Excessive Alcohol Consumption Increases Mortality in Later Life: A Genetic Analysis of the Health In Men Cohort Study

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ABSTRACT

We designed this cohort study of men aged 70-89 years to determine if excessive alcohol use increases mortality. They reported history of alcohol use (never, past, ≤ 2 daily drinks, 2-4 daily drinks, 4-6 daily drinks, > 6 daily drinks) and donated a blood sample in 2001-2004. We determined the *ADH1B* rs1229984 G>A polymorphism and retrieved mortality data from the Western Australian Data Linkage System. Other study measures included age, education, body mass index, smoking, and history of hypertension, diabetes, chronic respiratory diseases, coronary heart disease and stroke. Of the 3496 participants, 225 (6.4%) carried the *ADH1B* rs1229984 G>A polymorphism. Carriers consumed significantly less alcohol than non-carriers. The adjusted mortality hazard ratio (MHR, 95% confidence interval - 95%CI) over 8.0 years (range: 10 weeks to 11.2 years) relative to never drinkers was 1.15 (95%CI=0.86, 1.55) for past drinkers, 0.98 (95%CI=0.76, 1.25) for men consuming ≤ 2 daily drinks, 1.13 (95%CI=0.85, 1.49) for 2-4 drinks, 1.18 (95%CI=0.81, 1.71) for 4-6 drinks, and 1.87 (95%CI=1.11, 3.12) for those consuming more than 6 daily drinks on a regular basis. The MHR associated with the *ADH1B* rs1229984 G>A polymorphism was 0.68 (95%CI=0.54, 0.87). Excessive alcohol use in later life is associated with increased mortality, and this association is likely to be causal. We found no evidence that light to moderate alcohol use decreases the mortality of older men. Health messages regarding the safe use of alcohol in older age may benefit from taking these findings into account.

Keywords: ageing, alcohol, alcohol dehydrogenase, Mendelian randomisation, mortality, rs1229984 polymorphism.

INTRODUCTION

Epidemiological studies consistently report a J-shaped association between increasing alcohol intake and mortality (Di Castelnuovo *et al.*, 2006, Jayasekara *et al.*, 2014, McCaul *et al.*, 2010). A recent systematic review of 9 cohort studies published between 1991 and 2010 identified a slight decrease in the risk of death relative to non-drinkers amongst adults consuming less than 30 g/day, and a progressive increase in risk for those using more than 30 g/day (Jayasekara *et al.*, 2014). The association is biologically plausible because excessive alcohol use has been associated with the occurrence of malignancies of the mouth, oesophagus, liver and breasts; diabetes mellitus, cirrhosis of the liver, ischaemic heart disease and stroke, seizures, several neuropsychiatric disorders, and unintentional and intentional injury (Room *et al.*, 2005).

However, there is a paucity of information about what might constitute safe or healthy drinking in later life, and this is important given that older adults are at increased risk of health events and death. The results of previous observational studies have indicated that light to moderate alcohol consumption reduces the risk of cardiovascular events (Ronksley *et al.*, 2011) and mortality (Di Castelnuovo *et al.*, 2006, Muntwyler *et al.*, 1998, Scherr *et al.*, 1992), suggesting that this level of use might be desirable to maintain good health as the population ages. Nevertheless, this association could be due to bias or confounding, particularly residual confounding (Bergmann *et al.*, 2013). For example, people who drink alcohol in moderation may engage in other risk factors for mortality in moderation, whereas people who abstain from drinking or drink in excess, may also expose themselves to other risk factors for mortality to excess. To establish a causal association between exposure to various levels of alcohol use and mortality, the ideal study design would be a large randomized controlled trial, but this would be difficult to undertake and arguably unethical.

Mendelian randomisation is another potentially useful approach to infer causal links between exposures and clinical outcomes (Davey Smith *et al.*, 2005). The rationale, in this case, is that

genetic variations that increase or decrease the activity of certain enzymes involved in the metabolism of relevant exposures should also be associated with the clinical outcome of interest if the relationship between the exposure and the outcome is truly causal. For example, the rs1229984 G>A (Arg to His) variant of the alcohol dehydrogenase 1B (*ADH1B*) gene increases the oxidation of ethanol to acetaldehyde and make the experience of drinking less pleasant (Edenberg, 2007). We and others have shown that carriers of this particular polymorphism consume much less alcohol than non-carriers, have 20% lower risk of binge drinking, and 30% greater odds of being abstainers (Bierut *et al.*, 2012, Holmes *et al.*, 2014, Li *et al.*, 2011). Hence, the *ADH1B* rs1229984 G>A polymorphism produces a natural randomisation, with individuals allocated to a higher (GG) or lower (GA/AA) probability of excessive alcohol consumption and related disorders according to a random assortment of alleles that takes place during gamete production and fertilization. These groups should not differ systematically in any other way, as the distribution of unrelated (confounding) variables, including other genetic polymorphisms associated with the metabolism of alcohol, would be expected to be random. Consequently, if light to moderate alcohol use in later life decreases the risk of death, the *ADH1B* rs1229984 G>A polymorphism should also be associated with increased survival (i.e., lower mortality hazard).

As the *ADH1B* rs1229984 G>A polymorphism has a frequency of less than 7% amongst Caucasians (Holmes *et al.*, 2014), the exposure (in this case, alcohol use) and the outcome (i.e., mortality) must be frequent enough to ensure that a medium size study (thousands rather than tens of thousands participants) has adequate power to investigate these associations. We used data from the Health In Men Study (HIMS), a large cohort study of older Western Australians, to investigate if excessive alcohol use increases mortality. Accordingly, we hypothesised that there would be an independent triangular association between the *ADH1B* rs1229984 G>A polymorphism, regular alcohol intake, and mortality, and that (1) carriers of the *ADH1B* rs1229984 G>A polymorphism would consume less alcohol than non-carriers, (2) carriers of the *ADH1B* rs1229984 G>A polymorphism would

have lower mortality hazard than non-carriers, (3) older men who consumed less than 4 standard drinks per day would have lower mortality than never drinkers.

METHODS

Study design, setting and participants

HIMS is a cohort study that enrolled a community representative sample of 12203 Western Australian men aged 65 to 84 years during 1996-1998. At the second wave of assessments in 2001-2004, 4246 men older than 70 years consented to donate a blood sample, which we then used to extract DNA for genotypic analysis. We excluded from these analyses 554 men for whom data on alcohol consumption or the *ADH1B* rs1229984 G>A genotype were missing, as well as 196 men of possible non-Caucasian origin. Hence, the study sample consisted of 3496 older men.

HIMS has been conducted in accordance with the principles outlined in the Declaration of Helsinki for Human Rights. Study activities were approved by the Human Research Ethics Committee of the University of Western Australia and of the Western Australian Department of Health, and all men provided written informed consent to participate.

Outcome of interest

All cause mortality was the outcome of interest of this study. We retrieved these data from the Western Australian Data Linkage System (WADLS), which collects information from the Australian Bureau of Statistics about all deaths in Australia (Holman *et al.*, 2008). For the purposes of this study, time at risk started at the second assessment for HIMS (2001-2004), which was when the blood sample was collected, and finished on the 31st December 2012.

Exposures

During the 1996-1998 assessment, we asked participants if they had ever drunk alcohol (yes/no). Those who answered 'yes' were then asked if they had consumed alcoholic beverages during the preceding year (yes/no), and those who responded in the affirmative were then required to indicate how many standard drinks of alcohol they consumed each day of a usual week (from Monday to Sunday). A standard drink was defined as 285 ml of full strength beer (5%) or the corresponding volume of reduced alcohol beer, 1 pub measure of spirits, sherry or port, or 1 glass of wine (approximately 10g of alcohol). We added the total number of drinks consumed during a usual week and divided the result by 7 to calculate the average number of drinks consumed per day. Based on these data, we created a 'daily alcohol use variable' and ascribed the value of 0 to never drinkers, 1 to past drinkers, 2 for those consuming ≤ 2 drinks per day, 3 for those consuming > 2 but ≤ 4 drinks per day, 4 for those consuming > 4 but ≤ 6 drinks per day, and 5 for those consuming > 6 daily drinks.

We extracted DNA from blood samples collected during the 2001-2004 assessment and used the TaqMan Drug Metabolism Genotyping assay to determine the allelic distribution at the single nucleotide polymorphism (SNP) rs1229984, which was associated with a call rate of 98.5% for the entire sample (Life Technologies Corporation, California, USA). We considered that older participants born in Europe, Australia and New Zealand were likely to be of Caucasian origin – all other men were excluded from the present analyses.

Other study measures

We calculated the age of participants at the time of the 2001-2004 assessment by subtracting the date of birth from the date of the assessment and dividing the result by 365.25. We also asked participants the highest level of education that they had completed and assigned them to a group with less than high school education or with at least high school education. In addition, participants answered the question 'Have you ever smoked cigarettes, cigars or a pipe regularly?' (yes/no), and

those who answered ‘yes’ were asked ‘How often do you smoke now?’ (every day / not every day / not at all). We used these answers to classify men as ‘never a regular smoker’, ‘past smoker’ and ‘current smoker’. Finally, we asked participants ‘Have you ever been told by a doctor that you have or have had hypertension (yes/no), diabetes (yes/no), emphysema or chronic bronchitis (yes/no), angina or a heart attack (yes/no), or a stroke (yes/no)?’

We used standard procedures to measure participants’ height (to 0.5 cm) and weight (to 0.2 Kg) and calculated the body mass index (BMI) in Kg/m^2. Men with BMI<18.5 were classified as underweight, between 18.5 and 24.9 normal, 25 and 29.9 overweight, and 30 or above obese.

Bias

HIMS participants originated from a community-representative sample of older Western Australian older men (Norman *et al.*, 2009). We have previously shown that those who did not respond to our invitation had greater health morbidity and mortality (Almeida *et al.*, 2014c, Almeida *et al.*, 2015), so that people who completed the 2001-2004 assessment were healthier than those who were not available. The implication for the proposed analyses is that less alcohol users with poor health would be available, and this could potentially reduce the power of the study to detect the expected differences in mortality (type II error).

Study size

Based on past analyses of the study cohort (Almeida *et al.*, 2014c), we estimated that at least 30% of participants would have died by the end of 2012 (i.e., about 1050 men). A study with 3496 participants would have 80% power to declare as statistically significant a mortality hazard ratio of 0.84 associated with a protective effect of the *ADH1B* rs1229984 G>A polymorphism (two-sided alpha of 5%).

Statistical analyses

We used the statistical software Stata v.13.1 (StataCorp LP, College Station, Texas, USA) to manage and analyse the data. Analysis of contingency tables using the Hardy-Weinberg test determined if the distribution of *ADH1B* rs1229984 genotypes was in equilibrium. We used descriptive statistics (mean, standard deviation of the mean (SD) and proportions) to summarise our data, Pearson's chi-square statistic (X^2) to compare the distribution of alcohol use among carriers and non-carriers, and t-test to compare their age. Mann-Whitney U test was used to compare the alcohol consumption of carriers and non-carriers (z statistic). Cox regression was used to calculate the crude and adjusted mortality hazard ratio (MHR) according to daily alcohol use and the *ADH1B* rs1229984 G>A allelic distribution. We also plotted the Kaplan-Meir survival curve for carriers and non-carriers of the *ADH1B* rs1229984 A allelic polymorphism. Alpha was set at 5% and all tests reported are two-tailed.

TABLE 1

RESULTS

The age of participants at the time of the collection of the blood sample ranged from 70 to 89 years (mean=77.1, SD=3.6). Of the 3496 participants, 225 (6.4%) were carriers of the *ADH1B* rs1229984 G>A polymorphism. Their genotypic distribution was in Hardy-Weinberg equilibrium (Table 1). The data outlining the consumption of alcohol among carriers and non-carriers appear in table 2. Two hundred men (5.7%) reported having never consumed alcohol, and 280 (8.0%) were past drinkers. Among current drinkers, non-carriers consumed more alcohol than carriers (z=3.19, p=0.001).

TABLE 2

TABLE 3

One thousand three hundred and twenty-nine men (38.0%) died during the 8.0 (SD=2.5) years of follow up (range, 10 weeks to 11.2 years). The sociodemographic, lifestyle and clinical characteristics of these men at the time of the blood collection are summarised in table 3. Compared with survivors, those who died were older, had less education, were more likely to be past or current smokers and less likely to be overweight, and reported greater frequency of diabetes, chronic respiratory diseases, coronary heart disease and stroke. The adjusted MHR was nearly twice as large for men who consumed more than 6 drinks per day compared with never drinkers (MHR=1.76, 95%CI=1.17, 2.66). (The adjusted analyses included all variables that independently contributed to increase or decrease mortality.) Carriers of the *ADH1B* rs1229984 G>A polymorphism had a 32% lower MHR than non-carriers (MHR=0.68, 95%CI=0.54, 0.87) (Figure 1). We found evidence of a statistically significant interaction between alcohol use group and being a carrier (table 4).

TABLE 4

FIGURE 1

DISCUSSION

The results of this study confirmed the triangular association between the *ADH1B* rs1229984 G>A polymorphism, regular alcohol intake, and mortality. First, carriers of the *ADH1B* rs1229984 G>A polymorphism consumed less alcohol than non-carriers and none of the carriers were regular excessive drinkers. Second, men with the *ADH1B* rs1229984 G>A polymorphism had a lower mortality hazard than non-variant participants. Third, older men whose daily consumption of alcohol exceeded 6 standard drinks had an 8-year mortality hazard that was nearly twice as large as that of never drinkers, while the use of less then 6 standard drinks had no obvious effect on mortality.

Strengths and limitations

Participants were part of a large community-representative and well-characterised cohort study of older men that started in 1996 (Norman *et al.*, 2009). In addition, the analyses were restricted to those most likely to be of European descent, thereby enhancing the ethnic homogeneity of the sample. Mortality data covered a period of over 11 years and information on alcohol use was available from the time of entry into the study. Notwithstanding the relatively low frequency of carriers of the *ADH1B* rs1229984 G>A polymorphism in the sample (6.4%), the high mortality during follow up (38.0%) ensured that the study was sufficiently powered to investigate its association with death. We concede, however, that the study sample was most likely subject to healthy participant bias (McCaul *et al.*, 2015), and that heavy alcohol users with poor health had lower probability of completing the second wave of assessments for HIMS during 2001-2004 (Almeida *et al.*, 2014c). A consequence of such bias would have been loss of power to investigate the association between excessive alcohol use and death. This may explain, at least in part, the relatively small numbers of heavy drinkers in our sample, which in turn may create some uncertainty about the validity of the results. Despite this potential caveat, we were still able to demonstrate an increase of 86% in mortality hazard associated with daily consumption of more than 6 standard drinks, a finding that is consistent with previously published data (Di Castelnuovo *et al.*, 2006).

Two potential sources of error may have been introduced as part of the assessment of alcohol use. First, we cannot be certain that the amount of alcohol consumption that men reported was accurate, although previous surveys have shown that the approach we used generates reliable and valid information (Greenfield *et al.*, 2014, Sobell *et al.*, 1979). Second, there was time lag of 5.7 (SD=0.9) years between the collection of alcohol-related data and the blood samples, which we then used to extract DNA and determine the *ADH1B* rs1229984 G>A polymorphism. As a result, those

who died between these two assessments could not donate a blood sample and were not available for analysis. For this reason, follow up had to start at the date when the blood sample was donated rather than when information about alcohol use was collected. This raises the question of whether the pattern of alcohol use could have changed between the first and second assessments (no data on the use of alcohol were collected at the second assessment). As heavy drinkers tend to be censored early from longitudinal studies (Vaillant, 2003), a decreasing number of people consume large amounts of alcohol with increasing age. Hence, it is conceivable that the number of heavy alcohol users would have declined by the time we started follow up. Following this same line of reasoning, one would expect participants to have consumed even larger amounts of alcohol before they joined the study in 1996-1998, which was the time when we assessed their use of alcohol. Consequently, while our alcohol measure may have been inflated by the time we started the follow up, it is unlikely to over-represent the lifetime drinking behaviour of participants.

We also acknowledge that investigating the effect of one single polymorphism of the alcohol dehydrogenase gene may fail to provide a comprehensive picture of this metabolic pathway. For example, other genetic polymorphisms have been associated with increased alcohol consumption and risk of oesophageal cancer (Cui *et al.*, 2009, Hashibe *et al.*, 2008, Rivera-Meza *et al.*, 2010), thereby providing additional persuasive evidence that excessive alcohol use increases mortality.

HIMS has the merit of having collected information on several exposures associated with poor health outcomes, and this allowed us to adjust our analyses for other relevant measures when assessing the association between alcohol use and mortality (Knott *et al.*, 2015).

Interpretation of the findings

The results of this study indicate that excessive alcohol consumption causes an increase in the 8-year mortality of older men, and that light to moderate drinking neither decreases nor increases the

risk of death compared with never drinkers. Consistent with our findings, the European Prospective Investigation into Cancer and nutrition reported a 53% increase in mortality hazard among men with a lifetime consumption of 6 or more standard drinks per day, although their participants were 20 years younger than those in the HIMS cohort and their reference group consisted of men who consumed less than half a drink per day (Ferrari *et al.*, 2014). The most frequent causes of death in this group of men were alcohol-related cancers, injuries and other external causes (e.g., violence) (Ferrari *et al.*, 2014). Our findings add a novel degree of certainty about the harmful effects of excessive alcohol use by demonstrating that the *ADH1B* rs1229984 G>A polymorphism, which is associated with lower alcohol consumption or abuse, decreases the mortality hazard of older men.

An underlying assumption of Mendelian randomisation studies is that the relevant genetic polymorphism under investigation does not have other physiological effects in addition to the one associated with the exposure of interest. In the present case, the *ADH1B* rs1229984 G>A polymorphism should have no action other than its hindering effect on the function of the alcohol dehydrogenase enzyme, which leads to an increase in unpleasant experiences associated with drinking (Quertemont and Didone, 2006). We are not aware of any evidence suggesting that this may not be the case, but concede that other unmeasured factors and residual confounding could, theoretically, have played some role. If we accept, however, that the pleiotropic effect of this polymorphism is minimal, then this genetic variant would act by creating a predisposition to lower consumption of alcohol, which in turn enhances survival. Our results show that older men who regularly consume excessive amounts of alcohol have higher mortality, but they do not clarify whether lower regular intake is either harmful or protective. We found no evidence of a progressive increase in mortality hazard associated with increasing amounts of alcohol use up to 6 daily drinks (adjusted analyses), although we also noticed that nearly 9 in every 10 carriers of the A allelic polymorphism consumed 2 or less daily drinks or were abstainers. A large Mendelian randomization study that included over 260000 adults and older adults raised doubts about the

potential protective cardiovascular effect of light to moderate alcohol use (Holmes *et al.*, 2014). In contrast, similar studies have found no evidence to support the association between excessive alcohol use and depression or dementia, suggesting that alcohol is not likely to be a direct cause of either of these prevalent disorders in later life (Almeida *et al.*, 2014a, Almeida *et al.*, 2014b, Kumari *et al.*, 2014). Nonetheless, the most relevant question to address is whether the *ADH1B* rs1229984 G>A polymorphism is associated with lower mortality due to alcohol-related cancers, which is the most frequent cause of death among heavy users (Ferrari *et al.*, 2014).

A longitudinal study of 801 patients with a first primary diagnosis of cancer of the head, neck and oesophagus found that excessive alcohol use decreased survival, whereas the mortality hazard associated with the *ADH1B* rs1229984 G>A variant was borderline non-significant (MHR = 0.64, 95%CI=0.40, 1.03) (Leoncini *et al.*, 2015). However, currently available evidence also suggests that for the same level of alcohol use, this polymorphism increases rather than decreases the risk of oesophageal carcinoma (Lee *et al.*, 2008), possibly because of the increased exposure to alcohol and its byproducts arising from suboptimal function of the alcohol dehydrogenase enzyme. This can be viewed as an example of genetic-environment interaction.

We acknowledge that that our data are limited to older men and that our results may not apply equally to women or to younger men. However, we see no compelling reason to believe these groups would behave differently in relation to the effects of alcohol and the genetic variation that we investigated in this study.

In summary, our data confirmed that regular excessive alcohol use (> 6 daily drinks) increases mortality in later life, but failed to show that light to moderate consumption increases survival. These findings would benefit from replication by studies using larger and ethnically diverse cohorts. In the meantime, there may be merit in promoting conservative health messages regarding the safe

use of alcohol in the community. Current guidelines suggest that the regular consumption of more than 2 drinks for women and 3-4 drinks for men could be harmful (O'Flynn, 2011), with the National Health and Medical Research Council of Australia advising against the consistent use of more than 2 daily drinks for both men and women (Bowden *et al.*, 2014).

AUTHORS CONTRIBUTION

Conceived and designed the experiments: Almeida.

Performed the experiments: all authors.

Analyzed the data: Almeida.

Drafted the manuscript: Almeida.

Other: all authors reviewed the manuscript for important intellectual content and approved its submission for publication.

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Table 1. Frequency distribution of rs1229984 genotypes among 3496 older men.

Genotype	Observed number	Expected number	Hardy-Weinberg equilibrium test
AA	5	4	$X^2(1)=0.419$, p=0.518 (disequilibrium coefficient D=0.0003)
GA	220	222	
GG	3271	3270	

Table 2. Alcohol consumption and other study measures at the time of collection of the DNA sample among carriers of the *ADH1B* rs1229984 G>A allele polymorphism.

		Non-carrier N=3271 n (%)	Carrier N=225 n (%)	X^2 statistic (degrees of freedom)	p-value
Daily alcohol use	Never	185 (5.7)	15 (6.7)	12.13 (5)	0.033
	Past drinker	264 (8.1)	16 (7.1)		
	≤ 2 drinks	2173 (66.4)	169 (75.1)		
	2-4 drinks	487 (14.9)	20 (8.9)		
	4-6 drinks	128 (3.9)	5 (2.2)		
	> 6 drinks	34 (1.0)	0		
Age (years)	70-74	1194 (36.5)	83 (36.9)	4.09 (2)	0.129
	75-79	1414 (43.2)	85 (37.8)		
	≥ 80	663 (20.3)	57 (25.3)		
High school education		1487 (45.5)	93 (41.3)	1.46 (1)	0.227
BMI group	Normal	1069 (33.2)	75 (34.1)	0.46 (3)	0.927
	Underweight	21 (0.6)	1 (0.4)		
	Overweight	1629 (50.6)	113 (51.4)		
	Obese	499 (15.5)	31 (14.1)		
Smoking history	Never	1090 (33.3)	72 (32.0)	1.65 (2)	0.438
	Past	2008 (61.4)	145 (64.4)		
	Current	173 (5.3)	8 (3.6)		
Hypertension		2652 (81.1)	181 (80.4)	0.05 (1)	0.815
Diabetes		453 (13.8)	37 (16.4)	1.17 (1)	0.279
Chronic respiratory diseases		377 (11.5)	31 (13.8)	1.04 (1)	0.309
Coronary heart disease		850 (26.0)	51 (22.7)	1.21 (1)	0.271
Stroke		334 (10.2)	18 (8.0)	1.15 (1)	0.285

Table 3. Clinical characteristics of older men at study entry and for those deceased during an average follow up period of 8 years.

		Population N=3496 n (%)	Deceased N=1329 n (%)	Risk Ratio of death (95%CI)
Age (years)	70-74	1277 (36.5)	316 (23.8)	1
	75-79	1499 (42.9)	552 (41.5)	1.49 (1.32, 1.67)
	≥80	720 (20.6)	461 (34.7)	2.59 (2.32, 2.89)
High school education		1580 (45.2)	562 (42.3)	0.89 (0.81, 0.97)
BMI group	Normal	1144 (33.3)	462 (35.8)	1
	Underweight	22 (0.6)	12 (0.9)	1.35 (0.92, 1.99)
	Overweight	1742 (50.7)	608 (47.1)	0.86 (0.79, 0.95)
	Obese	530 (15.4)	208 (16.1)	0.97 (0.86, 1.10)
Smoking history	Never	1162 (33.2)	362 (27.2)	1
	Past	2153 (61.6)	876 (65.9)	1.31 (1.18, 1.44)
	Current	181 (5.2)	91 (6.8)	1.61 (1.36, 1.91)
Hypertension		2833 (81.0)	1082 (81.4)	1.03 (0.92, 1.14)
Diabetes		490 (14.0)	213 (16.0)	1.17 (1.05, 1.31)
Chronic respiratory diseases		408 (11.7)	215 (16.2)	1.46 (1.32, 1.62)
Coronary heart disease		901 (25.8)	448 (33.7)	1.46 (1.35, 1.59)
Stroke		352 (10.1)	197 (14.9)	1.56 (1.40, 1.73)

95%CI: 95% confidence interval of the risk ratio.
BMI: body mass index.

Table 4. Crude and adjusted mortality hazard ratios according to alcohol use and among carriers of the *ADH1B* rs1229984 G>A allele polymorphism.

		Crude MHR (95%CI)	Adjusted MHR (95%CI)
Daily alcohol use	Never	1	1*
	Past	1.35 (1.01, 1.80)	1.15 (0.86, 1.55)
	≤ 2 drinks	1.03 (0.81, 1.32)	0.98 (0.76, 1.25)
	2-4 drinks	1.26 (0.96, 1.65)	1.13 (0.85, 1.49)
	4-6 drinks	1.39 (0.96, 1.99)	1.18 (0.81, 1.71)
	> 6 drinks	2.42 (1.46, 4.02)	1.87 (1.11, 3.12)
rs1229984 G>A allele carrier		0.68 (0.54, 0.87)	0.88 (0.32, 2.42)**

MHR: mortality hazard ratio.

95%CI: 95% confidence interval of the mortality hazard ratio.

*Analyses adjusted for age (in days), educational attainment, body mass index and smoking groups, physical activity, and prevalent coronary heart disease and stroke.

**Analyses investigating the interaction between alcohol consumption group membership and being a carrier. The results report the independent effect of the *ADH1B* rs1229984 G>A polymorphism on mortality. The protective crude effect amongst carriers was no longer statistically significant when we investigated its interaction with alcohol use group, suggesting that the protective effect of the allele is only present when alcohol is not. (It was not possible to estimate the interaction between the *ADH1B* rs1229984 variant and regular consumption of 6 or more drinks because no carriers consumed this amount of alcohol.)

FIGURE LEGEND

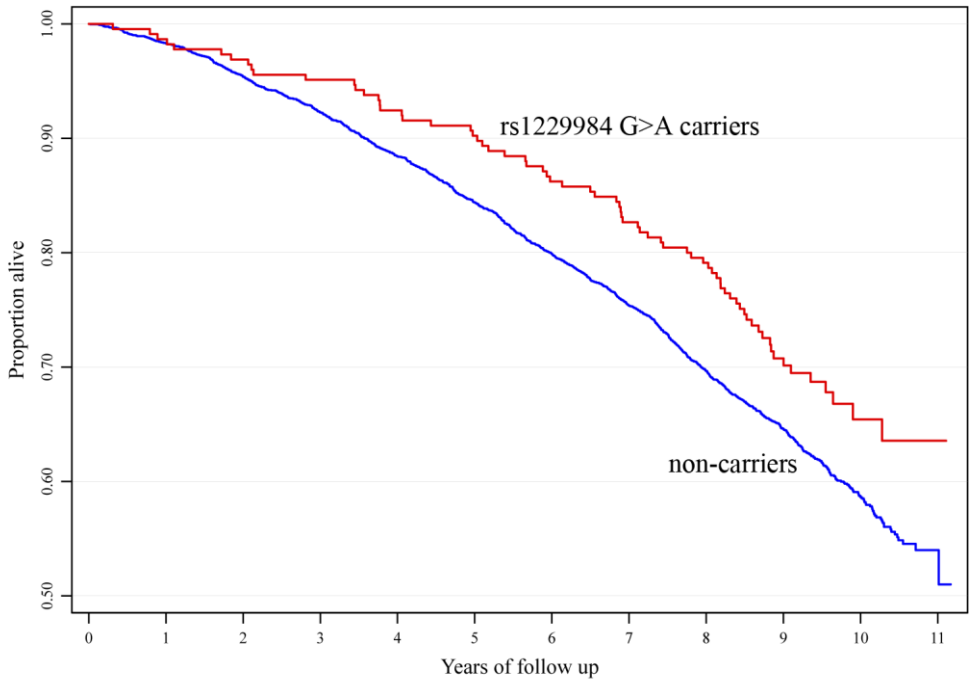

Figure 1. Kaplan-Meier survival curve for older men according to whether or not they carried the *ADH1B* rs1229984 G>A polymorphism (carriers depicted in orange and non-carriers in blue). The mean follow up period was 8.0±2.5 years (range: 0.2 to 11.2).