PLoS one

The Association of Alcohol and Alcohol Metabolizing Gene Variants with Diabetes and Coronary Heart Disease Risk Factors in a White Population

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Abstract

Background: Epidemiological studies have shown a J- or U-shaped relation between alcohol and type 2 diabetes and coronary heart disease (CHD). The underlying mechanisms are not clear. The aim was to examine the association between alcohol intake and diabetes and intermediate CHD risk factors in relation to selected *ADH* and *ALDH* gene variants.

Methodology/Principal Findings: Cross-sectional study including 6,405 Northern European men and women aged 30–60 years from the general population of Copenhagen, Denmark. Data were collected with self-administered questionnaires, a physical examination, a 2 hour oral glucose tolerance test, and various blood tests. J shaped associations were observed between alcohol and diabetes, metabolic syndrome (MS), systolic and diastolic blood pressure, triglyceride, total cholesterol, and total homocysteine. Positive associations were observed with insulin sensitivity and HDL cholesterol, and a negative association with insulin release. Only a few of the selected *ADH* and *ALDH* gene variants was observed to have an effect. The *ADH1c* (rs1693482) fast metabolizing CC genotype was associated with an increased risk of impaired glucose tolerance (IGT)/diabetes compared to the CT and TT genotypes. Significant interactions were observed between alcohol and *ADH1b* (rs1229984) with respect to LDL and between alcohol and *ALDH2* (rs886205) with respect to IGT/diabetes.

Conclusions/Significance: The selected *ADH* and *ALDH* gene variants had only minor effects, and did not seem to markedly modify the health effects of alcohol drinking. The observed statistical significant associations would not be significant, if corrected for multiple testing.

Introduction

Epidemiological studies have consistently shown that light to moderate drinkers compared to abstention are at lower risk of type 2 diabetes (T2D) and coronary heart disease (CHD) whereas heavy and excessive drinkers are at increased risk or has a risk equal to that of non-consumers [1–7]. The potential mechanisms of this so-called U or J-shaped association include beneficial effects on insulin sensitivity, high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol, blood pressure and triglycerides [8–11]. However it has been argued that the observed inverse association between moderate alcohol consumption and diabetes and CHD is attributed to confounding factors such as a healthy lifestyle, misclassification of former alcoholics, or to constituents of alcohol other than ethanol, such as the antioxidants in grapes [12].

Alcohol is primarily metabolized in the liver. The major enzymes involved are alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). Firstly ethanol is oxidized to acetalde-hyde in a reversible reaction catalyzed by the class I ADH isoenzymes (ADH1a, ADH1b, ADH1c) located in the cytosol of hepatocytes. Functional relevant polymorphisms are found in the genes encoding ADH1b and ADH1c, affecting ethanol degrada-tion rates and alcohol intake in white populations [13–16]. These polymorphisms have been widely studied and related to various disease outcomes both in Asian and white populations [17–19]. Acetaldehyde is then oxidized to acetate and water in a non-reversible reaction catalyzed by the mitochondrial class II ALDH2 [20,21]. The *ALDH2* gene is also polymorphic and contains an inactive variant unable to metabolize acetaldehyde resulting in the Oriental flushing syndrome [22,23]. However, this variant is nearly absent in white populations [24]. Another less studied polymorphism in the promoter region of *ALDH2* gene has been reported in white populations, which may influence ALDH2 activity through effects on transcriptional activity [25,26]. In addition, various single nucleotide polymorphisms (SNP's) in other

ADH's and ALDH's such as the ADH7 involved in early metabolism of alcohol in the stomach mucosa and the class I ALDH1b1 (previously named as ALDHX and ALDH5) may also play a role, although their functional relevance and clinical importance is unknown [17,27].

Variations in the alcohol metabolizing genes may help to clarify whether the association is causal, since it is less likely that an individual's genetic composition is associated with confounding factors as genotypes are distributed randomly and thus mimic a randomized trial (a principle referred to as Mendelian Randomization) [28]. Moreover a gene-environment interaction effect will only be observed if ethanol is responsible for the association.

The aim of the study was to examine the association between weekly alcohol intake and diabetes and CHD risk factors in relation to various ADH and ALDH gene variants.

Materials and Methods

Ethics statement

Informed written consent was obtained from all participants before participation. The study was approved by the Ethical Committees of Copenhagen and was in accordance with the principle of the Helsinki Declaration II.

Study population

The current study is based on the Inter99 study, a population-based randomized controlled trial, investigating the effect of lifestyle intervention (smoking cessation, increased physical activity, and healthier dietary habits) on CVD. The present study was focused on the baseline data before any intervention had been offered. Data were collected with self-administered questionnaires, a physical examination, a 2 hour oral glucose tolerance test and various blood tests. Details on the study population, health examination, and the intervention program have been described elsewhere [29]. Briefly, the Inter99 study population were residents in the southern part of the former Copenhagen County. An age- and sex-stratified random sample of 13,016 men and women born in 1939–40, 1944–45, 1949–50, 1954–55, 1959–60, 1964–65, and 1969–70 was drawn from the Danish Civil Registration System and invited to participate in a health examination during 1999–2001, so that they were aged 30, 35, 40, 45, 50, 55, 60, and 65 years on the day of the examination. A total of 12,934 were eligible for invitation. The baseline participation rate was 52.5% (n = 6,784). Information on current and former nationalities of participants as well as their parents was obtained from Statistics Denmark and from the self-administered questionnaire. A Northern European origin was defined as a Danish, Norwegian, Swedish, Icelandic, or Faroese nationality. A non-Northern European origin was defined as nationalities other than the above mentioned. Both current and potential former nationalities of participants and their parents were considered. Only participants with a Northern European origin (Denmark, Norway, Sweden, Iceland, and Faroe Islands) were included in the current study (n = 6,405).

Glucose tolerance status

All participants without known diabetes underwent a 2 hour standardized 75 g oral glucose tolerance test (OGTT) in the morning after an overnight fast. Plasma glucose, serum insulin, and serum C-peptide were measured at time (t) 0, 30, and 120 min during the OGTT. Glucose concentrations were analyzed by hexokinase/glucose-6-phosphate dehydrogenase assay (Boehringer Mannheim, Germany). Insulin and C-peptide levels were measured by a fluoroimmunoassay technique (Dako Diagnostics Ltd., UK). Glucose tolerance status was defined according to WHO diagnostic criteria 1999 [30]. Impaired fasting glucose (IFG) was defined as: fasting plasma glucose ≥6.1 mmol/l and 2 hour plasma glucose <7.8 mmol/l. Impaired glucose tolerance (IGT) was defined as: fasting plasma glucose <7.0 mmol/l, and 2 hour plasma glucose ≥7.8 mmol/l and <11.1 mmol/l. Diabetes was defined as: fasting plasma glucose ≥7.0 and 2 hour plasma glucose ≥11.1 mmol/l. IGT and diabetes were combined in the statistical analyses to increase power.

Surrogate measures of Insulin release and insulin sensitivity

Estimates of insulin release and insulin sensitivity were estimated using both homeostasis model assessment (HOMA) based upon fasting circulating glucose and insulin levels [31].

Alcohol

The information on alcohol drinking was obtained from the self-administered questionnaire. The on average amount and type (beer, wine, dessert wine, spirits) of alcoholic beverage consumed per week in the last 12 months were recorded. One beer, one glass of wine, or one glass of spirit was approximated to one standard drink defined as 1.5 cl or 12 g of pure ethanol. Total weekly alcohol intake was calculated as the sum of weekly intakes of beer, wine, dessert wine and spirits. For the analyses of main effects, alcohol consumption was categorized in eight categories: 0, >0–2, >2–4, >4–7, >7–14, >14–21, >21–35, >35 standard drinks per week. For the analyses of interaction effects, weekly alcohol intake was categorized in three groups: non-drinkers (0 standard drinks), light/moderate drinkers (>0–14 for women; >0–21 for men), heavy drinkers (>14 for women; >21 for men).

ADH and ALDH gene variants

Based on previous reports on potential causal associations with disease outcomes, the following single nucleotide polymorphisms (SNPs) were examined: ADH1b Arg47His (rs1229984), ADH1c Arg271Gln (rs1693482) [15], ADH7 (rs1573496) [17], ALDH2 5′-UTR A-357G (rs886205) [25,26,32], ALDH1b1 Ala69Val (rs2228093) [33,34], and ALDH1b1 Arg90Leu (rs2073478) [33,34]. The SNP's were genotyped by KBiosciences allele-specific PCR (KASPar) (KBiosciences, Hoddesdon, UK). All genotyping success rates were above 96.6% with a mismatch rate of 0.0% for the above mentioned SNP's in 384 duplicate samples. Rs1229984 and rs886205 deviated significantly from the Hardy-Weinberg equilibrium (p<0.001 and p = 0.025, respectively).

Biological risk factors and metabolic syndrome

The physical examination included measurement of weight (wearing light clothes and no shoes) and height, waist circumference (in standing position at umbilical level), hip circumference, and systolic and diastolic blood pressure (measured twice in a sitting position after 5 minutes rest). Fasting triglyceride, cholesterols, homocysteine, urine albumin, and urine creatine were measured by standard techniques. Metabolic syndrome (MS) was defined according to the WHO diagnostic criteria 1999 with modifications as suggested by EGIR [30,35]. MS was defined as insulin resistance, diabetes, impaired glucose regulation, or impaired fasting glucose in combination with two or more of the following risk factor components: dyslipidemia, hypertension, obesity or microalbuminuria. Insulin resistance was defined as fasting plasma insulin in the upper 25% quartile (≥50.0 pmol/l) of the non-diabetic population [35]. Glucose tolerance status was defined above. Dyslipidemia was defined as high triglycerides

($\geq$1.7 mmol/l) and/or low HDL cholesterol (<0.9 mmol/l (men) and <1.0 mmol/l (women)). Hypertension was defined as high systolic blood pressure ($\geq$140 mmHg) and/or high diastolic blood pressure ($\geq$90 mmHg). Obesity was defined as high BMI ($\geq$30 kg/m^2) and/or high waist-hip ratio (>0.90 (men) and >0.85 (women)). Microalbuminuria was defined as albumin-creatinine ratio $\geq$30 mg/g.

The self-administered questionnaire

The self-administered questionnaire provided information on potential confounders such as socioeconomic factors, smoking status, physical activity, general dietary habits, menopause status and use of hormone replacement therapy. Smoking status was recorded as never smokers, ex-smokers, occasional smokers (<gram tobacco per day), and daily smokers. Total physical activity was calculated on the basis of a question on commuting physical activity and a question on leisure time physical activity including walking, gardening etc. and grouped into four categories as described by von Huth et al. [36]. Based on responses to qualitative questions about intake of fruit, vegetables, fish, and saturated fat, a dietary quality score was calculated as described by Toft et al. [37]. Social class was defined on the basis of questions regarding number of years of vocational training and employment status and categorised into five classes as described previously [38]. Postmenopausal hormone replacement therapy use was recorded in three categories: 1) premenopausal, 2) postmenopausal ever user, and 3) postmenopausal never user.

Statistical analyses

Statistics were computed with the statistical program SAS, version 9.1 (SAS Institute Inc, Cary, NC, USA). All p values reported are two-tailed and statistical significance was defined as $p<0.05$.

All continuous outcome variables were visually tested for approximation to the normal distribution by histograms and QQ-plots. HDL, triglyceride, homocysteine, HOMA-is, and HOMA-%B were log-transformed to achieve a normal distribution. Crude associations with continuous outcomes were examined by means and geometric means and tested for significant differences by one-way analysis of variance (F test). Crude association with dichotomous outcomes were examined in simple frequency tables and tested for significant differences by the chi-square test. Adjusted associations were evaluated in linear and logistic regression models using the PROC GLM (continuous outcomes) and the PROC GENMOD (dichotomous outcomes) procedures. Effects were reported as odds ratios (OR) and β coefficients with 95% confidence intervals (95% CI). β coefficients from models with log-transformed outcomes were back-transformed and reported as % with 95 CI. Regression models were adjusted for sex, age, BMI, dietary habits, physical activity, smoking status, socioeconomic status, and postmenopausal hormone replacement therapy use. Interaction effects were examined and evaluated in the regression models by including a product term. F-tests and Wald's tests for single parameters were used to test for significance in the regression analyses. Reported p values were not corrected for multiple testing. However, the large number of tests was taken into account in the interpretation of results. Persons receiving blood pressure and/or lipid lowering drugs were excluded in models including blood pressure and/or lipids. Known diabetics were excluded in analyses including HOMA estimates, since they may receive medication. Models with homocysteine included only half of the population, since only a sub-sample of the study population were a priori selected for homocysteine determination [39].

Results

General characteristics of the study population

The current study population consisted of 3.099 (48.4%) men and 3.306 (51.6%) women with a mean age of 46.3 (range: 29.7–61.3) years. The median weekly alcohol intake was 6.5 (range: 0–330) standard drinks including 578 (9.4%) abstainers. A total of 375 (6.2%) participants had diabetes and 1.409 (24.4%) were characterized with MS. The frequency of the *ADH* and *ALDH* minor alleles were: 0.02 (rs1229984), 0.42 (rs1693482), 0.11 (rs1573496), 0.17 (rs886205), 0.12 (rs2228093), and 0.40 (rs2073478). Further characteristics of the study population are given in table 1.

Association of alcohol intake with diabetes and intermediate CHD risk factors

Alcohol consumption was significantly associated with the risk of diabetes as well as MS in a J or U shaped manner. Abstainers and excessive drinkers (>35 standard drinks per week) had the highest risks, whereas light drinkers (>2–4 standard drinks per week) had the lowest risk (table S1). Alcohol was also significantly associated with surrogate measures of insulin sensitivity and insulin release. An increasing alcohol intake was associated with increasing insulin sensitivity and decreasing insulin release when applying the HOMA model (table S1). Significant associations were also found

Table 1. Characteristics of the Inter99 study population.

Characteristic	
Men (% (n))	48.4 (3099)
Age (mean (sd))	46.26 (7.91)
Standard drinks per week (median (min, max))	6.5 (0, 330)
Abstainers (% (n))	9.4 (578)
Binge drinkers (% (n))	37.1 (2225)
Daily smoking (% (n))	35.7 (2274)
BMI$\geq$30 kg/m^2 (% (n))	17.6 (1124)
Very low physical activity (% (n))	12.0 (720)
Less healthy dietary habits (% (n))	16.0 (990)
Lowest social class (% (n))	3.6 (212)
Postmenopausal women (% (n))[a]	52.9 (1709)
Hormone replacement therapy (% (n))[a]	16.9 (546)
Diabetes	
Impaired glucose tolerance (% (n))	11.4 (690)
Known diabetes (% (n))	4.1 (251)
Screen-detected diabetes (% (n))	2.1 (124)
Metabolic syndrome (% (n))	24.4 (1409)
Systolic blood pressure (mean (sd))	129.31 (17.05)
Diastolic blood pressure (mean (sd))	81.77 (11.01)
Hdl cholesterol (mean (sd))	1.4 4(0.40)
Ldl cholesterol (mean (sd))	3.51 (0.96)
Total cholesterol (mean (sd))	5.54 (1.08)
Triglyceride (mean (sd))	1.33 (1.31)
Homocysteine (mean (sd))	9.00 (4.49)

Data are % (n), mean (sd), or median (min, max). N$_{total}$ may differ due to missing information on some of the variables.
[a]Among women.
doi:10.1371/journal.pone.0011735.t001

between alcohol and all the examined biological CHD risk factors. The associations between alcohol intake and blood pressure, triglyceride, total cholesterol, and total homocysteine were J shaped, and a positive association was observed with HDL cholesterol (table S2). No significant interaction effects were observed between alcohol and sex with respect to the various outcomes (range of p values: 0.072–0.839).

Associations of ADH and ALDH gene variants with diabetes and intermediate CHD risk factors

The *ADH1c* (rs1693482) polymorphism was significantly associated with diabetes/IGT both in crude analyses (table 2) and after adjustment for potential confounders (p = 0.011) in a co-dominant model. The fast metabolizing CC genotype were associated with an increased risk of IGT/diabetes compared to CT and TT genotypes although no clear dose-response relationship was observed (table 2). The *ADH1b* fast metabolizing AA

genotype also seemed to increase the risk of IGT/diabetes compared to the GG and GA genotypes. However, only very few subjects (n = 9) were AA homozygous and the results were not statistically significant (table 2). In addition the ADH1b and ADH1c fast metabolising alleles seemed to be associated with decreased insulin sensitivity and increased insulin release (table 2). However none of the associations were statistically significant. Moreover, the *ADH1b* (rs1229984) and *ALDH1b1* (rs2073478) variants also seemed to be associated with HDL and LDL, respectively, in crude analyses (table 2, table 3), but not after adjustment for confounders (data not shown).

Interaction effects between alcohol and ADH and ALDH gene variants

In crude analyses, interaction effects were observed between alcohol and *ADH1b* (rs1229984) with respect to LDL ($p_{interaction}$ = 0.009) and between alcohol and *ADH7* (rs1573496) with respect

Table 2. Association between *ADH* and *ALDH* gene variants and diabetes related phenotypes.

	n	Insulin sensitivity (geometric mean (95% CI))	Insulin release (geometric mean (95% CI))	Metabolic syndrome (% (n)) n_{cases} = 1409	IGT/diabetes (% (n)) n_{cases} = 1065
ADH1b (rs1229984)					
GG, *slow*	5744	0.82 (0.81;0.83)	52.05 (51.24;53.87)	24.0 (1253)	17.1 (942)
GA	230	0.78 (0.72;0.85)	52.53 (48.64;56.74)	26.1 (55)	17.0 (36)
AA, *fast*	9	0.61 (0.33;1.10)	61.36 (34.26;109.89)		22.2 (2)
		p = 0.291	p = 0.733	p = 0.486	p = 0.92
ADH1c (rs1693482)					
CC, *fast*	2016	0.80 (0.80;0.83)	52.36 (50.96;53.79)	24.6 (457)	19.4 (373)
CT	2886	0.82 (0.80;0.84)	52.07 (50.94;53.22)	23.6 (614)	15.6 (428)
TT, *slow*	1031	0.83 (0.80;0.87)	51.45 (49.60;53.37)	23.8 (222)	17.3 (172)
		p = 0.229	p = 0.755	p = 0.712	P = 0.003
ADH7 (rs1573496)					
CC	4881	0.82 (0.81;0.84)	51.98 (51.10;52.87)	24.2 (1073)	17.3 (807)
GC	1169	0.79 (0.76;0.83)	52.40 (50.62;54.24)	24.3 (260)	17.7 (198)
GG	72	0.73 (0.62;0.87)	59.30 (51.86;67.81)	25.4 (16)	16.7 (11)
		p = 0.125	P = 0.168	p = 0.969	P = 0.943
ALDH2 (rs886205)					
TT	4075	0.82 (0.80;0.83)	52.26 (51.29;53.25)	24.6 (910)	17.6 (685)
CT	1709	0.82 (0.79;0.85)	51.55 (50.12;53.03)	23.1 (357)	16.3 (266)
CC	144	0.88 (0.79;0.98)	49.15 (44.36;54.47)	19.7 (26)	18.0 (25)
		p = 0.389	p = 0.394	p = 0.258	P = 0.512
ALDH1b1 (rs2228093)					
CC	4586	0.82 (0.80;0.84)	51.93 (51.02;52.86)	23.9 (995)	17.2 (754)
CT	1303	0.81 (0.78;0.84)	52.74 (51.05;54.47)	24.7 (292)	17.2 (214)
TT	87	0.91 (0.80;1.04)	45.20 (40.26;50.74)	23.4 (18)	16.7 (14)
		p = 0.263	p = 0.068	p = 0.834	P = 0.992
ALDH1b1 (rs2073478)					
TT	2142	0.82 (0.79;0.84)	52.02 (50.67;53.41)	24.7 (478)	17.7 (361)
GT	2869	0.82 (0.80;0.84)	51.93 (50.81;53.07)	23.6 (615)	16.9 (463)
GG	930	0.81 (0.77;0.84)	53.10 (51.04;55.25)	24.5 (209)	17.1 (153)
		p = 0.909	p = 0.605	p = 0.689	P = 0.746

N are the maximum number of participants in each category. N may be lower due to missing information on some variables. Data are geometric means with 95% confidence intervals (CI) or % (n). P values are F tests or Chi-square test.
doi:10.1371/journal.pone.0011735.t002

Table 3. Association between *ADH* and *ALDH* gene variants and CHD related phenotypes.

	n	Systolic blood pressure (mmHg) (Mean (95% CI))	Diastolic blood pressure (mmHg) (Mean (95% CI))	Hdl cholesterol (mmol/l) (Geometric mean (95% CI))	Ldl cholesterol (mmol/l) (Mean (95% CI))	Triglyceride (mmol/l) (Geometric mean (95% CI))	Homocysteine (μmol/l) (Geometric mean (95% CI))
ADH1b (rs1229984)							
GG, *slow*	5744	129.3 (128.8;129.8)	81.7 (81.4;82.0)	1.39 (1.38;1.40)	3.51 (3.48;3.54)	1.12 (1.10;1.13)	8.42 (8.30;8.53)
GA	230	128.8 (126.6;131.1)	82.4 (80.8;84.0)	1.37 (1.33;1.42)	3.42 (3.29;5.54)	1.16 (1.08;1.25)	8.24 (7.77;8.74)
AA, *fast*	9	128.7 (115.8;141.5)	83.9 (77.5;90.3)	1.08 (0.92;1.26)	4.52 (2.82;4.22)	1.49 (1.05;2.12)	7.18 (4.46;11.55)
		p = 0.932	p = 0.560	p = 0.018	p = 0.382	p = 0.142	p = 0.604
ADH1c (rs1693482)							
CC, *fast*	2016	129.6 (128.8;130.4)	81.9 (81.4;82.4)	1.39 (1.37;1.40)	3.53 (3.48;3.57)	1.14 (1.11;1.17)	8.51 (8.33;8.70)
CT	2886	129.2 (128.6;129.9)	81.7 (81.2;82.1)	1.38 (1.37;1.40)	3.49 (3.45;3.52)	1.11 (1.09;1.13)	8.38 (8.22;8.54)
TT, *slow*	1031	128.6 (127.5;129.7)	81.7 (81.0;82.4)	1.40 (1.37;1.42)	3.51 (3.45;3.57)	1.10 (1.06;1.13)	8.29 (8.03;8.57)
		p = 0.321	p = 0.808	p = 0.624	p = 0.353	p = 0.096	p = 0.382
ADH7 (rs1573496)							
CC	4881	129.4 (128.9;129.9)	81.9 (81.5;82.2)	1.39 (1.38;1.40)	3.51 (3.49;3.54)	1.12 (1.11;1.14)	8.35 (8.24;8.48)
GC	1169	129.3 (128.2;130.3)	81.6 (80.9;82.3)	1.39 (1.37;1.41)	3.50 3.45;3.56)	1.11 (1.07;1.14)	8.57 (8.31;8.83)
GG	72	125.7 (121.5;130.0)	79.4 (76.9;81.9)	1.38 (1.29;1.48)	3.36 (3.12;3.59)	1.12 (0.99;1.26)	9.18 (7.56;11.14)
		p = 0.227	p = 0.177	p = 0.978	p = 0.368	p = 0.669	p = 0.141
ALDH2 (rs886205)							
TT	4075	129.5 (128.9;130.0)	81.9 (81.5;82.3)	1.39 (1.37;1.40)	3.50 (3.47;3.53)	1.12 (1.10;1.14)	8.45 (8.32;8.58)
CT	1709	129.0 (128.1;129.8)	81.4 (80.9;82.0)	1.39 (1.37;1.41)	3.53 (3.48;3.57)	1.12 (1.10;1.15)	8.31 (8.10;8.52)
CC	144	130.1 (127.0;133.3)	81.7 (79.8;83.5)	1.40 (1.33;1.46)	3.40 (3.26;3.54)	1.09 (1.00;1.20)	8.24 (7.70;8.83)
		p = 0.551	p = 0.339	p = 0.941	p = 0.298	p = 0.793	p = 0.498
ALDH1b1 (rs2228093)							
CC	4586	129.3 (128.8;129.9)	81.8 (81.4;82.1)	1.39 (1.38;1.40)	3.51 (3.48;3.53)	1.12 (1.10;1.13)	8.44 (8.31;8.57)
CT	1303	129.4 (128.4;130.3)	81.8 (81.1;82.4)	1.38 (1.36;1.40)	3.52 (3.46;3.57)	1.12 (1.09;1.16)	8.32 (8.10;8.55)
TT	87	129.5 (125.9;133.2)	81.7 (79.4;84.0)	1.45 (1.36;1.55)	3.41 (3.20;3.62)	1.11 (0.99;1.24)	8.43 (7.41;9.59)
		p = 0.993	p = 0.999	p = 0.244	p = 0.602	p = 0.929	p = 0.684
ALDH1b1 (rs2073478)							
TT	2142	129.4 (128.6;130.2)	81.7 (81.2;82.2)	1.38 (1.37;1.40)	3.50 (3.46;3.55)	1.12 (1.08;1.15)	8.52 (8.33;8.72)
GT	2869	129.6 (128.9;130.2)	81.9 (81.5;82.3)	1.38 (1.37;1.40)	3.54 (3.51;3.58)	1.11 (1.09;1.14)	8.39 (8.24;8.54)
GG	930	128.3 (127.1;129.5)	81.3 (80.6;82.1)	1.39 (1.36;1.41)	3.43 (3.36;3.49)	1.12 (1.08;1.16)	8.23 (7.96;8.51)
		p = 0.185	p = 0.424	p = 0.940	p = 0.007	p = 0.948	p = 0.203

N are the maximum number of participants in each category. N may be lower due to missing information on some variables. Data are geometric mean or mean with 95% confidence intervals (CI). P values are F tests.
doi:10.1371/journal.pone.0011735.t003

triglyceride ($p_{interaction} = 0.021$) (data not shown). However, only the interaction between alcohol and the *ADH1b* (rs1229984) variant with respect to LDL remained statistically significant after adjustment for confounders (table 4). Heavy drinking was associated with lower LDL levels among participants with the fast metabolizing A allele (table 4). Moreover, a significant interaction effect was observed between ALDH2 (rs886205) and IGT/diabetes in the adjusted model (table 5).

Discussion

In this study we examined the association between alcohol and diabetes and intermediate CHD risk factors in relation to selected *ADH* and *ALDH* gene variants in an adult general population sample.

We observed a strong association between alcohol intake and diabetes, MS and several CHD risk factors. The *ADH* and *ALDH* gene variants on the other hand had only minor effects, and did not seem to markedly modify the health effects of alcohol drinking.

Our results confirm previous studies showing a U- or J-shaped relation between alcohol and type 2 diabetes and CHD [1–7]. Meta analyses have shown that light-moderate alcohol consumption is associated with a protective effect in the order of 30–40% with respect to type 2 diabetes and 20–30% with respect to CHD [2,6,7,40].

The finding of positive association with surrogate measures of insulin sensitivity and a negative relation with insulin release, suggest that the J-shaped relation may be explained by a beneficial effect of moderate alcohol intake on the insulin

Table 4. Joint interaction effects between alcohol and ADH and ALDH gene variants with respect to CHD related phenotypes.

Genotype	Alcohol drinking	n	Systolic blood pressure (mmHg) (β (95% CI))	Diastolic blood pressure (mmHg) (β (95% CI))	Hdl cholesterol (mmol/l) (% (95% CI))	Ldl cholesterol (mmol/l) (β (95% CI))	Triglyceride (mmol/l) (% (95% CI))	Homocysteine (μmol/l) (% (95% CI))
ADH1b (rs1229984)								
GG (slow)	Non	493	0	0	0	0	0	0
GG (slow)	Light/ moderate	4140	−0.35 (−1.99;1.29)	−0.75 (−1.81;0.32)	8.15 (5.67;10.70)	0.00 (−0.09;0.09)	−2.20 (−6.82;2.65)	−5.81 (−10.44;−0.95)
GG (slow)	Heavy	915	4.31(2.38;6.23)	1.59 (0.34;2.84)	21.93 (18.61;25.34)	−0.01 (−0.12;0.09)	3.10 (−2.66;9.19)	−3.44 (−9.10;2.58)
GA and AA (fast)	Non	37	−1.37 (−6.95;4.21)	0.29 (−3.32;3.91)	3.28 (−5.02;12.30)	0.04 (−0.29;0.37)	3.23 (−13.29;22.90)	−5.59 (−24.14;17.50)
GA and AA (fast)	Light/ moderate	161	−1.16 (−4.29;1.97)	−0.42 (−2.45;1.61)	9.63 (4.88;14.58)	−0.14 (−0.31;0.04)	−3.29 (−11.80;6.04)	−7.57 (−16.19;1.94)
GA and AA (fast)	Heavy	23	1.19 (−6.01;8.39)	−1.09 (−5.75;3.58)	14.32 (3.50;26.29)	−0.69 (−1.09;−0.30)	27.68 (3.80;57.06)	−12.46 (−28.97;7.87)
			$P_{interaction} = 0.836$	$P_{interaction} = 0.483$	$P_{interaction} = 0.287$	$P_{interaction} = 0.017$	$P_{interaction} = 0.132$	$P_{interaction} = 0.766$
ADH1c (rs1693482)								
CC (fast)	Non	170	0	0	0	0	0	0
CC (fast)	Light/ moderate	1479	0.30 (−2.41;3.00)	−0.05 (−1.80;1.71)	4.77 (0.79;8.90)	−0.03 (−0.18;0.12)	0.77 (−7.04;9.23)	−7.52 (−15.25;0.91)
CC (fast)	Heavy	282	4.16 (0.93;7.38)	2.95 (0.85;5.04)	19.02 (13.59;24.71)	−0.03 (−0.21;0.15)	3.43 (−615;13.99)	−0.88 (−10.85;10.21)
CT	Non	274	0.52 (−2.77;3.80)	0.89 (−1.25;3.02)	−2.19 (−6.66;2.49)	−0.09 (−0.28;0.09)	−1.87 (−10.97;8.15)	−4.93 (−14.19;5.34)
CT	Light/ moderate	2057	−0.13 (−2.80;2.53)	−0.24 (−1.97;1.49)	5.34 (1.39;9.43)	−0.06 (−0.21;0.09)	−3.90 (−11.25;4.05)	−7.79 (−15.37;0.48)
CT	Heavy	458	5.10 (2.09;8.11)	1.87 (−0.09;3.83)	16.97 (12.00;22.15)	−0.13 (−0.30;0.04)	4.72 (−4.32;14.63)	−7.80 (−16.36;1.65)
TT (slow)	Non	85	0.55 (−3.83;4.94)	0.99 (−1.86;3.83)	−7.14 (−12.81;−1.10)	0.02 (−0.23;0.27)	1.51 (−10.97;15.74)	−4.37 (−17.02;10.22)
TT (slow)	Light/ moderate	721	−0.17 (−3.01;2.68)	0.22 (−1.62;2.07)	5.31 (1.10;9.70)	−0.06 (−0.23;0.10)	−3.62 (−11.47;4.92)	−10.31 (−18.18;−1.68)
TT (slow)	Heavy	191	4.62 (1.10;8.14)	1.81 (−0.48;4.09)	19.30 (13.44;25.46)	−0.03 (−0.23;0.17)	2.05 (−8.09;13.31)	−10.67 (−20.38;0.22)
			$P_{interaction} = 0.878$	$P_{interaction} = 0.566$	$P_{interaction} = 0.131$	$P_{interaction} = 0.692$	$P_{interaction} = 0.619$	$P_{interaction} = 0.515$
ADH7 (rs1573496)								
CC	Non	421	0	0	0	0	0	0
CC	Light/ moderate	3507	−0.13 (−1.92;1.57)	−0.33 (−1.50;0.83)	6.54 (3.87;9.27)	−0.02 (−0.12;0.08)	−2.03 (−7.08;3.29)	−7.07 (−11.98;−1.88)
CC	Heavy	781	4.43 (2.33;6.54)	2.15 (0.78;3.51)	19.58 (16.05;23.22)	−0.02 (−0.13;0.10)	5.85 (−0.56;12.68)	−4.15 (−10.17;2.28)
GC and GG	Non	121	−0.76 (−4.20;2.68)	−0.05 (−2.28;2.18)	−0.87 (−5.61;4-10)	−0.07 (−0.26;0.13)	−4.73 (−13.98;5.52)	−1.38 (−11.64;10.06)
GC and GG	Light/ moderate	878	−0.43 (−2.47;1.61)	−0.72 (−2.04;0.60)	6.47 (3.44;9.60)	0.01 (−0.11;0.12)	−1.41 (−7.18;4.72)	−1.89 (−7.79;4.39)
GC and GG	Heavy	195	4.82 (1.90;7.75)	0.87 (−1.03;2.77)	20.49 (15.49;25.69)	−0.11 (−0.27;0.06)	−0.77 (−9.16;8.40)	−3.94 (−12.70;5.70)
			$P_{interaction} = 0.853$	$P_{interaction} = 0.597$	$P_{interaction} = 0.872$	$P_{interaction} = 0.314$	$P_{interaction} = 0.219$	$P_{interaction} = 0.344$
ALDH2 (rs886205)								
TT	Non	345	0	0	0	0	0	0
TT	Light/ moderate	2953	0.40 (−1.54;2.35)	−0.59 (−1.84;0.67)	7.45 (4.54;10.45)	−0.03 (−0.13;0.08)	−3.39 (−8.78;2.31)	−5.21 (−10.74;0.66)
TT	Heavy	644	5.63 (3.35;7.91)	1.79 (0.31;3.26)	20.57 (16.72;24.55)	−0.08 (−0.20;0.05)	0.76 (−5.81;7.84)	−3.07 (−9.68;4.04)
CT and CC	Non	179	2.34 (−0.82;5.50)	0.01 (−2.03;2.06)	−0.78 (−5.12;3.75)	−0.08 (−0.26;0.10)	−4.81 (−13.27;4.47)	2.32 (−7.12;12.73)
CT and CC	Light/ moderate	1305	0.40 (−1.65;2.46)	−0.98 (−2.31;0.35)	8.41 (5.29;11.63)	−0.01 (−0.13;0.11)	−4.63 (−10.26;1.36)	−5.37 (−11.22;0.86)
CT and CC	Heavy	287	3.55 (0.84;6.27)	1.05 (−0.70;2.81)	22.20 (17.52;27.06)	0.00 (−0.16;0.15)	−4.63 (−10.26;1.36)	−2.45 (−10.90;6.81)
			$P_{interaction} = 0.080$	$P_{interaction} = 0.843$	$P_{interaction} = 0.743$	$P_{interaction} = 0.412$	$P_{interaction} = 0.224$	$P_{interaction} = 0.889$

Table 4. Cont.

Genotype	Alcohol drinking	n	Systolic blood pressure (mmHg) (β (95% CI))	Diastolic blood pressure (mmHg) (β (95% CI))	Hdl cholesterol (mmol/l) (% (95% CI))	Ldl cholesterol (mmol/l) (β (95% CI))	Triglyceride (mmol/l) (% (95% CI))	Homocysteine (μmol/l) (% (95% CI))
ALDH1b1 (rs2228093)								
CC	Non	411	0	0	0	0	0	0
CC	Light/moderate	3290	−0.19 (−2.00;1.63)	−0.68 (−1.85;0.50)	7.67 (4.95;10.46)	0.02 (−0.08;0.12)	−5.14 (−10.06;0.05)	−3.57 (−8.90;2.08)
CC	Heavy	715	3.48 (1.34;5.63)	1.20 (−0.19;2.59)	21.02 (17.38;24.78)	−0.02 (−0.14;0.10)	−0.04 (−6.20;6.52)	−2.26 (−8.71;4.64)
CT and TT	Non	114	−0.01 (−3.57;3.56)	−0.32 (−2.63;1.99)	−2.45 (−7.30;2.64)	0.09 (−0.11;0.29)	−8.13 (−17.36;2.14)	5.26 (−5.24;16.93)
CT and TT	Light/moderate	1003	−0.84 (−2.85;1.18)	−0.99 (−2.29;0.31)	6.95 (3.94;10.04)	0.00 (−0.11;0.12)	−3.09 (−8.68;2.83)	−4.95 (−10.73;1.21)
CT and TT	Heavy	225	6.12 (3.34;8.90)	2.17 (0.37;3.97)	20.56 (15.81;25.51)	0.01 (−0.15;0.16)	5.49 (−2.97;14.69)	−0.23 (−8.76;9.09)
			$P_{interaction} = 0.066$	$P_{interaction} = 0.364$	$P_{interaction} = 0.782$	$P_{interaction} = 0.570$	$P_{interaction} = 0.105$	$P_{interaction} = 0.423$
ALDH1b1 (rs2073478)								
TT	Non	190	0	0	0	0	0	0
TT	Light/moderate	1545	−0.18 (−2.81;2.46)	−1.20 (−2.90;0.50)	5.96 (2.06;10.02)	0.00 (−0.15;0.15)	−1.91 (−9.28;6.07)	−3.78 (−11.34;4.42)
TT	Heavy	326	3.22 (0.10;6.35)	0.95 (−1.07;2.98)	18.97 (13.76;24.42)	−0.08 (−0.26;0.10)	0.83 (−8.14;10.67)	−2.80 (−11.83;7.15)
GT	Non	254	0.41 (−2.85;3.68)	0.16 (−1.95;2.27)	−2.58 (−6.97;2.02)	0.00 (−0.18;0.19)	0.05 (−9.11;10.13)	−1.41 (−10.78;8.95)
GT	Light/moderate	2066	−0.37 (−2.96;2.23)	−0.84 (−2.52;0.84)	6.39 (2.52;10.41)	0.02 (−0.12;0.17)	−3.89 (−11.02;3.82)	−6.72 (−13.95;1.11)
GT	Heavy	442	4.95 (2.01;7.90)	1.54 (−0.37;3.44)	18.79 (13.87;23.93)	−0.01 (−0.18;0.16)	5.82 (−3.10;15.57)	−4.68 (−13.24;4.74)
GG	Non	85	−1.52 (−5.93;2.89)	−1.81 (−4.66;1.05)	−1.90 (−7.95;4.55)	−0.05 (−0.30;0.20)	−3.89 (−15.82;9.71)	2.71 (−10.68;18.10)
GG	Light/moderate	656	−1.14 (−3.95;1.67)	−1.18 (−3.00;0.63)	5.71 (1.54;10.04)	−0.08 (−0.24;0.07)	−0.15 (−8.16;8.56)	−9.13 (−16.74;−0.82)
GG	Heavy	161	3.87 (0.22;7.52)	0.75 (−1.61;3.12)	21.53 (15.34;28.04)	−0.02 (−0.23;0.18)	0.86 (−9.52;12.43)	−5.22 (−15.30;6.06)
			$P_{interaction} = 0.673$	$P_{interaction} = 0.804$	$P_{interaction} = 0.593$	$P_{interaction} = 0.745$	$P_{interaction} = 0.282$	$P_{interaction} = 0.837$

N are the maximum number of participants in each category. N may be lower due to missing information on some variables. Data are β coefficients with 95% confidence intervals (CI) from adjusted regression analyses. The category "wildtype non drinkers" was set as the joint reference group. β coefficients from models with log-transformed outcomes were back-transformed and reported as % with 95% CI. All p values are F tests.

doi:10.1371/journal.pone.0011735.t004

sensitivity and an adverse effect of heavy alcohol intake on insulin release perhaps caused by a toxic alcohol effect on the pancreatic β cells. However the insulin release may also decrease with increasing alcohol intake due to lower demands caused by the increasing sensitivity. In this context, misclassification of alcohol exposure should also be considered (see below).

Furthermore, our results support previous findings of beneficial effects of alcohol drinking on insulin sensitivity and HDL cholesterol levels [9–11]. Also in accordance with our results elevated blood pressure, triglyceride, total and LDL cholesterol in heavy-excessive drinkers have been reported previously [8,9]. Studies on the relationship between total alcohol intake and circulating homocysteine levels have been inconsistent, but several studies have shown a lowering effect of beer drinking on plasma homocysteine concentrations, which has also been reported previously for this cohort [38].

In the current study we observed significant associations between *ADH1c* (rs1693482) and IGT/diabetes (co-dominant model). The fast metabolizing C allele was related to a higher risk of IGT/diabetes. This is supportive of a protective role of alcohol, since individuals with genotypes coding for fast alcohol degradation have lower blood

alcohol concentrations. Moreover, the *ADH1c* C allele has been associated with a lower alcohol intake also contributing to lower blood alcohol concentrations [13,14]. Beulens et al. also reported in a nested case-control study of 1.023 white men and women with incident diabetes and 1.382 controls that the *ADH1c* genotype modified the association between alcohol consumption and diabetes. In this study the slow metabolizing allele seemed to attenuate the lower risk of diabetes among moderate to heavy drinkers [41]. Although we did observe an association between the *ADH1c* (rs1693482) variant and IGT/diabetes as well as insulin sensitivity, the direction was opposite, and we could not confirm the results. However in accordance with our results, Hines et al. showed in a nested case-control study of 1166 U.S. male physicians (396 patients and 777 controls) that the slow oxidizing *ADH1c* allele is associated with reduced risk of myocardial infarct in moderate drinkers [42]. This has been confirmed in other populations [43,44]. In addition an interaction between *ADH1c* and the level of alcohol consumption in relation to HDL has been reported in several studies [42,45] although not in all [43,46].

The *ADH1b* rs1229984 GG slow genotype has been associated with elevated blood pressure, triglycerides, and uric acid in one

Table 5. Joint interaction effects between alcohol and *ADH* and *ALDH* gene variants with respect to diabetes related phenotypes.

Genotype	Alcohol drinking	n	Insulin sensitivity (% (95% CI))	Insulin release (% (95% CI))	Metabolic syndrome (OR (95% CI)) $n_{cases} = 1409$	IGT/diabetes (OR (95% CI)) $n_{cases} = 1065$
ADH1b (rs1229984)						
GG (slow)	Non	493	0	0	1	1
GG (slow)	Light/moderate	4140	13.11 (6.67;19.94)	−12.66 (−17.42;−7.63)	0.63 (0.47;0.85)	0.67 (0.50;0.88)
GG (slow)	Heavy	915	22.66 (14.45;31.47)	−25.28 (−30.06;−20.17)	0.93 (0.66;1.31)	1.05 (0.76;1.45)
GA and AA (fast)	Non	37	−11.60 (−27.68;8.04)	2.35 (−15.48;23.95)	1.31 (0.48;3.56)	0.76 (0.26;2.22)
GA and AA (fast)	Light/moderate	161	14.55 (2.39;28.16)	−14.14 (−22.86;−4.43)	0.60 (0.33;1.08)	0.63 (0.35;1.13)
GA and AA (fast)	Heavy	23	8.74 (−14.75;38.72)	−13.94 (−31.78;8.55)	0.35 (0.08;1.45)	0.90 (0.28;2.91)
			$P_{interaction} = 0.353$	$P_{interaction} = 0.449$	$P_{interaction} = 0.326$	$P_{interaction} = 0.929$
ADH1c (rs1693482)						
CC (fast)	Non	170	0	0	1	1
CC (fast)	Light/moderate	1479	10.37 (0.14;21.66)	−8.43 (−16.56;0.49)	0.76 (0.46;1.26)	0.75 (0.48;1.19)
CC (fast)	Heavy	282	20.06 (6,84;34.91)	−20.32 (−28.78;−10.93)	1.21 (0.67;2.18)	1.08 (0.64;1.85)
CT	Non	274	−3.42 (−14.16;8.66)	7.40 (−4.03;20.18)	1.45 (0.80;2.63)	0.95 (0.55;1.64)
CT	Light/moderate	2057	14.44 (3.97;25.96)	−9.50 (−17.43;−0.80)	0.72 (0.44;1.19)	0.53 (0.34;0.83)
CT	Heavy	458	21.83 (9.28;35.81)	−21.31 (−29.07;−12.71)	1.04 (0.60;1.80)	1.02 (0.62;1.69)
TT (slow)	Non	85	−0.37 (−15.01;16.81)	3.77 (−10.83;20.76)	1.11 (0.51;2.44)	0.65 (0.29;1.43)
TT (slow)	Light/moderate	721	13.78 (2.69;26.06)	−9.77 (−18.19;−0.48)	0.84 (0.49;1.42)	0.70 (0.43;1.15)
TT (slow)	Heavy	191	22.27 (7.67;38.85)	−24.79 (−33.38;−15.08)	1.10 (0.59;2.07)	0.94 (0.53;1.68)
			$P_{interaction} = 0.855$	$P_{interaction} = 0.632$	$P_{interaction} = 0.614$	$P_{interaction} = 0.315$
ADH7 (rs1573496)						
CC	Non	421	0	0	1	1
CC	Light/moderate	3507	13.56 (6.55;21.04)	−12.42 (−17.59;−6.92)	0.63 (0.46;0.88)	0.78 (0.57;1.06)
CC	Heavy	781	20.02 (11.34;29.38)	−23.08 (−28.41;−17.36)	1.03 (0.71;1.50)	1.19 (0.83;1.70)
GC and GG	Non	121	−2.80 (−14.07;9.95)	1.59 (−9.68;14.28)	1.07 (0.58;1.97)	1.46 (0.83;2.56)
GC and GG	Light/moderate	878	9.94 (2.26;18.20)	−10.36 (−16.35;−3.93)	0.72 (0.50;1.05)	0.82 (0.57;1.18)
GC and GG	Heavy	195	23.49 (11.10;37.26)	−28.41 (−35.28;−20.80)	0.74 (0.44;1.26)	1.25 (0.77;2.02)
			$P_{interaction} = 0.526$	$P_{interaction} = 0.180$	$P_{interaction} = 0.227$	$P_{interaction} = 0.577$
ALDH2 (rs886205)						
TT	Non	345	0	0	1	1
TT	Light/moderate	2953	18.59 (10.67;27.06)	−14.73 (−20.15;−8.94)	0.53 (0.37;0.74)	0.54 (0.39;0.74)
TT	Heavy	644	25.56 (15.76;36.18)	−25.56 (−31.09;−19.59)	0.78 (0.52;1.16)	0.83 (0.58;1.20)
CT and CC	Non	179	7.60 (−3.86;20.44)	−4.99 (−14.67;5.79)	0.59 (0.33;1.05)	0.43 (0.24;0.76)
CT and CC	Light/moderate	1305	17.35 (9.06;26.27)	−14.79 (−20.52;−8.64)	0.43 (0.30;0.62)	0.49 (0.35;0.69)
CT and CC	Heavy	287	31.78 (19.56;45.25)	−29.09 (−35.36;−22.22)	0.62 (0.38;1.00)	0.79 (0.51;1.22)
			$P_{interaction} = 0.224$	$P_{interaction} = 0.440$	$P_{interaction} = 0.591$	$P_{interaction} = 0.038$
ALDH1b1 (rs2228093)						
CC	Non	411	0	0	1	1
CC	Light/moderate	3290	15.14 (7.98;22.78)	−13.69 (−18.81;−8.23)	0.59 (0.42;0.81)	0.63 (0.46;0.86)
CC	Heavy	715	24.91 (15.71;34.84)	−25.64 (−30.87;−20.02)	0.81 (0.55;1.19)	0.99 (0.70;1.42)
CT and TT	Non	114	0.79 (−11.56;14.86)	−4.00 (−15.24;8.73)	1.06 (0.56;2.01)	0.84 (0.45;1.57)
CT and TT	Light/moderate	1003	13.48 (5.66;21.87)	−12.88 (−18.61;−6.74)	0.67 (0.46;0.97)	0.84 (0.45;1.57)
CT and TT	Heavy	225	19.21 (7.81;31.81)	−25.16 (−31.99;−17.64)	1.14 (0.70;1.85)	1.11 (0.70;1.75)
			$P_{interaction} = 0.756$	$P_{interaction} = 0.754$	$P_{interaction} = 0.673$	$P_{interaction} = 0.699$
ALDH1b1 (rs2073478)						
TT	Non	190	0	0	1	1
TT	Light/moderate	1545	16.98 (6.57;28.41)	−15.55 (−22.75;−7.67)	0.56 (0.35;0.88)	0.75 (0.48;1.19)
TT	Heavy	326	29.77 (16.08;45.07)	−30.03 (−37.10;−22.16)	0.77 (0.45;1.32)	1.03 (0.61;1.74)
GT	Non	254	1.17 (−9.87;13.57)	−2.45 (−12.65;8.94)	0.86 (0.48;1.53)	0.98 (0.56;1.71)

Table 5. Cont.

Genotype	Alcohol drinking	n	Insulin sensitivity (% (95% CI))	Insulin release (% (95% CI))	Metabolic syndrome (OR (95% CI)) $n_{cases} = 1409$	IGT/diabetes (OR (95% CI)) $n_{cases} = 1065$
GT	Light/moderate	2066	16.75 (6.50;27.98)	−15.18 (−22.31;−7.38)	0.54 (0.35;0.85)	0.67 (0.43;1.06)
GT	Heavy	442	22.26 (10.09;35.77)	−25.49 (−32.60;−17.64)	0.86 (0.48;1.53)	1.11 (0.67;1.83)
GG	Non	85	5.49 (−9.94;23.57)	−6.90 (−19.95;8.29)	0.94 (0.44;2.02)	1.40 (0.68;2.86)
GG	Light/moderate	656	11.93 (1.33;23.64)	−12.45 (−20.40;−3.71)	0.64 (0.39;1.05)	0.77 (0.47;1.26)
GG	Heavy	161	22.61 (7.69;39.59)	−24.27 (−33.10;−14.27)	0.72 (0.38;1.37)	1.32 (0.72;2.40)
			$P_{interaction} = 0.574$	$P_{interaction} = 0.460$	$P_{interaction} = 0.745$	$P_{interaction} = 0.855$

N are the maximum number of participants in each category. N may be lower due to missing information on some variables. Data are β coefficients or odds ratios (OR) with 95% confidence intervals (CI) from adjusted regression analyses. The category "wildtype non drinkers" was set as the joint reference group. β coefficients from models with log-transformed outcomes were back-transformed and reported as % with 95% CI. All p values are F tests.
doi:10.1371/journal.pone.0011735.t005

study of Japanese [47], whereas another study on Europeans found no relation to HDL [46]. We observed a decreased fasting serum LDL level among heavy drinkers with the intermediate/fast *ADH1b* (rs1229984) GA/AA genotype.

An interaction effect was also observed between *ALDH2* (rs886205) and IGT/diabetes in the current study. The relationship of this *ALDH2* variant as well as the *ADH7* and *ALDH1b1* variants with diabetes and CHD related phenotypes have not been studied previously, except from a previous study (n = 1,216) from our group [13]. In this study we observed an association between the *ALDH1B1* (rs2228093) gene variant and diastolic blood pressure, which was not confirmed in the current study. Besides that we did not observe any effects of *ADH1b* (rs1229984), *ADH1c* (rs1693482), *ALDH1b1* (rs2228093, rs2073478), and *ALDH2* (rs886205) with respect to blood pressure, cholesterols, and triglyceride in this previous study [13]. Studies on the inactive *ALDH2* Asian variant have found no association with neither cholesterols [48] nor blood pressure [49,50].

Taken as a whole the studies on the effects of genetic variation in *ADH* and *ALDH* on the risk of type 2 diabetes and CHD have been inconsistent. One explanation could be that drinking patterns and levels of intake differ between Danes and e.g. the US population. Moreover, our results with the genetic variants would not be significant after correction for multiple testing, and we believe that many of the inconsistencies between studies may be due to chance findings, although we cannot exclude that they are real. Nonetheless, these results do not exclude a causal relationship between alcohol and diabetes and CHD, but they do suggest that the influence of genetic variation in the alcohol metabolizing enzymes is relatively small.

Previously, we showed that the *ADH1b* (rs1229984), *ADH1c* (rs1693482) and *ALDH1b1* (rs2228093) genotypes is associated with amount of alcohol intake, which may have interfered with the principles of Mendelian randomization and influenced the results. Individuals with *ADH1b* and *ADH1c* slow metabolizing genotypes were drinking more [51]. Thus *ADH1b* and *ADH1c* slow metabolizers have higher blood ethanol concentrations due to both the lower activity of the enzyme and to a higher alcohol intake. Both effects stem directly from the genotype and cannot be separated. If ethanol is responsible for the adverse/beneficial effects of alcohol drinking the observed associations would have been intensified. However, if a more downstream metabolite e.g. acetaldehyde is responsible for the effects of alcohol, the observed associations may have been attenuated

towards the null value due to opposing effects of enzyme activity and alcohol intake (low ADH enzyme activity results in low acetaldehyde peak levels and a high alcohol intake results in high acetaldehyde levels). The *ALDH1b1* (rs2228093) genotypes have also been shown to influence alcohol drinking habits, but the effects of this polymorphism on enzyme activity is unknown.

Several other potential limitations of the study should be considered. Firstly, despite the relatively large number of participants in the current study, it is possible that the study may have missed important effects of the genetic variants due to low statistical power. In contrast, a large number of statistical tests have been performed in this study which increases the risk of chance findings. The observed statistical significant associations involving the *ADH* and *ALDH* gene variants would not be significant after correction for multiple testing. Moreover, rs1229984 and rs886205 were not in Hardy-Weinberg equilibrium. However the prevalence of the gene variants was similar to other studies on European populations, suggesting that this has perhaps happened by chance [14,26,32,34,52]. Also, among 384 replicate samples, we found no genotype errors for the two SNPs. In addition, the alcohol intake was estimated on the basis of a self-administered questionnaire and not by an objective method. Due to social desirability bias the participants may have underreported their actual intake. However, the ranking of participants were probably quite accurate, as total weekly alcohol intake as assessed by this method in another population-based study has previously been found to be positively associated with markers of high alcohol intake [13,53]. However, the J-shape might also be explained by the possibility that some previous or current heavy drinkers are misclassified as non-drinkers. Finally, the cross-sectional study design may not allow us to draw firm conclusions about the causal direction of associations between alcohol and e.g. diabetes. Thus, we cannot exclude the possibility that persons with diabetes have changed their alcohol intake due to the disease. However, associations with genetic variants may favour a causal relationship, since an individual's genetic composition does not change over time and is less likely to be associated with confounding factors.

In conclusion, strong associations between weekly alcohol intake and diabetes, MS and several intermediate CHD risk factors were observed. The *ADH* and *ALDH* gene variants on the other hand had only minor effects, and did not seem to modify the health effects of alcohol drinking greatly in this study.

Supporting Information

Acknowledgments

Author Contributions

Conceived and designed the experiments: LLNH AL. Analyzed the data: LLNH AL. Wrote the paper: LLNH. Interpretation of results: LLNH. Principal investigator of the Inter99 study and responsible for data collection: TJ KBJ. Interpretation of data: TJ KBJ TH OP AL. Revision of the paper: TJ KBJ TH OP AL. Responsible for the genotype analyses: TH OP.

References

1. Stampfer MJ, Colditz GA, Willett WC, Speizer FE, Hennekens CH (1988) A prospective study of moderate alcohol consumption and the risk of coronary disease and stroke in women. N Engl J Med 319: 267–273.

2. Corrao G, Rubbiati L, Bagnardi V, Zambon A, Poikolainen K (2000) Alcohol and coronary heart disease: a meta-analysis. Addiction 95: 1505–1523.

3. Gronbaek M, Becker U, Johansen D, Gottschau A, Schnohr P, et al. (2000) Type of alcohol consumed and mortality from all causes, coronary heart disease, and cancer. Ann Intern Med 133: 411–419.

4. Rimm EB, Williams P, Fosher K, Criqui M, Stampfer MJ (1999) Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. BMJ 319: 1523–1528.

5. Sesso HD, Gaziano JM (1999) Alcohol intake and cardiovascular morbidity and mortality. Curr Opin Nephrol Hypertens 8: 353–357.

6. Carlsson S, Hammar N, Grill V (2005) Alcohol consumption and type 2 diabetes Meta-analysis of epidemiological studies indicates a U-shaped relationship. Diabetologia 48: 1051–1054.

7. Koppes LL, Dekker JM, Hendriks HF, Bouter LM, Heine RJ (2005) Moderate alcohol consumption lowers the risk of type 2 diabetes: a meta-analysis of prospective observational studies. Diabetes Care 28: 719–725.

8. Cushman WC (2001) Alcohol consumption and hypertension. J Clin Hypertens (Greenwich) 3: 166–170.

9. Savolainen MJ, Kesaniemi YA (1995) Effects of alcohol on lipoproteins in relation to coronary heart disease. Curr Opin Lipidol 6: 243–250.

10. Davies MJ, Baer DJ, Judd JT, Brown ED, Campbell WS, et al. (2002) Effects of moderate alcohol intake on fasting insulin and glucose concentrations and insulin sensitivity in postmenopausal women: a randomized controlled trial. JAMA 287: 2559–2562.

11. Sierksma A, Patel H, Ouchi N, Kihara S, Funahashi T, et al. (2004) Effect of moderate alcohol consumption on adiponectin, tumor necrosis factor-alpha, and insulin sensitivity. Diabetes Care 27: 184–189.

12. Kannel WB, Ellison RC (1996) Alcohol and coronary heart disease: the evidence for a protective effect. Clin Chim Acta 246: 59–76.

13. Husemoen LL, Fenger M, Friedrich N, Tolstrup JS, Beenfeldt FS, et al. (2008) The association of ADH and ALDH gene variants with alcohol drinking habits and cardiovascular disease risk factors. Alcohol Clin Exp Res 32: 1984–1991.

14. Tolstrup JS, Nordestgaard BG, Rasmussen S, Tybjaerg-Hansen A, Gronbaek M (2008) Alcoholism and alcohol drinking habits predicted from alcohol dehydrogenase genes. Pharmacogenomics J 8: 220–227.

15. Eriksson CJ, Fukunaga T, Sarkola T, Chen WJ, Chen CC, et al. (2001) Functional relevance of human adh polymorphism. Alcohol Clin Exp Res 25: 157S–163S.

16. Bosron WF, Li TK (1987) Catalytic properties of human liver alcohol dehydrogenase isoenzymes. Enzyme 37: 19–28.

17. Hashibe M, McKay JD, Curado MP, Oliveira JC, Koifman S, et al. (2008) Multiple ADH genes are associated with upper aerodigestive cancers. Nat Genet 40: 707–709.

18. Homann N, Stickel F, Konig IR, Jacobs A, Junghanns K, et al. (2006) Alcohol dehydrogenase 1C*1 allele is a genetic marker for alcohol-associated cancer in heavy drinkers. Int J Cancer 118: 1998–2002.

19. Borras E, Coutelle C, Rosell A, Fernandez-Muixi F, Broch M, et al. (2000) Genetic polymorphism of alcohol dehydrogenase in europeans: the ADH2*2 allele decreases the risk for alcoholism and is associated with ADH3*1. Hepatology 31: 984–989.

20. Gemma S, Vichi S, Testai E (2006) Individual susceptibility and alcohol effects:biochemical and genetic aspects. Ann Ist Super Sanita 42: 8–16.

21. Crabb DW, Matsumoto M, Chang D, You M (2004) Overview of the role of alcohol dehydrogenase and aldehyde dehydrogenase and their variants in the genesis of alcohol-related pathology. Proc Nutr Soc 63: 49–63.

22. Yoshida A, Huang IY, Ikawa M (1984) Molecular abnormality of an inactive aldehyde dehydrogenase variant commonly found in Orientals. Proc Natl Acad Sci U S A 81: 258–261.

23. Hempel J, Kaiser R, Jornvall H (1984) Human liver mitochondrial aldehyde dehydrogenase: a C-terminal segment positions and defines the structure corresponding to the one reported to differ in the Oriental enzyme variant. FEBS Lett 173: 367–373.

24. Brennan P, Lewis S, Hashibe M, Bell DA, Boffetta P, et al. (2004) Pooled analysis of alcohol dehydrogenase genotypes and head and neck cancer: a HuGE review. Am J Epidemiol 159: 1–16.

25. Harada S, Okubo T, Nakamura T, Fujii C, Nomura F, et al. (1999) A novel polymorphism (−357 G/A) of the ALDH2 gene: linkage disequilibrium and an association with alcoholism. Alcohol Clin Exp Res 23: 958–962.

26. Chou WY, Stewart MJ, Carr LG, Zheng D, Stewart TR, et al. (1999) An A/G polymorphism in the promoter of mitochondrial aldehyde dehydrogenase (ALDH2): effects of the sequence variant on transcription factor binding and promoter strength. Alcohol Clin Exp Res 23: 963–968.

27. Stewart MJ, Malek K, Xiao Q, Dipple KM, Crabb DW (1995) The novel aldehyde dehydrogenase gene, ALDH5, encodes an active aldehyde dehydrogenase enzyme. Biochem Biophys Res Commun 211: 144–151.

28. Smith GD, Ebrahim S (2004) Mendelian randomization: prospects, potentials, and limitations. Int J Epidemiol 33: 30–42.

29. Jorgensen T, Borch-Johnsen K, Thomsen TF, Ibsen H, Glumer C, et al. (2003) A randomized non-pharmacological intervention study for prevention of ischaemic heart disease: baseline results Inter99. Eur J Cardiovasc Prev Rehabil 10: 377–386.

30. [Anonymous] (1999) Definition, diagnosis and classification of diabetes mellitus and its complications. Report of a WHO Consultation, Part 1: Diagnosis and classification of diabetes mellitus.

31. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, et al. (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412–419.

32. Fischer M, Wetherill LF, Carr LG, You M, Crabb DW (2007) Association of the aldehyde dehydrogenase 2 promoter polymorphism with alcohol consumption and reactions in an American Jewish population. Alcohol Clin Exp Res 31: 1654–1659.

33. Hsu LC, Chang WC (1991) Cloning and characterization of a new functional human aldehyde dehydrogenase gene. J Biol Chem 266: 12257–12265.

34. Sherman D, Dave V, Hsu LC, Peters TJ, Yoshida A (1993) Diverse polymorphism within a short coding region of the human aldehyde dehydrogenase-5 (ALDH5) gene. Hum Genet 92: 477–480.

35. Balkau B, Charles MA (1999) Comment on the provisional report from the WHO consultation. European Group for the Study of Insulin Resistance (EGIR). Diabet Med 16: 442–443.

36. von Huth SL, Borch-Johnsen K, Jorgensen T (2007) Commuting physical activity is favourably associated with biological risk factors for cardiovascular disease. Eur J Epidemiol 22: 771–779.

37. Toft U, Kristoffersen LH, Lau C, Borch-Johnsen K, Jorgensen T (2007) The Dietary Quality Score: validation and association with cardiovascular risk factors: the Inter99 study. Eur J Clin Nutr 61: 270–278.

38. Husemoen LL, Thomsen TF, Fenger M, Jorgensen T (2004) Effect of lifestyle factors on plasma total homocysteine concentrations in relation to MTHFR(C677T) genotype. Inter99 (7). Eur J Clin Nutr 58: 1142–1150.

39. Husemoen LL, Thomsen TF, Fenger M, Jorgensen HL, Jorgensen T (2003) Contribution of thermolabile methylenetetrahydrofolate reductase variant to total plasma homocysteine levels in healthy men and women. Inter99 (2). Genet Epidemiol 24: 322–330.

40. Fagrell B, De Faire U, Bondy S, Criqui M, Gaziano M, et al. (1999) The effects of light to moderate drinking on cardiovascular diseases. J Intern Med 246: 331–340.

41. Beulens JW, Rimm EB, Hendriks HF, Hu FB, Manson JE, et al. (2007) Alcohol consumption and type 2 diabetes: influence of genetic variation in alcohol dehydrogenase. Diabetes 56: 2388–2394.

42. Hines LM, Stampfer MJ, Ma J, Gaziano JM, Ridker PM, et al. (2001) Genetic variation in alcohol dehydrogenase and the beneficial effect of moderate alcohol consumption on myocardial infarction. N Engl J Med 344: 549–555.

43. Younis J, Cooper JA, Miller GJ, Humphries SE, Talmud PJ (2005) Genetic variation in alcohol dehydrogenase 1C and the beneficial effect of alcohol intake on coronary heart disease risk in the Second Northwick Park Heart Study. Atherosclerosis 180: 225–232.

44. Djousse L, Levy D, Herbert AG, Wilson PW, D'Agostino RB, et al. (2005) Influence of alcohol dehydrogenase 1C polymorphism on the alcohol-cardiovascular disease association (from the Framingham Offspring Study). Am J Cardiol 96: 227–232.

45. Hines LM, Hunter DJ, Stampfer MJ, Spiegelman D, Chu NF, et al. (2005) Alcohol consumption and high-density lipoprotein levels: the effect of ADH1C genotype, gender and menopausal status. Atherosclerosis 182: 293–300.

46. Whitfield JB, O'Brien ME, Nightingale BN, Zhu G, Heath AC, et al. (2003) ADH genotype does not modify the effects of alcohol on high-density lipoprotein. Alcohol Clin Exp Res 27: 509–514.

47. Hashimoto Y, Nakayama T, Futamura A, Omura M, Nakarai H, et al. (2002) Relationship between genetic polymorphisms of alcohol-metabolizing enzymes and changes in risk factors for coronary heart disease associated with alcohol consumption. Clin Chem 48: 1043–1048.

48. Nakamura Y, Amamoto K, Tamaki S, Okamura T, Tsujita Y, et al. (2002) Genetic variation in aldehyde dehydrogenase 2 and the effect of alcohol consumption on cholesterol levels. Atherosclerosis 164: 171–177.

49. Tsuritani I, Ikai E, Date T, Suzuki Y, Ishizaki M, et al. (1995) Polymorphism in ALDH2-genotype in Japanese men and the alcohol-blood pressure relationship. Am J Hypertens 8: 1053–1059.

50. Okayama A, Ueshima H, Yamakawa M, Kita Y (1994) Low-Km aldehyde dehydrogenase deficiency does not influence the elevation of blood pressure by alcohol. J Hum Hypertens 8: 205–208.

51. Linneberg A, Gonzalez-Quintela A, Vidal C, Jorgensen T, Fenger M, et al. (2009) Genetic determinants of both ethanol and acetaldehyde metabolism influence alcohol hypersensitivity and drinking behaviour among Scandinavians. Clin Exp Allergy 40: 123–130.

52. Peterson RJ, Goldman D, Long JC (1999) Effects of worldwide population subdivision on ALDH2 linkage disequilibrium. Genome Res 9: 844–852.

53. Linneberg A, Hertzum I, Husemoen LL, Johansen N, Jorgensen T (2006) Association between alcohol consumption and aeroallergen sensitization in Danish adults. Clin Exp Allergy 36: 714–721.