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

Lise Lotte N. Husemoen1*, Torben Jørgensen1,2, Knut Borch-Johnsen3, Torben Hansen3,6, Oluf Pedersen3,4,5, Allan Linneberg1

1 Research Centre for Prevention and Health, Glostrup, Denmark, 2 Faculty of Health Science, University of Copenhagen, Copenhagen, Denmark, 3 Hagedorn Research Institute and Steno Diabetes Center, Gentofte, Denmark, 4 Faculty of Health Science, University of Aarhus, Aarhus, Denmark, 5 Institute of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark, 6 Faculty of Health Science, University of Southern Denmark, Odense, Denmark

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.

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* E-mail: lloh@glo.regionh.dk

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 alcohol intake and diabetes and intermediate CHD risk factors in relation to selected ADH and ALDH gene variants.

Alcohol is primarily metabolized in the liver. The major enzymes involved are alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). Firstly ethanol is oxidized to acetaldehyde 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 degradation 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 (SNPs) 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 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 standardised 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 fluorimunoassay 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 $\geq 6.1$ 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 $\geq 7.8$ mmol/l and $<11.1$ mmol/l. Diabetes was defined as: fasting plasma glucose $\geq 7.0$ and 2 hour plasma glucose $\geq 11.1$ 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, >21–33, >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 Arg90Leu (rs1573496) [17], ALDH2 5’-UTR A-357G (rs886205) [25,26,32], ALDH1b1 Ala69Val (rs2228093) [33,34], and ALDH1b1 Arg90Leu (rs2073478) [35,34]. The SNPs were genotyped by KBiosciences allele-specific PCR (KASP) (KBiosciences, Hoddesdon, UK). All genotyping success rates were above 96.6% with a mismatch rate of 0.0% for the above mentioned SNPs 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, cholesterol, 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
creatinine ratio (≥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 (≥140 mmHg) and/or high diastolic blood pressure (≥90 mmHg). Obesity was defined as high BMI (≥30 kg/m²) and/or high waist-hip ratio (>0.90 (men) and >0.85 (women)). Microalbuminuria was defined as albumin-creatinine ratio ≥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

| Characteristic | % (n)       |
|---------------|------------|
| Men           | 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 ≥30 kg/m² (% (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)) | 52.9 (1709) |
| Hormone replacement therapy (% (n)) | 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) |
| Total cholesterol (mean (sd)) | 5.31 (0.96) |
| 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. *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 was 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 (pinteraction = 0.009) and between alcohol and **ADH7** (rs1573496) with respect to IGT/diabetes (pinteraction = 0.003).

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

| Gene | Variant | n | Insulin sensitivity (geometric mean (95% CI)) | Insulin release (geometric mean (95% CI)) | Metabolic syndrome (% (n)) | IGT/diabetes (% (n)) |
|------|---------|---|---------------------------------------------|------------------------------------------|--------------------------|------------------------|
| **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) | 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) | 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) | 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) | 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) | 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) | 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
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 sensitivity.
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) | Diastolic blood pressure (mmHg) | HDL cholesterol (mmol/l) | LDL cholesterol (mmol/l) | Triglyceride (mmol/l) | Homocysteine (μmol/l) |
|-----------|------------------|---|--------------------------------|---------------------------------|-------------------------|------------------------|----------------------|----------------------|
| ADH1b     |                  |   | (β (95% CI)) | (β (95% CI)) | (% (95% CI)) | (β (95% CI)) | (% (95% CI)) | (% (95% CI)) |
| GG (slow) | Non              | 493 | 0 | 0 | 0 | 0 | 0 | 0 |
| GG (slow) | Light/           | 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) |
| GA and AA (fast) | 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) |
| Pinteraction  | 0.836 | | | | | | | |
sensitivity and an adverse effect of heavy alcohol intake on insulin release perhaps caused by a toxic alcohol effect on the pancreatic $\beta$ 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 intake due to lower demands caused by the increasing sensitivity and an adverse effect of heavy alcohol intake on insulin sensitivity and an adverse effect of heavy alcohol intake on insulin release perhaps caused by a toxic alcohol effect on the pancreatic $\beta$ 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 [30].

In the current study we observed significant associations between 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 [42,43] although not in all [45,46]. The ADH1b rs1229984 GG slow genotype has been associated with elevated blood pressure, triglycerides, and uric acid in one table.

### Table 4. Cont.

| Genotype | Alcohol drinking | n | Systolic blood pressure (mmHg) $\beta$ (95% CI) | Diastolic blood pressure (mmHg) $\beta$ (95% CI) | HDL cholesterol (mmol/l) $\%$ (95% CI) | LDL cholesterol (mmol/l) $\%$ (95% CI) | Triglyceride (mmol/l) $\%$ (95% CI) | Homocysteine ($\mu$mol/l) $\%$ (95% CI) |
|----------|------------------|---|---------------------------------------------|---------------------------------------------|------------------------------------------|------------------------------------------|------------------------------------------|-------------------------------------------|
| **ADH1b1 (rs2228093)** | | | | | | | | |
| CC Non | 411 | 0 | 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 | $1.20 (-0.19;2.59)$ | 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 | $6.12 (3.34;8.90)$ | 20.56 (15.81;25.51) | 0.01 ($-0.15;0.16$) | 5.49 ($-2.97;14.69)$ | $-0.23 (-8.76;0.90)$ | | |
| $P_{interaction} = 0.066$ & $P_{interaction} = 0.364$ & $P_{interaction} = 0.782$ & $P_{interaction} = 0.570$ & $P_{interaction} = 0.105$ & $P_{interaction} = 0.423$ | | | | | | | |
| **ADH1b1 (rs2073478)** | | | | | | | | |
| TT Non | 190 | 0 | 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) | 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 ($-0.18;0.10)$ | 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 ($-0.12;0.17$) | $-3.89 (-11.02;2.82)$ | $-6.72 (-13.95;1.11)$ | |
| GT Heavy | 442 | 4.95 (2.01;7.90) | 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 ($-0.30;0.20$) | $-3.89 (-15.82;7.91)$ | $-2.71 (-10.68;18.10)$ | |
| GG Light/ moderate | 656 | $-1.14 (-3.95;1.67)$ | $-1.18 (-3.00;0.63)$ | 5.71 (1.54;10.04) | $-0.08 (-0.24;0.07)$ | $-0.15 (-8.16;8.56)$ | $-9.13 (-16.74;0.52)$ | |
| GG Heavy | 161 | 3.87 (0.22;7.52) | 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 $\beta$ coefficients with 95% confidence intervals (CI) from adjusted regression analyses. The category “wildtype non drinkers” was set as the joint reference group. $\beta$ coefficients from models with log-transformed outcomes were back-transformed and reported as % with 95% CI. All p values are F tests.

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Alcohol, CHD and Diabetes
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 | Insulin release | Metabolic syndrome | IGT/diabetes |
|-----------------|------------------|-----|---------------------|----------------|-------------------|--------------|
|                 |                  |     | (% (95% CI))        | (% (95% CI))   | (OR (95% CI))     | (OR (95% CI)) |
| ADH1b (rs1229984) |                  |     |                     |                |                   |              |
| GG (slow)       | Non              | 493 | 0                   | 1              | 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) |
| CT and TT Heavy|                  | 225 | 19.21 (7.81;31.81)  | −24.79 (−33.38;15.08) | 1.10 (0.59;2.07)  | 0.94 (0.53;1.68) |
| CT and TT Light/moderate |       | 1545| 20.80 (6.55;34.91)  | −25.26 (−30.06;−20.02) | 0.74 (0.44;1.26)  | 1.25 (0.77;2.02) |
| CT and TT Non  |                  | 190 | 15.14 (7.96;22.78)  | −13.69 (−18.81;−8.23) | 0.59 (0.42;0.81)  | 0.63 (0.46;0.86) |
| CT and TT 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 Light/moderate |       | 114 | 15.14 (7.96;22.78)  | −13.69 (−18.81;−8.23) | 0.59 (0.42;0.81)  | 0.63 (0.46;0.86) |
| 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 Heavy|                  | 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 Light/moderate |       | 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) |

Joint interaction effects between alcohol and gene variants with respect to diabetes related phenotypes.

Plenary function: Pinteraction = 0.855 Pinteraction = 0.632 Pinteraction = 0.564 Pinteraction = 0.873 Pinteraction = 0.440 Pinteraction = 0.591 Pinteraction = 0.038 Pinteraction = 0.0577
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 and ALDH1b1 (rs2228093) gene variants is associated 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, rs2073478), and ADH1c genotypes is associated with 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, cholesterol, and triglyceride in this previous study [13]. Studies on the inactive ALDH2 Asian variant have found no association with neither cholesterol [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.

### Table 5. Cont.

| Genotype     | Alcohol drinking n | Insulin sensitivity (%) (95% CI) | Insulin release (%) (95% CI) | Metabolic syndrome (OR (95% CI)) | IGT/diabetes (OR (95% CI)) |
|--------------|---------------------|-----------------------------------|-------------------------------|----------------------------------|----------------------------|
| ALDH2        |                      |                                   |                               |                                  |                           |
|              | 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)           |
|              | 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)           |
|              | 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)           |
|              | 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)           |
|              | 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)           |

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.

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Supporting Information

Table S2  Associations between weekly alcohol intake and CHD related phenotypes.
Found at: doi:10.1371/journal.pone.0011735.s001 (0.06 MB DOC)

Table S1  Associations between weekly alcohol intake and diabetes related phenotypes.
Found at: doi:10.1371/journal.pone.0011735.s002 (0.05 MB DOC)

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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.

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