefits of alcohol on cognitive function although the clinical significance is uncertain.

Several traditional observational studies in western settings have observed better cognitive function among moderate alcohol users(1-3) but there are exceptions (4, 36). In contrast, in an under-studied population with a different confounding structure we found no association of moderate alcohol use with cognitive function in two complimentary study designs, as confirmed by the Hausman test (Table 3). The discrepancies could be due to failure to adjust fully for confounding factors, such as socioeconomic position, lifestyle, health status or cognitive potential, which are cognitively protective and difficult to assess comprehensively. Moreover, with aging and ill-health moderate alcohol users may be increasingly heavily selected healthy users compared to never users leading to apparently greater benefits of moderate alcohol use in older people (37). In western settings moderate alcohol users usually have higher socio-economic position, a healthier lifestyle, better health status and possibly greater cognitive potential than other users (7, 38-40). Moreover, in these settings regardless of the definition, moderate alcohol users usually have better cognitive function than abstainers (41). This suggests that the association is not biologically driven because different doses of alcohol would be expected to have different effects if alcohol were the causal agent.

Public health implications

Our study, which is potentially less biased than a traditional observational design (13), has not shown moderate alcohol use to be positively associated with cognitive function. From a public health perspective, alcohol use appears to have little role in protecting cognitive function, and should not be promoted as such. These findings also demonstrate that causality should be thoroughly verified in a variety of settings using different kinds of evidence including experimental or genetic studies rather than relying on simple observations in a particular setting.

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REFERENCES

1. Stampfer MJ, Kang JH, Chen J, et al. Effects of moderate alcohol consumption on cognitive function in women. *N Engl J Med*. 2005;352(3):245-253.
2. Bond GE, Burr RL, McCurry SM, et al. Alcohol and cognitive performance: a longitudinal study of older Japanese Americans. The Kame Project. *Int Psychogeriatr*. 2005;17(4):653-668.
3. Ruitenberg A, van Swieten JC, Witteman JC, et al. Alcohol consumption and risk of dementia: the Rotterdam Study. *Lancet*. 2002;359(9303):281-286.
4. Lobo E, Dufouil C, Marcos G, et al. Is there an association between low-to-moderate alcohol consumption and risk of cognitive decline? *Am J Epidemiol*. 2010;172(6):708-716.
5. Lawlor DA, Smith GD, Bruckdorfer KR, et al. Those confounded vitamins: what can we learn from the differences between observational versus randomised trial evidence? *Lancet*. 2004;363(9422):1724-1727.
6. Smith GD, Ebrahim S. Data dredging, bias, or confounding. *Bmj*. 2002;325(7378):1437-1438.
7. Naimi TS, Brown DW, Brewer RD, et al. Cardiovascular risk factors and confounders among nondrinking and moderate-drinking US adults. *Am J Prev Med*. 2005;28(4):369-373.
8. Brion MJ. Commentary: Assessing the impact of breastfeeding on child health: where conventional methods alone fall short for reliably establishing causal inference. *Int J Epidemiol*. 2010;39(1):306-307.
9. Au Yeung SL, Leung GM, Chan WM, et al. Moderate alcohol use and cognitive function in an elderly Chinese cohort. *J Am Geriatr Soc*. 2011;59(1):172-174.
10. Au Yeung SL, Jiang CQ, Zhang WS, et al. Moderate Alcohol Use and Cognitive Function in the Guangzhou Biobank Cohort Study. *Ann Epidemiol*. 2010;20(12):873-882.
11. Zhou HD, Deng J, Li JC, et al. Study of the relationship between cigarette smoking, alcohol drinking and cognitive impairment among elderly people in China. *Age Ageing*. 2003;32(2):205-210.
12. Boyle P, Levin B, International Agency for Research on Cancer., et al. *World cancer report 2008*. Lyon

Geneva: International Agency for Research on Cancer ;

Distributed by WHO Press; 2008.

13. Smith GD, Ebrahim S. Mendelian randomization: prospects, potentials, and limitations. *Int J Epidemiol*. 2004;33(1):30-42.
14. Muramatsu T, Wang ZC, Fang YR, et al. Alcohol and aldehyde dehydrogenase

genotypes and drinking behavior of Chinese living in Shanghai. *Hum Genet*. 1995;96(2):151-154.
15. Thomasson HR, Edenberg HJ, Crabb DW, et al. Alcohol and aldehyde dehydrogenase genotypes and alcoholism in Chinese men. *Am J Hum Genet*. 1991;48(4):677-681.
16. Janghorbani M, Ho SY, Lam TH, et al. Prevalence and correlates of alcohol use: a population-based study in Hong Kong. *Addiction*. 2003;98(2):215-224.
17. Schooling CM, Jiang CQ, Lam TH, et al. Alcohol use and fasting glucose in a developing southern Chinese population: the Guangzhou Biobank Cohort Study. *J Epidemiol Commun H*. 2009;63(2):121-127.
18. Hao W, Chen H, Su Z. China: alcohol today. *Addiction*. 2005;100(6):737-741.
19. Jiang C, Thomas GN, Lam TH, et al. Cohort profile: The Guangzhou Biobank Cohort Study, a Guangzhou-Hong Kong-Birmingham collaboration. *Int J Epidemiol*. 2006;35(4):844-852.
20. Welsh KA, Hoffman JM, Earl NL, et al. Neural correlates of dementia: regional brain metabolism (FDG-PET) and the CERAD neuropsychological battery. *Archives of Clinical Neuropsychology*. 1994;9(5):395-409.
21. Prince M, Acosta D, Chiu H, et al. Dementia diagnosis in developing countries: a cross-cultural validation study. *Lancet*. 2003;361(9361):909-917.
22. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiat Res*. 1975;12(3):189-198.
23. Xu GL, Meyer JS, Huang YG, et al. Adapting Mini-Mental State Examination for dementia screening among illiterate or minimally educated elderly Chinese. *Int J Geriatr Psych*. 2003;18(7):609-616.
24. Heys M, Jiang CQ, Schooling CM, et al. Is childhood meat eating associated with better later adulthood cognition in a developing population? *Eur J Epidemiol*. 2010;25(7):507-516.
25. Heys M, Jiang C, Cheng KK, et al. Does the age of achieving pubertal landmarks predict cognition in older men? Guangzhou Biobank Cohort Study. *Ann Epidemiol*. 2010;20(12):948-954.
26. Roerecke M, Rehm J. Ischemic heart disease mortality and morbidity rates in former drinkers: a meta-analysis. *Am J Epidemiol*. 2011;173(3):245-258.
27. Room R. Smoking and drinking as complementary behaviours. *Biomed Pharmacother*. 2004;58(2):111-115.
28. Palmer TM, Lawlor DA, Harbord RM, et al. Using multiple genetic variants as instrumental variables for modifiable risk factors. *Stat Methods Med Res*. 2011.
29. Rothman KJ, Greenland S, Lash TL, et al. Modern epidemiology. Philadelphia: Lippincott Williams & Wilkins, 2008:p.

30. Conen D, Vollenweider P, Rousson V, et al. Use of A Mendelian Randomization Approach to Assess the Causal Relation of gamma-Glutamyltransferase with Blood Pressure and Serum Insulin Levels. *Am J Epidemiol*. 2010;172(12):1431-1441.
31. Stock JH, Yogo M. *Testing for Weak Instruments in Linear IV Regression*. Cambridge, MA: National Bureau of Economic Research, Inc; 2002.
32. Kalmijn S, van Boxtel MP, Verschuren MW, et al. Cigarette smoking and alcohol consumption in relation to cognitive performance in middle age. *Am J Epidemiol*. 2002;156(10):936-944.
33. Smith GD. Mendelian Randomization for Strengthening Causal Inference in Observational Studies: Application to Gene x Environment Interactions. *Perspect Psychol Sci*. 2010;5(5):527-545.
34. Shin IS, Stewart R, Kim JM, et al. Mitochondrial aldehyde dehydrogenase polymorphism is not associated with incidence of Alzheimer's disease. *Int J Geriatr Psych*. 2005;20(11):1075-1080.
35. Pierce BL, Ahsan H, Vanderweele TJ. Power and instrument strength requirements for Mendelian randomization studies using multiple genetic variants. *Int J Epidemiol*. 2011;40(3):740-752.
36. Cooper C, Bebbington P, Meltzer H, et al. Alcohol in moderation, premorbid intelligence and cognition in older adults: results from the Psychiatric Morbidity Survey. *J Neurol Neurosurg Psychiatry*. 2009;80(11):1236-1239.
37. Thun MJ, Peto R, Lopez AD, et al. Alcohol consumption and mortality among middle-aged and elderly US adults. *New Engl J Med*. 1997;337(24):1705-1714.
38. Rozzini R, Trabucchi M. Re: "Association between reported alcohol intake and cognition: Results from the women's health initiative memory study". *Am J Epidemiol*. 2005;162(3):294-U293.
39. Cooper C, Bebbington P, Meltzer H, et al. Alcohol in moderation, premorbid intelligence and cognition in older adults: results from the Psychiatric Morbidity Survey. *J Neurol Neurosur Ps*. 2009;80(11):1236-1239.
40. Krahn D, Freese J, Hauser R, et al. Alcohol use and cognition at mid-life: The importance of adjusting for baseline cognitive ability and educational attainment. *Alcohol Clin Exp Res*. 2003;27(7):1162-1166.
41. Eckardt MJ, File SE, Gessa GL, et al. Effects of moderate alcohol consumption on the central nervous system. *Alcohol Clin Exp Res*. 1998;22(5):998-1040.

Table 1. Alcohol Consumption and Socio-demographic Characteristics by *ALDH2* Genotype Among Men From the Guangzhou Biobank Cohort Study (2003-8)

		ALDH2 genotype (from rs671)			
		Two inactive alleles (AA)	One inactive allele (AG/GA)	No inactive alleles (GG)	[a]P value
Alcohol units (10g ethanol) per day	n	416	2,023	2,428	
	mean (SD)	0.09 (0.79)	0.24 (1.22)	0.90 (2.52)	<0.001
Age group (%) years	n, years	417	2,053	2,457	
	50-54	11.0	10.0	9.2	0.41
	55-59	20.9	20.9	21.2	
	60-64	25.9	23.9	26.3	
	65-69	19.7	23.8	23.4	
	70-74	16.3	15.7	14.6	
	75-79	5.5	4.2	3.7	
	80+	0.7	1.5	1.4	
Education (%)	n	417	2,051	2,455	
	Less than primary	2.6	2.3	2.3	0.63
	Primary	24.7	27.3	26.2	
	Junior middle	29.0	30.3	31.1	
	Senior middle	27.1	25.1	23.5	
	Junior college	10.3	8.5	9.2	
	College	6.2	6.5	7.7	
[b]Longest held occupation (%)	n	415	2,035	2,435	
	Manual	54.5	56.1	53.5	0.22
	Non-manual	36.1	35.4	38.6	
	Unknown	9.4	8.6	7.9	
Smoking status (%)	n	416	2,045	2,444	
	Never	41.1	40.4	40.1	0.88
	Former	29.3	27.7	27.8	
	Current	29.6	31.9	32.2	
Physical activity (IPAQ) (%)	n	417	2,053	2,457	
	Inactive	9.1	8.5	8.1	0.23
	Minimally active	36.9	38.8	41.6	
	HEPA active	54.0	52.7	50.3	
[c]Hypertension	n	416	2,050	2,456	
	Present	39.4	42.7	44.8	0.08
[d]Diabetes	n	417	2,037	2,442	
	Present	10.6	11.0	11.3	0.87

HEPA: health-enhancing physical activity (i.e., vigorous activity at least 3 days a week achieving at least 1,500 metabolic equivalent (MET) minutes per week or activity on 7 days of the week, achieving at least 3,000 MET minutes per week; IPAQ: International Physical Activity Questionnaire; [a]P-value from ANOVA for continuous variables and from a χ^2 test for categorical variables, 2 sided; [b]Manual occupations are agricultural worker, factory work or sales and service; non-manual are administrator/manager, professional/technical, military/disciplined; [c]Hypertension defined as systolic blood pressure/diastolic blood pressure >140/90 or previous diagnosis of hypertension or use of anti-hypertensive medication; [d]Diabetes defined as fasting glucose>7mmol/L or previous diagnosis of diabetes or use of anti-diabetic medication

Table 2. Socio-demographic Characteristics by Alcohol Category Among Men From the Guangzhou Biobank Cohort Study (2003-8)

		Alcohol category					
		Never	Occasional <1/week	Moderate ≦210g /wk	Heavy >210g /wk	Former	[a]P value
[b]Main alcohol type	n	4,544	1,713	946	512	519	
(%)	Beer	0	17.6	5.1	0.8	0	<0.001
	Western table wine	0	10.6	16.0	0.4	0	
	Chinese rice wine (low strength)	0	42.9	62.1	53.9	0	
	Chinese rice wine (high strength)	0	18.7	8.3	24.2	0	
	Spirits	0	10.3	8.7	20.7	0	
Age group	n	4,544	1,839	946	512	519	
(%) year	50-54	7.2	12.0	8.9	9.6	7.6	<0.001
	55-59	18.1	23.7	18.0	20.7	20.3	
	60-64	25.1	25.9	23.7	24.0	23.6	
	65-69	26.0	21.2	24.7	25.4	24.0	
	70-74	18.2	13.3	19.0	17.4	18.6	
	75-79	4.6	2.9	4.1	1.8	3.7	
	80+	0.8	1.0	1.6	1.2	2.2	
Education	n	4,541	1,839	946	512	511	
(%)	Less than primary	2.3	2.2	3.5	4.7	3.7	<0.001
	Primary	26.0	23.0	30.8	41.6	35.8	
	Junior middle	29.4	30.1	29.5	27.3	29.6	
	Senior middle	24.3	25.5	21.1	18.0	18.0	
	Junior college	9.5	11.4	8.3	4.7	7.8	
	College	8.5	7.9	6.9	3.7	5.1	
[c]Longest held	n	4,527	1,823	942	510	509	
occupation (%)	Manual	49.4	52.8	52.6	62.4	56.0	<0.001
	Non-manual	39.0	39.0	38.1	28.4	30.7	
	Unknown	11.6	8.2	9.3	9.2	13.4	
Smoking status	n	4,540	1,838	946	512	512	
(%)	Never	48.0	38.5	27.8	10.0	24.6	<0.001
	Former	26.6	28.5	32.7	27.7	42.0	
	Current	25.4	33.1	39.5	62.3	33.4	
Physical activity	n	4,544	1,839	946	512	512	
(%) (IPAQ)	Inactive	10.0	4.2	9.5	10.9	4.3	<0.001
	Minimally active	46.4	39.4	44.5	44.9	52.2	
	HEPA active	43.7	56.3	46.0	44.1	43.6	
[d]Hypertension	n	4,535	1,838	942	510	512	
	Present	46.2	39.5	46.0	52.9	50.6	<0.001
[e]Diabetes	n	4,524	1,823	934	508	509	
	Present	12.5	10.2	11.8	13.4	13.2	0.07

HEPA: health-enhancing physical activity (i.e., vigorous activity at least 3 days a week achieving at least 1,500 metabolic equivalent (MET) minutes per week or activity on 7 days of the week, achieving at least 3,000 MET minutes per week; IPAQ: International Physical Activity Questionnaire; [a]P-value from a χ^2 test, 2 sided; [b]Chinese rice wine (low strength) was <38% ethanol while Chinese rice wine (high strength) was ≧38% ethanol; [c]Manual occupations are agricultural worker, factory work or sales and service; non-manual are administrator/manager, professional/technical, military/disciplined. [d]Hypertension defined as systolic blood pressure/diastolic blood pressure >140/90 or previous diagnosis of hypertension or use of anti-hypertensive medication; [e] Diabetes defined as fasting glucose>7mmol/L or previous diagnosis of diabetes or use of anti-diabetic medication

Table 3. Associations of One Alcohol Unit (10 g Ethanol) per Day With Delayed 10-word Recall Score and MMSE Score Using a Mendelian Randomization Design and an Observational Multivariable Linear Regression Analysis Among Men From the Guangzhou Biobank Cohort Study (2003-2008), With and Without Heavy Alcohol Users or Former Alcohol Users.

		Mendelian randomization - 2SLS				[a]Observational multivariable linear regression		
Sample	Cognitive test	n	β	95% CI	[b]Hausman P-value	n	β	95% CI
All	Delayed 10-word recall score	4,707	-0.03	-0.18, 0.13	0.61	7,934	0.02	-0.0001, 0.04
	MMSE score	2,284	0.06	-0.22, 0.34	0.55	2,458	0.006	-0.04, 0.05
Heavy alcohol users excluded	Delayed 10-word recall score	4,427	-0.12	-0.77, 0.52	0.56	7,450	0.12	0.03, 0.20
	MMSE score	2,125	0.27	-1.27, 1.82	0.79	2,279	0.15	-0.07, 0.36
Former alcohol users excluded	Delayed 10-word recall score	4,421	-0.008	-0.16, 0.14	0.77	7,446	0.02	-0.001, 0.04
	MMSE score	2,130	0.12	-0.14, 0.38	0.25	2,296	0.008	-0.03, 0.05

MMSE: Mini Mental State Examination; 2SLS: Two-stage least squares. [a]Adjusted for age, education, physical activity, longest held occupation and smoking; [b]The Hausman p-value indicates whether the 2SLS and unadjusted ordinary least squares estimates differ