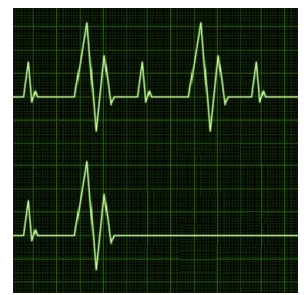
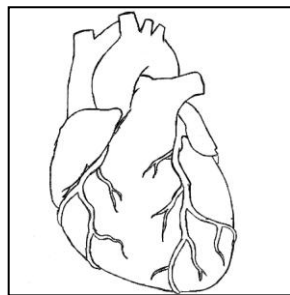
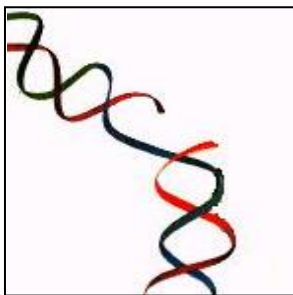


ALCOHOL DRINKING PATTERNS

Genetic predictors and associations with
coronary heart disease, obesity and mortality



By
JANNE SCHURMANN TOLSTRUP
National Institute of Public Health
Copenhagen, Denmark

May 2006

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PhD thesis

By

Janne Schurmann Tolstrup
National Institute of Public Health
Copenhagen, Denmark

Supervisors

Morten Grønbæk, professor, PhD, Dr Med Sci
National Institute of Public Health, Copenhagen, Denmark

Børge Grønne Nordestgaard, professor, Dr Med Sci
Department of Clinical Biochemistry, Herlev University Hospital, Herlev, Denmark

PhD defence to be held at Monday, November 20, 2006 at the National Institute of Public Health, Øster Farimagsgade 5a, Dk-1399 Copenhagen.

THIS THESIS IS BASED ON FOUR PUBLICATIONS:

Paper 1

Tolstrup JS, Nordestgaard BG, Rasmussen S, Tybjaerg-Hansen A and Grønbæk M: Alcoholism and drinking habits predicted from alcohol dehydrogenase genes. *Submitted*.

Paper 2

Tolstrup JS, Jensen MK, Tjønneland A, Overvad K, Mukamal KJ and Grønbæk M: A prospective study of alcohol drinking pattern and coronary heart disease in women and men. *British Medical Journal* 2006; 332: 1244-8.

Paper 3

Tolstrup JS, Heitmann BL, Tjønneland AM, Overvad K, Sørensen TIA, Grønbæk M: The relation between drinking pattern and body mass index, waist and hip circumference. *International Journal of Obesity* 2005; 29: 490-97

Paper 4

Tolstrup JS, Jensen MK, Tjønneland A, Overvad K, Grønbæk M: Drinking pattern and mortality in middle-aged men and women. *Addiction* 2004; 99, 324-30

P R E F A C E

The work of this thesis was carried out during my appointment as a phd-student at the National Institute of Public Health 2003 to 2006. It was supported by grants from the Danish Graduate School of Public Health, the Health Insurance Foundation and the Ministry of the Interior and Health.

I wish to express my sincere thanks to my supervisors *Morten Grøn­bæk* and *Børge Nordestgaard* for sharing their scientific insight and experience with me. Especially, I owe thanks to *Morten* for his inspiration and never-ending confidence in me, and to *Børge* for his enthusiasm and constructive criticism.

While preparing the papers for this thesis, I have had the opportunity to work with many excellent and creative persons (*Berit Heitmann, Majken Karoline Jensen, Kenneth Mukamal, Kim Overvad, Søren Rasmussen, Thorkild IA Sørensen, Anne Tjønneland, and Anne Tybjærg-Hansen*). For this, I am grateful.

Birgit Agerholm Larsen, Helle Koldkær and *Nina Dahl* are thanked for their helpfulness and patience during my stay at the Department of Clinical Biochemistry, Herlev University Hospital.

During the writing of my thesis, I have been much inspired by *Klaus Juul* who has served as a role model in the art of concise and simple scientific writing.

Special thanks go to all my colleagues and friends at the National Institute of Public Health for contributing to an open and stimulating workplace. Thanks and appreciation go to *Katrine Strandberg-Larsen, Laust Mortensen* and *Majken Karoline Jensen*, eminent young and funny epidemiologists, for reading and commenting my thesis, and to *Morten Hulvej Jørgensen* for reading and commenting my thesis, and for making days at the sunshine office pleasant. Also, *Naja Rod Nielsen* is thanked for sharing her brilliant epidemiological insight. *Loni Keil Brigsted* and *Trine Koefoed* are thanked for keeping administrative routines painless for the rest of us, and for invaluable help with English as well as with Danish grammar. I am much obliged to *Ditte Johansen* for generously sharing her statistical expertise and for her positive and constructive attitude. A special thanks to *Tine Gad* for doing the cover layout of this thesis.

Above all, I owe my thanks to every single man and women who participated in the Copenhagen City Heart Studies and in the Diet, Cancer and Health Cohort.

Janne Schurmann Tolstrup, May 2006.

LIST OF CONTENTS

INTRODUCTION	1
BACKGROUND AND AIMS	2
Genetic predictors of alcohol drinking patterns and alcoholism	2
Alcohol drinking pattern and coronary heart disease	3
Alcohol drinking pattern and obesity	3
Alcohol drinking pattern and all-cause mortality	3
DATA SOURCES AND ASSESMENT OF MEASUREMENTS.....	5
The Copenhagen City Heart Studies (data source for Study 1).....	5
The Diet, Cancer and Health Study (data source for Studies 2, 3 and 4)	5
Assessment of alcohol exposures by frequency questionnaires	6
Assessment of endpoints by linkage with national registers.....	6
Statistics for thesis.....	7
SUMMARY OF RESULTS.....	9
Study 1: ADH2 and ADH3, alcohol drinking patterns and alcoholism	9
Study 2: Alcohol drinking pattern and coronary heart disease	11
Study 3: Alcohol drinking pattern and obesity	12
Study 4: Alcohol drinking pattern and all-cause mortality	13
DISCUSSION.....	14
Can drinking patterns and alcoholism be predicted from genetic variation in ADH2 and ADH3?	14
Drinking pattern and coronary heart disease, evidence for sex-specific associations?	15
Is drinking pattern independently associated with obesity?	18
Is the J-shaped all-cause mortality curve influenced by drinking pattern?	18
Should public advice on sensible drinking include a message on drinking pattern?	19
Sources of bias - alternative explanations for obtained results?	21
CONCLUSIONS AND PERSPECTIVES	26
SUMMARY IN ENGLISH.....	28
SUMMARY IN DANISH.....	29
LIST OF CITED LITERATURE.....	30

PAPERS

- 1 Alcoholism and drinking habits predicted from alcohol dehydrogenase genes
- 2 A prospective study of alcohol drinking pattern and coronary heart disease in women and men
- 3 The relation between drinking pattern and body mass index, waist and hip circumference
- 4 Drinking pattern and mortality in middle-aged men and women

INTRODUCTION

Alcohol is used worldwide, as a legal drug by some, and as a natural part of the diet by others. The World Health Organization estimates that only five percent of the adult Danish population abstain from alcohol drinking.¹ On the individual level, drinking behaviour is influenced by environmental factors, such as culture and religion, and by heredity; for instance, twin studies have shown that approximately 50 percent of problem drinking and alcoholism can be explained by heritability.^{2,3} The contribution of specific genes in explaining drinking behaviour is sparsely studied, especially among Caucasians.

People who have experienced the malaise following an episode of heavy drinking may find it intuitively true that drinking pattern is important when studying risks associated with alcohol. Nevertheless, the majority of epidemiologic studies are based on a single measure summarising alcohol exposure into an average amount. Recently, however, evidence has emerged that this one-dimensional approach does not adequately account for health risks associated with alcohol drinking; important variation is comprised in the *drinking pattern*. In studies that focus on amount only, individuals who drink relatively small amounts on a number of drinking sessions are categorised with individuals consuming the same weekly amount of alcohol on one Saturday night. These two patterns of drinking may be associated with very different risks.

Drinking pattern is not unambiguously defined and has been characterised as drinking with meals, in weekends only, to intoxication, to a certain blood alcohol level, more than a certain amount per session (6 drinks, 13 drinks, ½ a bottle of spirits, etc.), and amount and frequency have been combined.^{4,9} A common feature of these approaches is that alcohol exposure is described in more than one dimension. Only few studies have sought to clarify the relative roles of amount and frequency of alcohol intake.¹⁰

Hypotheses tested in the present thesis relate to genetic predictors of drinking patterns and alcoholism, and to the influence of drinking patterns on different health endpoints. In *Study 1*, functional variation in main alcohol degrading enzymes is associated with the individual's drinking behaviour, such as weekly amount of alcohol intake and daily drinking, and with risk of alcoholism. In *Studies 2 to 4*, associations between drinking frequency and coronary heart disease, obesity and mortality, are examined to study if drinking frequency has independent effects on the different endpoints. Thus, in these studies, the main focus is not on studying differences between nondrinkers and drinkers, but rather on studying drinkers characterised by different drinking patterns.

The present thesis is structured as follows: First, background and specific aims of the thesis are presented, followed by a brief outline of the data sources on which it is based. Thereafter, results from Studies 1 to 4 are summarised, which is followed by discussions of results and of potential biases. Finally, main conclusions are presented and future perspectives are discussed.

BACKGROUND AND AIMS

Genetic predictors of alcohol drinking patterns and alcoholism

Whole genome screens have demonstrated linkage between phenotypes for problem drinking and chromosome 4, more precisely in the region of the alcohol dehydrogenase (*ADH*) gene cluster.¹¹ This region consists of seven loci that encode alcohol degrading enzymes (Figure 1).¹² *ADH1*, *ADH2* and *ADH3* are characterised by enzyme products mainly responsible for degrading ethanol (subsequently referred to as alcohol), while other *ADH* enzymes mainly degrade other types of alcohols.

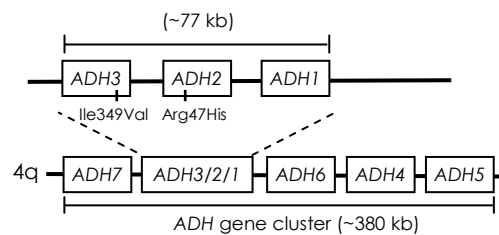


FIGURE 1. Map of the human alcohol dehydrogenase genes (*ADH*s). A detailed map of *ADH1*, *ADH2* and *ADH3* is expanded above the map of the gene cluster as a whole. Genetic variation affecting enzyme activity is found in *ADH2* and *ADH3* (the resulting amino acid changes are indicated). Reproduced from Osier 2002.¹²

Alcohol is passively absorbed from the stomach and duodenum and is distributed within the body's water compartment. The rate of alcohol degradation is 30 to 90 minutes per drink and is influenced by sex, frequency of alcohol intake, age and genetic factors.¹³ Degradation of alcohol into acetaldehyde is rate limiting for the reaction (Figure 2). Acetaldehyde is normally rapidly degraded to acetate and water by acetaldehyde dehydrogenase (*ALDH*).



FIGURE 2. Alcohol degradation. *ADH*; alcohol dehydrogenase, *ALDH*; acetaldehyde dehydrogenase.

Genetic variation with functional implications is found at *ADH2* and *ADH3* loci. At *ADH2*, alleles *ADH2·2* and *ADH2·1* produce enzymes with a 38 fold difference in *in vitro* alcohol degradation rate, and at *ADH3*, alleles *ADH3·1* and *ADH3·2* produce enzymes with a 2.5 fold difference.¹⁴

During normal alcohol degradation, the blood level of acetaldehyde is low. When concentrations of acetaldehyde become high, e. g. during treatment with disulfiram (Antabuse®) or in individuals with defective *ALDH* (not found in Caucasians¹⁵), individuals experience severe nausea and flushing and abstain from drinking alcohol. It is possible that individuals carrying the slow alcohol degradation *ADH2·1* and *ADH3·2* alleles are able to drink alcohol without experiencing discomfort due to elevated

acetaldehyde levels. If so, these individuals may be more likely to drink alcohol in larger amounts and more often, and may be at a higher risk of developing alcoholism compared with individuals carrying the fast alcohol degradation *ADH2*·2 and *ADH3*·1 alleles. This is only sparsely studied in Caucasians and only in relatively small case-control studies.

The purpose of Study 1 was to test the hypothesis that individuals with *ADH2* and *ADH3* slow compared with fast alcohol degradation drink more alcohol and more often, and are at higher risk of alcoholism.

Alcohol drinking pattern and coronary heart disease

Substantial epidemiological evidence suggests that alcohol has beneficial effects on the cardiovascular system.¹⁶⁻¹⁸ Plausible mediating factors such as increased high-density lipoprotein levels, lower plasma fibrinogen levels and reduced platelet aggregation have also been identified.¹⁹ However, important questions still remain. Among these is the role of drinking pattern, especially among women, who differ from men in both alcohol pharmacokinetics and in absolute risk of coronary heart disease. An episodic drinking pattern with large consumptions of alcohol per drinking session (*binge drinking*) has been associated with a higher risk of coronary heart disease,^{6,7,9,20} but few studies have sought to clarify the relative roles of amount and frequency of alcohol intake.

In Study 2, associations between amount and frequency of alcohol intake and risk of coronary heart disease are examined. The main purpose was to test the hypothesis that the risk of coronary heart disease is lower among individuals drinking frequently compared with individuals drinking less frequently for the same weekly amount of alcohol intake

Alcohol drinking pattern and obesity

Alcohol intake may be associated with obesity for several reasons; alcoholic beverages are energy dense and are generally not substituting food but rather added to the total energy intake.²¹ Furthermore, metabolites from alcohol degradation may inhibit fat oxidation.²² However, research in this area remains inconclusive, with some studies showing amount of alcohol to be positively associated with obesity,²³⁻²⁵ others showing no association²⁶ and still others showing an inverse association.²⁷ Little is known about the relationship between alcohol drinking pattern and obesity. Obese individuals are at increased risk of coronary heart disease and any association between drinking pattern and obesity could hypothetically explain part of an association between drinking pattern and coronary heart disease.²⁸⁻³¹

The main purposes of Study 3 were to 1) examine the association between alcohol drinking frequency and general obesity (measured as body mass index) and 2) examine the association between alcohol drinking frequency and fat distribution (measured as waist and hip circumference).

Alcohol drinking pattern and all-cause mortality

A J-shaped association between amount of alcohol intake and all-cause mortality has been found in many prospective studies.³²⁻³⁵ This is thought to reflect a reduced risk of coronary heart disease among light to moderate drinkers, and an increased risk of

conditions like liver cirrhosis, chronic pancreatitis, cancers and injuries among more heavy drinking individuals. For public health purposes, this association is important because it reflects net loss of life attributable to alcohol consumption and thus constitutes the scientific basis for creating guidelines on sensible drinking. Other studies have found that a binge-like drinking pattern is associated with a higher risk of mortality, but to our knowledge, no studies have sought examine if the J-shaped curve between alcohol and all-cause mortality depends upon the alcohol drinking frequency.^{4,6,36}

In Study 4, the main purpose was to test the hypothesis that the J-shaped association between amount of alcohol intake and mortality is modified by drinking frequency, so that for each level of weekly amount, the risk of mortality is higher among individuals with a non-frequent intake compared with individuals with a more frequent intake.

DATA SOURCES AND ASSESMENT OF MEASUREMENTS

The Copenhagen City Heart Studies (data source for Study 1)

In 1976, a random sample of the Danish general population above 20 years living in the Copenhagen area was invited to participate in the Copenhagen City Heart Study (number of participants 14,223; response rate 74%). This examination was followed by three more examinations; a second examination in 1981-83, where all previously invited plus 500 new individuals aged 20-24 years were invited (number of participants 12,698; response rate 70%); a third examination in 1991-94 where all previously invited plus 3000 new individuals aged 20-49 years were invited (number of participants 10,135; response rate 61%); and a fourth examination in 2001-03 where all previously invited plus an additional sample of 1040 individuals aged 20 to 29 years were invited (number of participants 6,238; response rate 50%). All participants gave informed consent and the ethics committee for Copenhagen and Frederiksberg approved the study (100.2039/91).

Before visiting the study clinic, participants completed a questionnaire (including questions on alcohol intake). At the clinic visit, physical examinations were performed and questionnaires were checked for missing information and any uncertainties were clarified. More particularly, blood samples were taken for DNA purification in 1991-94 and thus individuals participating in this examination constitute the study sample for Study 1. Enrolment and examination procedures have been described in more detail elsewhere.^{37,38}

Ethnicity: Distributions of *ADH2* and *ADH3* genotypes vary considerably according to ethnicity,¹² and ethnicity is likely to be associated with alcohol drinking patterns (*population stratification*). Hence, knowledge of the ethnic composition of the study population is essential. Eligibility criterion for participation in any of the examinations was Danish citizenship and therefore, the Copenhagen City Heart Study does not reflect the ethnic admixture of Copenhagen (the proportion of inhabitants with foreign citizenship was eight percent in 1994).³⁹ However, even a few participants of foreign ethnicity could potentially confound our results since the fast alcohol degradation *ADH2:2* allele is rare among Caucasians but frequent in other populations. Information on ethnicity was not assessed at the examinations, and hence information on birthplace was obtained from the Civil Registration System. Participants born in Asia, Africa, the Middle East, South America or Greenland were excluded from further study (n=211).

The Diet, Cancer and Health Study (data source for Studies 2, 3 and 4)

During 1993 to 1997, a random sample of Danish men and women aged 50 to 65 years living in the Copenhagen and Aarhus areas were invited to participate in the Diet, Cancer and Health Study (number of participants 57,053; response rate 35%). All participants gave informed consent and the ethics committee for Copenhagen and Frederiksberg approved the study (KF 01-116-96).

Eligible cohort members were born in Denmark and had no previous cancers at the time of inclusion. Participants completed a food-frequency questionnaire (including questions on amount of alcohol intake) before visiting a study clinic, where another

questionnaire concerning lifestyle factors (including questions on alcohol drinking frequency) was completed. Trained personnel checked for missing information and clarified uncertainties in the questionnaires with every participant. Enrolment and examination procedures have been described in detail elsewhere.⁴⁰ A description of the development and validation of the food frequency questionnaire has been published previously.^{41,42}

Assessment of alcohol exposures by frequency questionnaires

Information on amount of alcohol was obtained by frequency questionnaires in the Copenhagen City Heart Study and in the Diet, Cancer and Health Study. The validity of this method has been examined in the Danish part of the MONICA project.^{43,44} Here, information on alcohol intake obtained by frequency questionnaire was compared with information on alcohol intake obtained by dietary interview. A close agreement between the two information sources was observed. Although this comparison does not represent a true validation of frequency questionnaires, dietary interviews are considered to convey more accurate information than frequency questionnaires. Dietary interviews are time consuming and expensive, and assessing information by this method in large cohorts such as the Copenhagen City Heart Study and the Diet, Cancer and Health Study would not be feasible.

It would be informative to validate selfreported alcohol intake against a biochemical marker, because it is a more objective measure and potential errors of self-reports and markers are unlikely to be correlated. There is no perfect biochemical marker of alcohol intake, but the level of high-density lipoprotein cholesterol in the blood has been suggested.^{45,46} In the Copenhagen City Heart Study, a dose-response relation between alcohol and high-density lipoprotein cholesterol has been observed by others, also speaking in favour of the validity of assessing alcohol intake by frequency questionnaires.⁴⁷ The validity of obtaining information on drinking frequency from questionnaire has not been examined.

Assessment of endpoints by linkage with national registers

Participants were followed by linkage with central Danish registries using the unique person identification number. The Danish Hospital Discharge Register⁴⁸ and the Danish Register of Causes of Death⁴⁹ contain information on all admissions to Danish hospitals and causes of death, respectively. Diagnoses are classified according to the World Health Organization's *International Classification of Diseases*, using the eighth revision until 1994 and the tenth revision from 1994 and onward. Advantages of assessing endpoints from central registers include the ease by which large study populations can be followed continuously for various endpoints, and that loss to followup is almost negligible (in the present studies, less than one percent). For comparison, studies like the Health Professionals Follow-up Study in the USA have to rely on selfreport, which is more time-consuming and generally implies more loss to followup. By combining data from the Danish Hospital Discharge Register and the Danish Register of Causes of Death, information on endpoints (alcoholism and coronary heart disease) was assessed, defining a case as either hospital admission with the respective endpoint as the primary or secondary diagnosis, or the respective endpoint as the cause or contributing cause of

death. Admissions coded by ICD-8 modification codes 1 ('Observational') and 2 ('Not found') were excluded. Modification codes are not used in the ICD-10 system.

The diagnosis of coronary heart disease which consists of myocardial infarction and stable and unstable angina pectoris, has not been validated as an entity. With respect to myocardial infarction, the validity of the ICD-8 diagnosis has been analysed by others. Thus, 94 percent of myocardial infarction diagnoses in the Danish Hospital Discharge Register and the Danish Register of Causes of Death were later confirmed in the DANMONICA study.⁵⁰ The diagnostic sensitivity for myocardial infarction in that study was 78 percent. The diagnosis of alcoholism has not been validated.

Information on vital status was obtained from the Civil Registration System and this information is considered to convey almost perfect sensitivity and specificity.⁵¹ Information on deaths is registered with a delay of approximately four days or one month after the incident, depending on whether the person died in Denmark or abroad.

Statistics for thesis

(For other statistics, please refer to Papers 1 to 4)

Calculation of odds ratios for alcoholism and confidence intervals from previous studies of ADH2 and ADH3 (Table 1): In order to compare our results on ADH genotypes and alcoholism with results of others, we calculated odds ratios on the basis of presented results in previous studies. Sample sizes of some of these data were small and hence usual asymptotic methods are unreliable. Thus, exact logistic regression was applied⁵² (proc logistic with the exact statement invoked [SAS 8.2]). Pooled odds ratios were estimated by logistic regression, applying fixed effects for ADH2 and ADH3 genotypes, and random effects to account for between-study heterogeneity (proc nlmixed [SAS 8.2]).

Sensitivity analysis of misclassifications of coronary heart disease (Table 4): In order to evaluate the impact of possible misclassification of coronary heart disease diagnoses, sensitivity analyses were performed. Different combinations of false positive rates (Fpr) and sensitivity (Se) were assumed, and corrected incidence rates of coronary heart disease were calculated by applying the following equation:⁵³

$$A' = \text{Se} \cdot A + \text{Fpr} \cdot T,$$

where A' is the number of participants classified with coronary heart disease, A is the true number of participants with disease, and T is the true person time at risk. Assuming that false-negatives are adding negligible person time, $T \approx T'$, where T' is the observed person time, and hence:

$$A = (A' - \text{Fpr} \cdot T') / \text{Se} \Rightarrow A/T' = (A'/T' - \text{Fpr}) / \text{Se},$$

where A/T' is the corrected incidence rate in the respective category. Corrected incidence rate ratios were subsequently calculated by dividing corrected incidence rates in exposed categories with the corrected incidence rate in the reference category.

Comparison of participants and nonparticipants among those invited (Figure 9): In order to compare rates of all-cause mortality, alcoholism and alcoholic liver cirrhosis among participants and nonparticipants of the 1991-94 examination of the Copenhagen City Heart Study, risk estimates were computed by means of Cox proportional hazard regression. Information on vital status was obtained from the Civil Registration System, and information on alcoholism and alcoholic liver cirrhosis was obtained from the Danish

Hospital Discharge Register. In the Cox model, age was used as the time scale and analyses were corrected for delayed entry and adjusted for sex. The followup time for each individual was the period from date of the 1991-94 examination of the Copenhagen City Heart Study until date of the respective endpoint, death, emigration, or January 1, 2004, whichever came first. Data to perform similar analyses for participants of the Diet, Cancer and Health Study were not available.

SUMMARY OF RESULTS

Study 1: *ADH2* and *ADH3*, alcohol drinking patterns and alcoholism

Among the 9080 participants from the Copenhagen City Heart Study who were eligible for this study, allele frequencies coding for slow alcohol degradation were 0.98 (*ADH2*·1) and 0.42 (*ADH3*·2). Participants with *ADH2* slow compared with participants with *ADH2* fast alcohol degradation were two to three times more likely to drink alcohol, and among alcohol drinkers, they had an approximately 30% higher alcohol intake (data not shown). Also, they were more often daily, heavy and excessive drinkers (Figure 3A, B, and C).

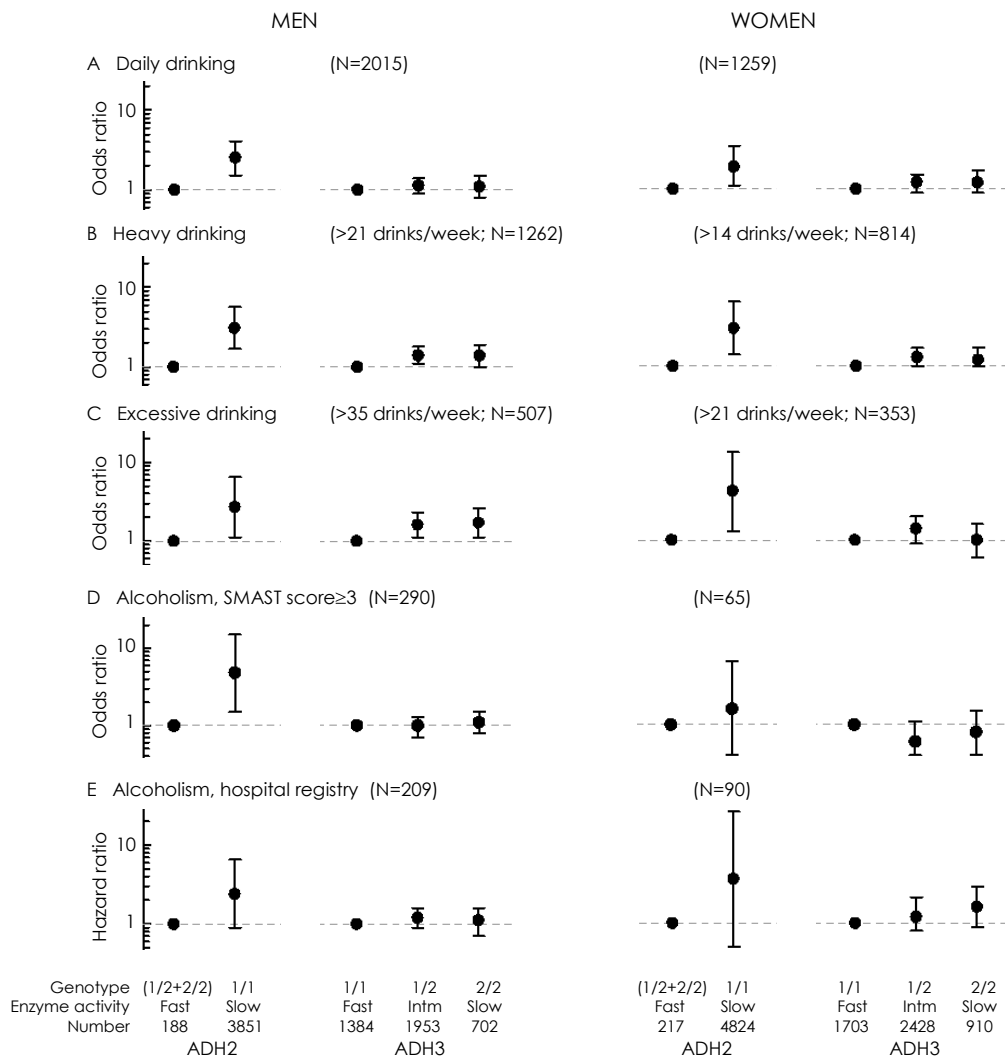


FIGURE 3. Sex-specific odds ratios for daily, heavy and excessive drinking and alcoholism (SMAST score ≥ 3) and hazard ratios for alcoholism (hospital registry information) by *ADH2* and *ADH3* genotypes. Number of cases in the respective analyses is noted beside each endpoint. Relative enzyme activity and total number of participants is noted at the bottom lines. Reference categories were *ADH2* 2/2+1/2 and *ADH3* 1/1. Estimates were adjusted for age, examination year, school education and other genotype.

Furthermore, there was a tendency that participants with *ADH2* slow versus fast alcohol degradation had a higher risk of alcoholism, as estimated from the *Short Michigan Alcoholism Screening Test* (SMAST) and hospital registry information (Figure 3D and E).

For *ADH3*, odds for heavy and excessive drinking were 40% to 70% higher among men who were heterozygous or homozygous for the slow alcohol degrading *ADH3*:2 allele than among men who were homozygous for the fast alcohol degrading *ADH3*:1 allele (Figure 3B and C). Similar results were found among women; however, effect sizes were slightly smaller and only statistically significant for heavy drinking.

Alleles of *ADH2* and *ADH3* are differently distributed in various ethnic groups. Frequencies of *ADH2*:1 and *ADH3*:2, coding for slow alcohol degradation are approximately 98% and 40% among Caucasians and only 30% and 10% among East Asians.⁵⁴ Hence, the genotype frequency of *ADH2*:1/1 is 95% among Caucasians and 9% among East Asians (Figure 4A). Odds ratios of heavy drinking and alcoholism according to *ADH2*:1/1 in the two populations are comparable (Figure 4B and C), but due to the different genotype distributions in the two populations, population attributable risks for heavy drinking and alcoholism are much higher among Caucasians than among East Asians. Therefore, population risks of heavy drinking and alcoholism attributable to the *ADH2*:1/1 genotype was 67% and 70% among Caucasians compared with 9% and 24% among East Asians (Figure 4D and E).

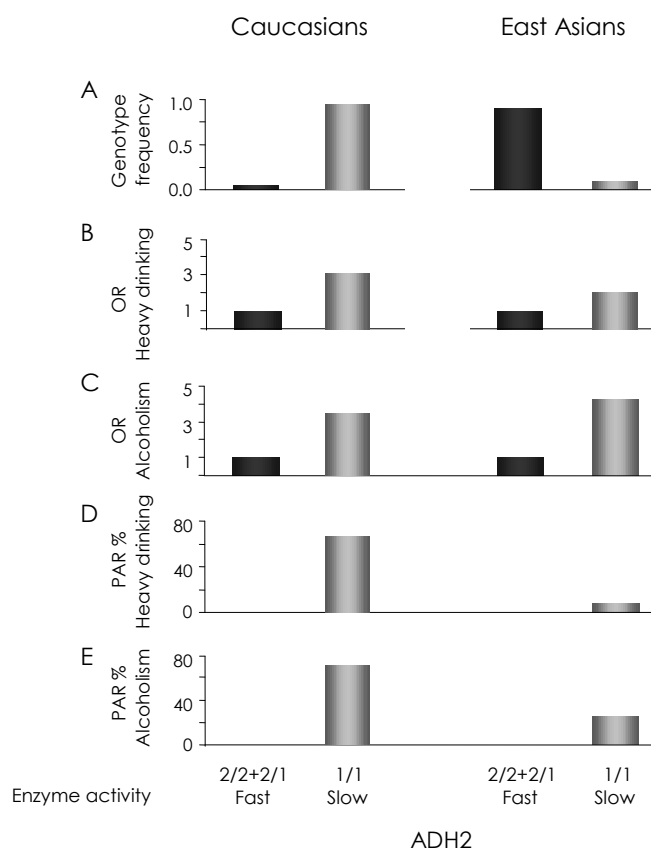


FIGURE 4. Genotype frequencies, odds ratios and population attributable risks of heavy drinking and alcoholism according to *ADH2* genotypes among Caucasians and East Asians (men and women combined). For East Asians, genotype frequencies and odds ratios were calculated from previous studies.^{54,55} Reference category was *ADH2*:2/2+1/2. OR; odds ratio, PAR; population attributable risk.

Study 2: Alcohol drinking pattern and coronary heart disease

During 302,857 person-years of follow up, 1283 men and 749 women from the Diet, Cancer and Health Study developed coronary heart disease. Among men, drinking frequency was inversely associated with risk of coronary heart disease over the whole range of drinking frequencies and the lowest risk was observed among daily drinkers (hazard ratio 0.59, 95% confidence interval 0.48 to 0.71, compared with men drinking on less than 1 day/week). Compared with women who drank alcohol on less than 1 day/week, women who drank alcohol on 1 day/week had a lower risk of coronary heart disease (0.64, 0.51 to 0.81); however, there was little difference between women who drank alcohol on 1 day/week, 2 to 4 days/week (0.63, 0.52 to 0.77), 5 or 6 days/week (0.79, 0.61 to 1.03), and 7 days/week (0.65, 0.51 to 0.84).

Exploring associations between drinking frequency and coronary heart disease within strata of amount of alcohol intake, inverse associations were consistently observed among men (Figure 5A), but not among women (Figure 5B). In contrast, exploring associations between amount of alcohol intake within strata of drinking frequency, inverse associations were consistently observed among women (Figure 5D), but not among men (Figure 5C).

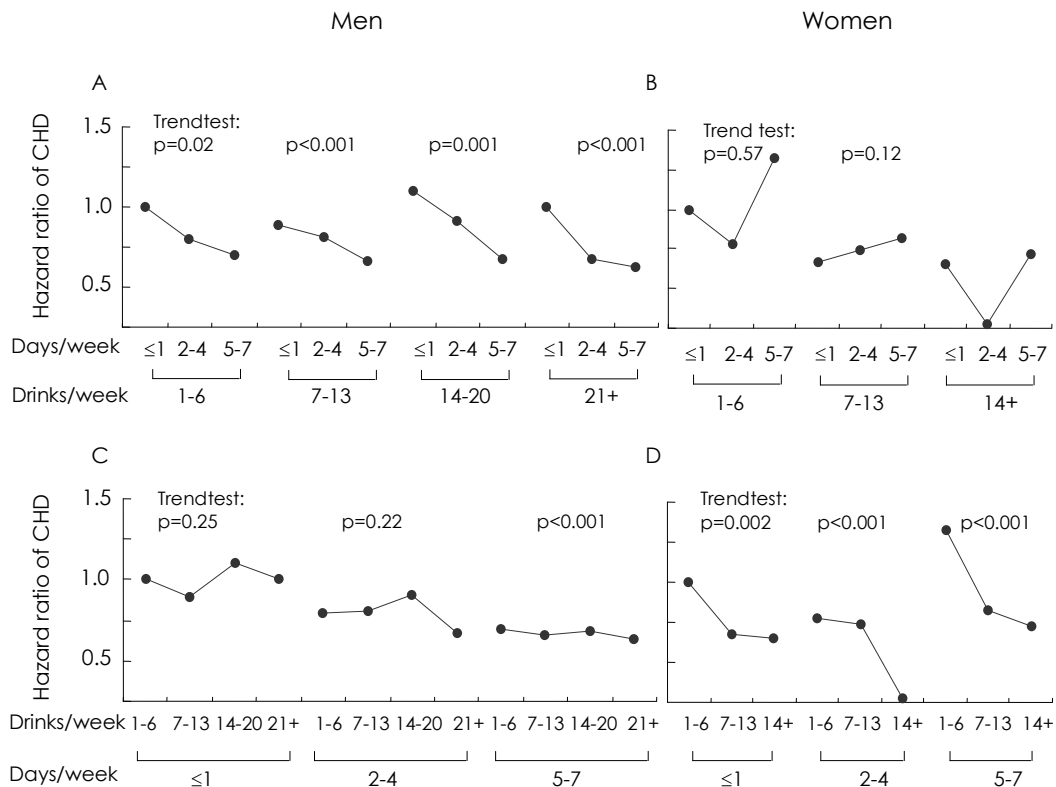


FIGURE 5. Sex-specific hazard ratios of coronary heart disease according to combinations of drinking frequency and amount of alcohol intake. Hazard ratios were adjusted for age, education, smoking, physical activity, body mass index, and intake of vegetables, fruit, fish and saturated fat. Participants drinking 1-6 drinks/week on ≤ 1 day/week were reference.

Study 3: Alcohol drinking pattern and obesity

Among 25,325 men and 24,552 women from the Diet, Cancer and Health Study who were eligible for this study, 15% of men and 12% of women were obese (body mass index ≥ 30 kg/m²), 25% of men and 25% of women had large waist circumference (≥ 102 centimetres, men; ≥ 88 centimetres, women) and 47% of men and 45% of women had small hip circumference (< 100 centimetres).

Drinking frequency was inversely associated with obesity (Figure 6A and B) and with large waist circumference (Figure 6C and D), meaning that the most frequent drinkers had the lowest probability of being obese and the lowest probability of having large waist. A high drinking frequency was associated with small hip circumference (Figure 6E and F). Results were similar for men and women, and were consistent within strata of the weekly amount of alcohol intake (data not shown).

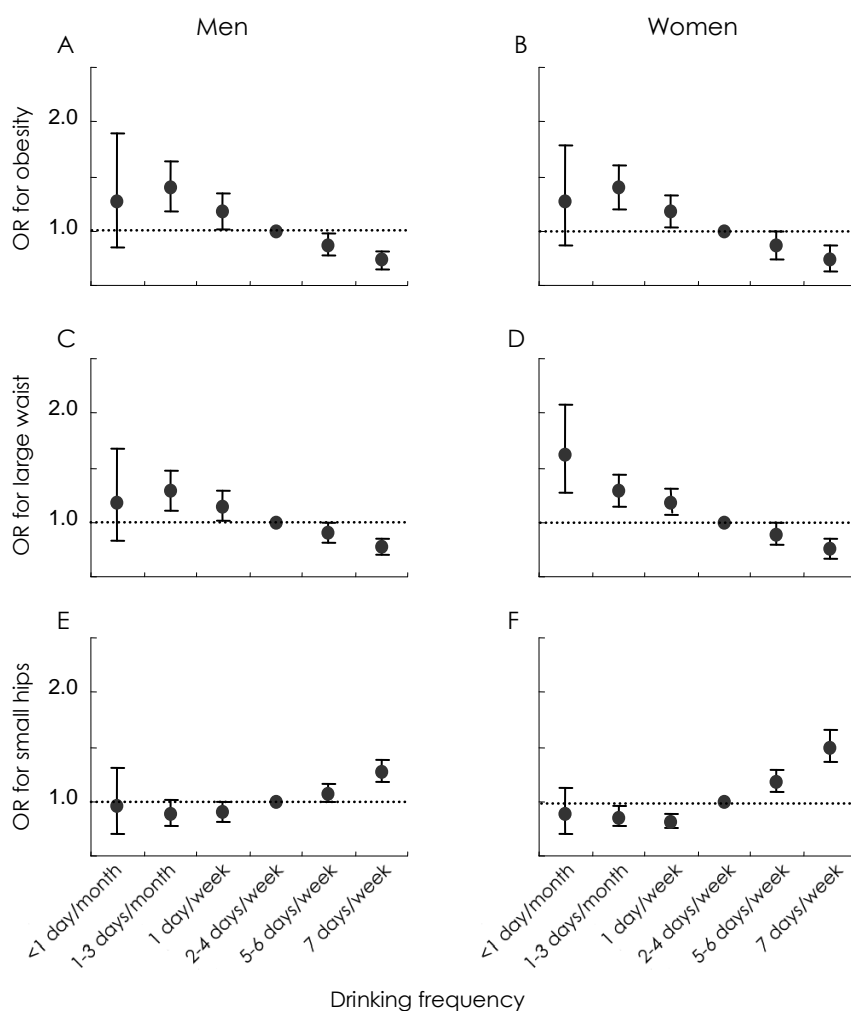


FIGURE 6. Sex-specific odds ratios for obesity, large waist and small hips according to alcohol drinking frequency. Odds ratios were adjusted for age, amount of alcohol intake, education, smoking, physical activity, and diet. Odds ratios for large waist and for small hips were also adjusted for BMI residuals. Obesity was defined as BMI ≥ 30 kg/m², large waist was defined as ≥ 102 centimeters for men and ≥ 88 centimeters for women, and small hip circumference was defined as < 100 centimeters. Reference category was participants drinking on 2 to 4 days/week. OR; odds ratio, BMI; body mass index.

Study 4: Alcohol drinking pattern and all-cause mortality

During 386,638 person-years of follow up in the Diet, Cancer and Health Study, 1528 men and 915 women died. Among both men and women, the well-known J-shaped curve between amount of alcohol intake and risk of all-cause mortality was observed (Figure 7). Furthermore, among men drinking more than 14 drinks/week and among women drinking more than 7 drinks/week, non-frequent drinkers had a higher risk of mortality than frequent drinkers (Figure 7). Hazard ratios also tended to increase with amount of alcohol intake among frequent drinkers. An overall test comparing frequent and non-frequent drinkers with a weekly intake of more than 1 drink/week was statistical significant (men: $p=0.03$, women: $p=0.05$).

Exploring combinations of amount and frequency of alcohol intake in more detail, men drinking on 5 to 6 days/week and a weekly amount of 7 to 13 drinks or 14 to 21 drinks had the lowest hazard ratios (0.51, 95% confidence interval 0.36 to 0.73; and 0.52, 0.35 to 0.76), compared with men drinking less than 1 drink/week. For women, the lowest, although not statistical significant, hazard ratios were for drinking on 5 to 6 days/week and a weekly amount of 1 to 6 drinks (0.72, 0.32 to 1.64) and for drinking on 5 to 6 days/week and a weekly amount of 7 to 13 drinks/week (0.84, 0.56 to 1.27).

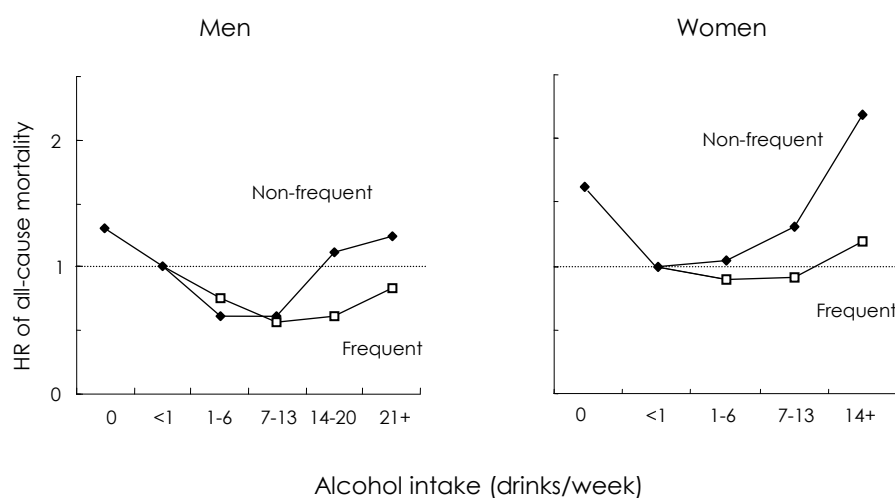


FIGURE 7. Sex-specific hazard ratios (HR) of all-cause mortality according to amount and frequency of alcohol intake. Non-frequent drinking was defined as drinking on less than two days/week and frequent drinking was defined as drinking on at least two days/week. Hazard ratios were adjusted for education, smoking, body mass index, physical activity, diet, and diseases before baseline. Reference category was participants drinking more than 0 and less than 1 drink/week ('<1').

DISCUSSION

Can drinking patterns and alcoholism be predicted from genetic variation in *ADH2* and *ADH3*?

In our study of 9080 Caucasians, participants with *ADH2* slow versus fast alcohol degradation had a higher alcohol intake, were more often daily, heavy and excessive drinkers and had higher risk of alcoholism. Furthermore, individuals with *ADH3* slow versus fast alcohol degradation were more often heavy and excessive drinkers. In agreement with these results, a previous study in 334 Australian Caucasians found that *ADH2* slow versus fast alcohol degradation was associated with a higher alcohol intake in men, but not in women; for *ADH3*, no differences were found.⁵⁶ However, the Australian study had much less statistical power than ours.

Among men, we found relative estimates for alcoholism ranging from 2.1 to 4.8 among *ADH2*:1 homozygotes, which is comparable with results among East Asians (meta-analysis pooled odds ratio 4.3, 95% confidence interval 2.9 to 6.5).⁵⁴ Previous studies among Caucasians have found more modest effect sizes of alcoholism, however, the majority of these studies were underpowered and were not adjusted for sex and age (Table 1). Previous studies among Caucasians of *ADH3*, in agreement with our results, have not found associations between *ADH3* polymorphism and alcoholism (Table 1).

TABLE 1. Meta-analysis of previous studies of *ADH2* and *ADH3* genotype and alcoholism in Caucasians

	Country	Study design	Cases/control	Odds ratio (95% confidence interval)		
				<i>ADH2</i> :1/1*	<i>ADH3</i> :1/2†	<i>ADH3</i> :2/2†
Vidal 2004 ¹⁵	Spain	Case-control	♂ 264/255	0.9 (0.5-1.6)	0.8 (0.6-1.3)	0.9 (0.6-1.6)
Ogurtsov 2001 ⁵⁷	Russia	Case-control	♂♀ 110/50	2.4 (1.1-5.3)	NA	NA
Rodrigo 1999 ⁵⁸	Spain	Case-control	♂ 150/280	1.2 (0.6-2.4)	NA	NA
Grove 1998 ⁵⁹	England	Case-control	♂♀ 264/121	NA	1.0 (0.6-1.6)	1.8 (0.9-3.7)
Whitfield 1998 ⁵⁶	Australia	General population study	♂ 37/119 ♀ 27/151	6.4 (0.9-274) 0.4 (0.1-2.5)	NA	NA
Espinos 1997 ⁶⁰	Spain	Case-control	♂♀ 71/71	1.5 (0.6-4.4)	0.4 (0.2-1.0)	0.6 (0.2-1.7)
Sherman 1994 ⁶¹	England	Case-control	♂♀ 26/16	NA	3.8 (0.5-48)	15 (1.0-∞)
Vidal 1993 ⁶²	Spain	Case-control	♂♀ 107/115	1.2 (0.6-2.6)	NA	NA
Gilder 1993 ⁶³	England	Case-control	♂♀ 82/84	1.2 (0.3-4.4)	1.0 (0.5-2.1)	1.5 (0.6-4.0)
Poupon 1992 ⁶⁴	France	Case-control	♂♀ 81/60	NA	0.8 (0.4-1.8)	0.5 (0.1-1.7)
Day 1991 ⁶⁵	England	Case-control	♂♀ 72/79	NA	0.6 (0.3-1.4)	0.6 (0.2-1.5)
Couzigou 1990 ⁶⁶	France	Case-control	♂♀ 46/39	0.6 (0.0-12)	NA	NA
Previous studies			♂♀ 894/1164 ♂♀ 858/686	1.2 (0.9-1.7)	0.8 (0.6-1.1)	1.0 (0.7-1.5)
Previous studies+ our study			♂♀ 1249/9889 ♂♀ 1213/9411	1.4 (1.1-1.9)	0.9 (0.7-1.1)	1.1 (0.8-1.3)

Odds ratios for each specific study were calculated using exact logistic regression on the basis of presented results in the original papers. Pooled odds ratios were calculated by logistic regression with a random effect for study. NA indicates that data were not available. Four studies reporting genotype frequencies not in Hardy-Weinberg equilibrium were omitted.

*With *ADH2*:1/2+2/2 as reference. †With *ADH3*:1/1 as reference.

A likely explanation of our findings is that differences in enzyme activity from the *ADH2* and *ADH3* polymorphisms result in intra-individual differences in alcohol degradation and that, for a given level of alcohol intake, individuals with fast alcohol degradation have higher levels of acetaldehyde and thus more unpleasant symptoms compared with individuals with slow alcohol degradation. Thus, with slow alcohol degradation, individuals can enjoy the pleasure of high blood alcohol levels without the uncomfortable symptoms seen with high acetaldehyde levels.

Our results also suggest that *ADH2* and *ADH3* genotypes may partly explain why Caucasians generally drink more alcohol than East Asians. The population risk of heavy drinking and alcoholism attributable to the *ADH2*·1/1 genotype was 67% and 70% among Caucasians in the present study, and only 9% and 24% among East Asians

In conclusion, the magnitude and consistency of observed associations and the biological plausibility adds to the evidence that *ADH2* and *ADH3* polymorphisms are causally related to alcohol drinking patterns and alcoholism.

Drinking pattern and coronary heart disease, evidence for sex-specific associations?

Our data suggest that drinking frequency is inversely and independently associated with risk of coronary heart disease among men. In contrast, results among women suggest that the weekly amount of alcohol intake is more important than drinking frequency for the inverse association with coronary heart disease.

Previous studies have addressed associations between drinking pattern and coronary heart disease (Table 2). A study with comparable measures of drinking pattern as our study also emphasizes frequency as the primary determinant among men.¹⁰ Among women, a recent case-control study found inverse associations with both amount and drinking frequency, although there was a tendency that amount was more strongly associated than frequency.⁶⁷ Most studies with a measure of binge drinking (often defined as drinking more than a certain number of drinks per session as for instance eight)²⁰, found increased risk among both men^{5,7,20} and women^{8,20} (Table 2).

Several explanations may account for sex specific differences in the association between drinking pattern and coronary heart disease. One explanation is sex-specific drinking habits, such as drinking with meals, which may contribute to a greater risk reduction than drinking outside meals.⁵ It is possible that frequently drinking men are more likely to drink with meals than frequently drinking women. However, a favourable effect of meal-related alcohol intake is not found in all populations.¹⁰ Another explanation could be a greater degree of residual confounding among women than among men. Interestingly, biomarkers suggested to explain the association between alcohol and decreased risk of coronary heart disease, such as high-density lipoprotein and fibrinogen, were found to explain a larger proportion of the association among men than among women, suggesting that alcohol has particular effects on mediators according to sex.⁶⁷ Other biological explanations for sex-specific associations include differences in alcohol pharmacokinetics and effects of alcohol on sex hormones. Some results suggest that men have more efficient first-pass metabolism for alcohol in the liver, while women may be eliminating alcohol faster than men.⁶⁸ Further, alcohol drinking is thought to increase oestrogen levels,^{69,70} and endogenous oestrogen may have beneficial effects on the cardiovascular system, protecting women from coronary heart disease until menopause,

whereupon the incidence approaches the incidence among men.⁷¹ It remains to be proven if any of these putative mechanisms depends upon the drinking pattern. Few women in this study were pre-menopausal and our findings may be limited to postmenopausal women.

In summary, our results suggest that there may be sex-specific associations between drinking frequency and coronary heart disease. These findings could be due to unobserved sex-specific drinking habits or to sex-specific associations between drinking frequency and cardiovascular mediators.

TABLE 2. Previous studies of drinking pattern and coronary heart disease

Author	Country	Study design	Population N (N _{Endpoints})	Measure of drinking pattern	Findings
Mäkelä 2006 ⁴	Finland	Cohort	♂ 3481 (561) ♀ 2913 (263)	Amount divided into amount consumed on heavy and non-heavy drinking sessions.*	Inverse association for amount consumed on non-heavy drinking sessions and no association for amount consumed on heavy drinking sessions.
Mukamal 2005 ⁶⁷	USA	Nested case-control	♂ 798 (266) ♀ 747 (249)	Frequency Drinking with meals	Drinking 3-4 or 5-7 days/week implied lower risk than drinking <1 or 1-2 days/week (♂ and ♀).
Murray 2005 ^{72†}	Canada	Cohort	♂ 2526 (376)	Binge drinking (≥8 drinks per session).	No association
Trevisan 2004 ⁵	Italy	Case-control	♂ 1332 (427)	Drinking outside meals Weekend drinking	Drinking outside meals and only in weekends implied higher risks compared with drinking mainly with meals or during the week [OR=1.5 (1.0-2.3) and 1.9 (1.2-3.0), respectively].
Mukamal 2003 ¹⁰	USA	Cohort	♂ 38077 (1418)	Frequency and weekly amount combined. Drinking with meals.	Inverse association with frequency, and frequency seemed to be stronger associated than amount. Compared with drinking <1 day/week the RR for drinking 5-7 days/week was 0.6 (0.5-0.8). Findings were independent of the proportion of alcohol consumed with meals.
Laatikainen 2003 ^{6‡}	Finland	Cohort	♂ 5092 (123)	Binge drinking (≥6 drinks per session)	Binge drinking increase risk [HR 1.8 (1.0-3.1)].
Murray 2002 ²⁰	USA	Cohort	♂ 580 (59) ♀ 574 (28)	Binge drinking (≥8 drinks per session)	Binge drinking increase risk [♂: HR 2.3 (1.2-4.2), ♀: HR 1.1 (1.0-1.2)]
Malyutina 2002 ^{7‡}	Russia	Cohort	♂ 6502 (384)	Binge drinking (≥13 drinks per session compared with <7 drinks per session). Frequency and amount per session combined.	Binge drinking implies increased risk [HR 1.3 (0.8-2.0)]. Increased risk among frequent heavy drinkers. HR for drinking ≥3 days/week and ≥10 drinks/session was 1.8 (0.9-3.7) compared with drinking <1/week and ≥10 drinks/session.
Hammar 1997 ⁸	Sweden	Case-referent	♂ 1569 (289) ♀ 760 (140)	Binge drinking (1/2 a bottle of spirits or intoxication).	♂: No association. ♀: HR 1.8 (0.9-3.7) for binge drinking.
McElduff 1997 ⁹	Australia	Case-control	♂ 9712 (6685) ♀ 5918 (2880)	Frequency and amount per session combined.	Both frequency and amount per session are associated. ♂: Lowest risk for drinking 5-6 days/week and 1-4 drinks/session. ♀: Lowest risk for drinking 5-6 days/week and 1-2 drinks/session.

* Heavy drinking sessions defined as blood alcohol concentration ≥ 0.1% † Heavy drinkers excluded at study entry ‡ Endpoint was deaths from coronary heart disease. Cohort; general population cohort, OR; odds ratio, RR; relative risk, HR; hazard ratio.

Is drinking pattern independently associated with obesity?

We observed inverse associations between alcohol drinking frequency and odds ratios of obesity and large waist; frequently drinking participants were less likely to be obese and to have large waists than less frequently drinking participants. Associations were similar among men and women and were independent of the amount of alcohol intake. In agreement with these results, one other study has found that waist circumference was inversely associated with drinking frequency (reported as monthly, weekly and daily drinking).⁷³

The most important limitation of this study is the cross-sectional design; information on alcohol and anthropometric measures were obtained at the same time. Hence, it is not possible to determine the causal relationship for the observed associations. It cannot be excluded that being obese may cause different alcohol drinking patterns than being lean. However, if the observed associations between drinking frequency and obesity represent a causal relation, a possible biological mechanism is differential induction of the *microsomal ethanol-oxidising system* by drinking frequency. While the bulk of ingested alcohol is degraded by alcohol dehydrogenase, microsomal ethanol-oxidising system is induced by heavy, regular alcohol intake.⁷⁴ It has been suggested that alcohol dehydrogenase and microsomal ethanol-oxidising system in conjunction constitute a futile cycle, so that energy from alcohol is resulting mostly in increased thermogenesis. If such a cycle is of any physiological significance, drinking frequency may be important for the degree of microsomal ethanol-oxidising system activation, and hence for the fraction of energy from alcohol that is lost as heat.⁷⁵ Another mechanism could be that low doses of alcohol stimulate energy expenditure because alcohol has an acute thermogenic effect.⁷⁶ It is possible that, for the same level of weekly alcohol intake, a frequent drinking pattern results in relatively more energy being converted to heat, compared with a less frequent intake.

In summary, we observed strong inverse associations between drinking frequency and obesity. If our results represent causal associations, obesity may explain part of the association between alcohol-drinking pattern and coronary heart disease, since obesity is a well-known risk factor for coronary heart disease.

Is the J-shaped all-cause mortality curve influenced by drinking pattern?

We found that drinking pattern influenced the J-shaped relation between alcohol intake and all-cause mortality. For the same amount of alcohol consumption, a non-frequent intake implied a higher risk of death than a frequent intake.

Previous studies have examined the association between drinking pattern and all-cause mortality (Table 3). Although other measures of drinking patterns are used, results consistently imply a hazardous effect of drinking large amounts of alcohol per session.^{4,6,36,77} We did not have the ability to identify participants with a binge-like drinking pattern, except for non-frequent drinkers with a high weekly intake, who logically must drink several drinks per session. In our study, two types of participants, the first of whom drinks two drinks each day and the second who drinks one drink each day plus seven additional drinks on Saturday nights, report the same weekly amount and drinking frequency, representing two different drinking patterns the latter of which may be associated with a higher risk of mortality than the former. Hence, the observed risks

among frequent drinkers are a mixture of risks for frequent drinkers with a binge-like drinking pattern and frequent drinkers without a binge-like drinking pattern. If we were able to adjust for binge drinking, risks among the frequent drinkers would possibly be lowered.

In summary, we observed the well-known J-shaped curve between amount of alcohol intake and risk of all-cause mortality, but the risk was generally higher among participants with a non-frequent intake than among participants with a frequent intake.

Should public advice on sensible drinking include a message on drinking pattern?

For public health purposes, the association between alcohol and all-cause mortality is relevant because it reflects net loss of life attributable to alcohol consumption and thus constitutes the scientific basis for creating guidelines on sensible drinking. In 1990, the Danish National Board of Health introduced the *sensible drinking limits*, advising the public not to exceed a certain amount of alcohol intake per week (14 drinks/week for women and 21 drinks/week for men). In the light of our and previous results,³²⁻³⁵ these guidelines seem reasonable; at these levels of alcohol consumption, the risk of mortality is not increased compared with non-drinkers, at least not among the frequent drinkers (Figure 7). Countries like for instance the United Kingdom have comparable guidelines for sensible amount of alcohol drinking, which since 1994 furthermore included advice on sensible drinking pattern, more precisely not to drink more than three and four drinks per session for women and men. In the autumn of 2005, the Danish guidelines for sensible drinking were expanded to also comprise drinking pattern, advising men and women not to drink more than five drinks on any session. Considering the emerging and consistent evidence that the beneficial effects of alcohol is not attained by episodic binge drinking, and that all-cause mortality is increased among individuals with a binge-like drinking pattern, this seem to be of considerable public health relevance. Another potential important factor however not further discussed in this thesis is age: among the young, the association between alcohol intake and all-cause mortality, and especially drinking pattern and all-cause mortality is sparsely examined. Our study population consisted of middle-aged men and women and results are thus conditional for having survived until 50 to 65 years. This age group is at high risk of coronary heart disease and qualifies for studying beneficial effects of alcohol. For younger individuals, the risk of coronary heart disease is low and beneficial effects of alcohol are probably negligible. Hence, the detrimental effects of alcohol, such as increased risk of traffic accidents and injuries most likely predominate. Therefore, the current guidelines for sensible drinking may not be sensible for the young.

TABLE 3. Previous studies of drinking pattern and all-cause mortality

Author	Country	Population N (N _{Endpoints})	Study design	Measure of drinking pattern	Findings
Mäkelä 2006 ⁴	Finland	♂ 3481 (746) ♀ 2913 (398)	Cohort	Amount divided into amount consumed on heavy and non-heavy drinking sessions*	♂ Increased risk in the highest category of alcohol consumed on heavy drinking sessions, and no increased risk for amount of alcohol consumed in non-heavy drinking sessions. Highest drinking category for heavy and non-heavy drinking sessions was ≥ 7 drinks/week. ♀ No association for alcohol consumed on heavy drinking sessions, and inverse association with amount of alcohol consumed in non-heavy drinking sessions. Highest drinking category for heavy and non-heavy drinking sessions was ≥ 1.5 drinks/week.
Laatikainen 2003 ⁶	Finland	♂ 5092 (347)	Cohort	Binge drinking (≥ 6 drinks per session)	Binge drinking increases risk [HR=1.6 (1.2-2.1)].
Malyutina 2002 ⁷	Russia	♂ 6502 (836)	Cohort	Binge drinking (≥ 13 drinks per session compared with < 7 drinks per session). Frequency and amount per session combined.	No association for binge drinking. Increased risk among frequent heavy drinkers. HR for drinking ≥ 3 days/week and ≥ 10 drinks/session was 1.6 (1.0-2.5) compared with drinking < 1 /week and ≥ 10 drinks/session.
Trevisan 2001 ⁷⁷	Italy	♂ 7688 (457) ♀ 4326 (107)	Cohort	Drinking outside meals	Drinking outside meals implied higher risk compared with drinking mainly with meals [♂ OR 1.5 (1.1-2.0) and ♀ 5.0 (1.5-11), respectively].
Rehm 2001 ³⁶	USA	♂ 2037 (272) ♀ 3035 (260)	Cohort	Binge drinking (≥ 8 drinks per session at least monthly or intoxication)	♂ Binge drinking implies increased risk [HR 1.6 (0.9-3.1)] ♀ No association.

* Heavy drinking sessions defined as blood alcohol concentration ≥ 0.1 %
Cohort; general population cohort, OR; odds ratio, HR; hazard ratio.

Sources of bias - alternative explanations for obtained results?

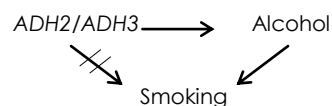
Confounding

An important potential confounder in a genetic association study is ethnic stratification, i.e. that genotype distribution varies across subgroups of the population and at the same time, baseline risk differs according to these subgroups.⁷⁸ Distributions of *ADH2* and *ADH3* genotypes show marked variation across ethnic groups,¹² and ethnicity is likely associated with alcohol drinking patterns and risk of alcoholism. Therefore, participants born outside Denmark were excluded. This procedure has limitations; it was not possible to differ between individuals of Danish and of non-Danish ethnicity born abroad meaning that some participants of Danish ethnicity were excluded. This exclusion is, however, most likely nondifferential according to genotype, and leads only to a minor reduction in effective study size. Another limitation is that, by this procedure, it was not possible to exclude participants of non-Danish ethnicity born in Denmark. However, considering the facts that genotypes were in Hardy-Weinberg equilibrium, and that the *ADH2:2* frequency was comparable with findings in other Caucasian populations,¹² speak against a major residual confounding from ethnic stratification.

An alternative explanation of our results on *ADH* genotypes is that untyped variants are in linkage disequilibrium with the *ADH2* and *ADH3* polymorphisms, meaning that our results stem from indirect associations. In that case, the causal variant would be localised within the *ADH2* and/or *ADH3* loci or within nearby genes (or regions regulating gene transcription), most likely within other genes in the *ADH* gene cluster (Figure 1). If so, the function of unidentified causal variants would probably alter alcohol degradation rate and results would still represent the same biological mechanism. Unless the typed *ADH2* and *ADH3* polymorphisms were perfect surrogates for the causal variants, obtained results would underestimate the direct association.

Estimates for *ADH2* and *ADH3* genotypes were not adjusted for smoking or other modifiable factors. Smoking and alcohol are associated lifestyle behaviours, but it is unlikely that *ADH2* and *ADH3* genes are associated with smoking through other pathways than alcohol (Figure 8). In this case, an association between genes and smoking would result from an effect of genes on alcohol, and smoking would thus not have the properties of a confounding factor.⁷⁹ Ultimately, adjustment for smoking could lead to attenuated estimates.

FIGURE 8. Alcohol and smoking are associated behaviours, but because any association between *ADH* genes and smoking is likely to result of an effect of genes on alcohol, smoking is not a confounder.



Residual confounding or confounding from uncontrolled risk factors could have caused bias in the observed associations between drinking pattern and health endpoints (Studies 2, 3, and 4). Suggestions of such factors include social factors since high volumes of alcohol per drinking session is shown to be associated with negative social circumstances.⁸⁰ School education was used as a proxy for social status, but more detailed

information on other social factors would have been desirable. For instance, marital status, social network and socio-economic position are likely linked with both drinking patterns and health status, and could hence be confounding results.^{81,82}

Adjustment had minor influence on results for drinking pattern and coronary heart disease. For example, adjustment for potential confounders changed hazard ratios from 0.65 to 0.71 among men drinking 5 to 6 days/week, and from 0.60 to 0.59 among daily drinking men. Most influence of adjustment was that due to adjustment by smoking. Residual confounding is possible; dimensions such as smoking duration and passive smoking were not accounted for. However, this would most likely have attenuated results, because smoking and alcohol are positively associated behaviours and smoking are positively associated with risk of coronary heart disease. It is not likely that wine drinking, which may be more beneficial than beer and spirits,¹⁸ are causing our results, because it has previously been shown that wine drinkers in this cohort actually drink less frequently than beer drinkers.⁸³ Thus, confounding from wine would most likely have attenuated results. In analyses of drinking frequency and obesity, adjustment also had limited influence.

In analyses of drinking pattern and all-cause mortality, adjustment for potential confounders (smoking, physical activity, body mass index, diet, school education and diseases) generally reduced the difference between frequent and non-frequent drinkers and hence the importance of drinking pattern (please refer to Figure 1 in Paper 4).

In conclusion, there is no reason to suspect that obtained results from the study of *ADH* genes is greatly influenced by confounding. Results from studies of drinking pattern, coronary heart disease, obesity and mortality may be confounded by social factors.

Misclassification of exposures

Misclassification of *ADH2* and *ADH3* genotypes could arise from preanalytical sources such as contamination by foreign DNA. To estimate the extent of such errors, DNA from new blood samples must be extracted and analysed, meaning that participants should be contacted and invited for reexamination. Due to the costs of such a validation, this was not feasible. Errors may arise during analyses because of contamination from one sample to another or because of incorrect interpretation of the signal from the chip. The latter error is more likely if the magnitude of the signal is relatively low compared to the background signal. Therefore, to minimise such errors, a relatively low value of the background signal was tolerated, and in the case of ambiguous signals, samples were rerun. Also, samples of known genotype were included on each chip to control the quality of each assay. Post analytical errors could arise due to incorrect registration into the database. To avoid this error, two independent laboratory technicians independently checked all results and database entries. Finally, mistaken identity of samples could occur through either of the analytical phases.

Any of the above mentioned errors in the assessment of *ADH2* and *ADH3* genotypes are probably nondifferential according to endpoint, and are thus unlikely to explain the obtained results. Furthermore, the observed genotype distribution complies with the expected distribution as predicted from the law of Hardy-Weinberg: in a population of random mating individuals with no selection pressure for either of the genotypes, the

distribution of genotypes follows this law. If not, genotyping errors are often responsible.

Drinking frequency and amount of alcohol intake could also be misclassified, which could lead to significant bias if the misclassification is differential. For instance, it is likely that participants have cut down on alcohol in response to early symptoms of coronary heart disease (so-called *sick quitters*⁸⁴), causing a falsely high incidence rate among participants in the low alcohol categories and resulting in an overall inverse association. In order to evaluate the influence of sick quitters, sensitivity analyses were performed where early cases were excluded. This did not change results, which argues against that the inverse association between drinking frequency and coronary heart disease is explained by this potential bias.

In conclusion, we do not have reason to believe that the obtained results are considerably affected by misclassification of any of the exposures.

Misclassification of endpoints

The various endpoints are probably subject to some misclassification, i.e. sensitivity and/or specificity less than 100 percent. For the study on *ADH2* and *ADH3* genotypes, measures of heavy and excessive drinking will be misclassified if amount of alcohol intake is under- or overreported. This error is likely independent of genotype, and because endpoints are binary, will lead to bias towards the null.⁷⁹ The same applies for error in the definition of alcoholism by questionnaire (SMAST score). For alcoholism defined by hospital registry information, sensitivity is likely considerably less than 100 percent because many alcoholics are untreated or treated at private clinics not registered in the national registers. However, specificity could be close to perfect; few non-alcoholics are presumably diagnosed as alcoholics. In this scenario, non-differential misclassification is not affecting the hazard ratio.⁸⁵

Misclassification of the coronary heart disease diagnosis occurs if patients fulfilling criteria for coronary heart disease were not diagnosed (that is, sensitivity less than 100 percent) or patients not fulfilling criteria for coronary heart disease were diagnosed as such (that is, a non-zero false positive rate). In order to explain our results, misclassification will have to be differential (i.e. to depend on drinking frequency); for example, a relatively lower sensitivity among frequent drinkers would cause an apparent inverse association between drinking frequency and coronary heart disease. This could occur if for instance frequent drinkers, who were more often smokers, were more likely to be misdiagnosed with lung diseases instead of correctly being diagnosed with heart disease.

The influence on hazard ratios of various scenarios of misclassifications of coronary heart disease is shown in Table 4.⁵³ In order to explain the decreased hazard ratio in daily drinking men, the nondifferential misclassification must be substantial. For example, assuming perfect sensitivity in the reference category and 60 percent among daily drinkers, and a false positive rate of zero in both groups, the true hazard ratio would be 1.0. Sensitivity analyses with similar scenarios were performed for other categories of drinking frequency and for women. Hazard ratios were generally robust unless a high degree of differential misclassification was assumed. Therefore, misclassification of disease status is unlikely to considerably have affected our results.

TABLE 4. Hazard ratios among men drinking 7 days/week compared with men drinking less than 1 day/week corrected for various scenarios of differential and nondifferential misclassification of the coronary heart disease diagnosis. Diagonal cells (underlined) represent scenarios with nondifferential misclassification.

<1 day/week (reference)		7 days/week							
Sensitivity	FP rate	Sensitivity	1	0.8	0.8	0.8	0.6	0.6	0.6
		FP rate	0	0	2	4	0	2	4
1	0		<u>0.60</u>	0.75	0.56	0.37	1.00	0.74	0.49
0.8	0		0.48	<u>0.60</u>	0.45	0.29	0.80	0.60	0.39
0.8	2		0.56	0.71	<u>0.53</u>	0.35	0.94	0.70	0.46
0.8	4		0.69	0.86	0.64	<u>0.42</u>	1.15	0.86	0.56
0.6	0		0.36	0.45	0.33	0.22	<u>0.60</u>	0.45	0.29
0.6	2		0.42	0.53	0.39	0.26	0.71	<u>0.53</u>	0.35
0.6	4		0.52	0.65	0.48	0.32	0.86	0.64	<u>0.42</u>

Note: Hazard ratios were calculated as incidence rate ratios. FP rate; false positive rate (events per 1000 person years; overall incidence rate among men was 9.2 per 1000 person years).

In the study of drinking pattern and obesity, endpoint measures (body mass index, waist and hip circumference), were obtained at the study clinic by trained personnel. Errors can be due to person-to-person variations in measurement method or incorret data entry. These errors are in all probability nondifferential and not likely to explain the observed associations.

For all-cause mortality, sensitivity and false positive rate were in all probability close to perfect and zero, respectively. Misclassification could be due to incorrect or lack of data entry into the Civil Registration System and is most likely nondifferential. Specificity is probably close to perfect and in that case, the non-differential misclassification is not affecting the hazard ratio.⁸⁵

In conclusion, we have no reason to believe that the obtained results are considerably affected by misclassification of any of the studied endpoints.

Selection bias

Selection bias can occur as a consequence of nonparticipation. Individuals, who choose not to participate in the Copenhagen City Heart Study had higher all-cause mortality, and higher incidence of alcoholism and alcoholic liver cirrhosis than individuals who participated (Figure 9). This indicates that participants were at better health than nonparticipants, and that alcoholism and heavy alcohol drinking are underestimated compared with the underlying population. In other words, alcohol drinking patterns and alcoholism were associated with nonparticipation (endpoints in Study 1). If genotypes were also associated with nonparticipation, this could have lead to selection bias.⁸⁶ However, as none of the invited persons were aware of their genotype, this is not likely, and associations between *ADH* genotypes and alcohol endpoints are unlikely to be affected by selection bias.⁸⁷

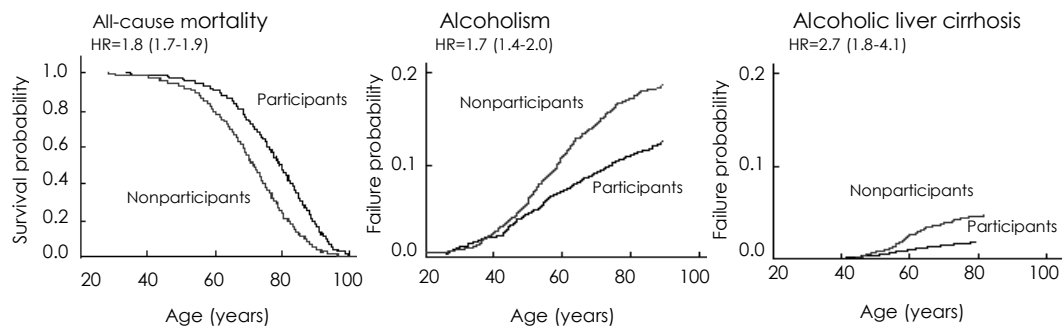


FIGURE 9. All-cause mortality, alcoholism and alcoholic liver cirrhosis among participants and nonparticipants of the Copenhagen City Heart Study 1991-1994. HR: Hazard ratios for non-participants compared with participants, adjusted for sex.

In the Diet, Cancer and Health Study, participation rate was 35 percent. Data to perform analyses on nonparticipants were not available, but a similar tendency as in the Copenhagen City Heart Study is likely. If so, heavy alcohol drinking and poor health were both causes of nonparticipation, and individuals with both causes are likely to be underrepresented. In other words, heavy drinkers who participate may be at better health than heavy drinkers in the population, whereas light and moderate drinkers who volunteer may be more representative of moderate and nondrinkers drinkers in the population. Ultimately, this may result in an apparent inverse association between alcohol and endpoint, even if there is no such association in the underlying population.⁸⁶ We observed inverse associations between drinking frequency and obesity and coronary heart disease and direct associations between alcohol intake and all-cause mortality. Hence, assuming selection bias were present as outlined above, results on mortality would probably have been biased towards the null, whereas results on coronary heart disease and obesity would have been biased away from the null. There is no definitive test for evaluating the presence or magnitude of this bias, but the facts that adjustment for diseases at baseline did not have major effects, that the observed number of coronary heart disease cases in the cohort during followup did not differ from the expected, that expected patterns for other risk factors such as smoking, body mass index and school education was observed, and that all-cause mortality as expected was increased among the most heavy drinking individuals, speaks against our results being caused by selection bias.⁸⁸

In conclusion, selection bias is not likely to explain the obtained results in studies of *ADH* genotypes and all-cause mortality. It cannot be entirely excluded that selection bias may have had some impact on results in studies of coronary heart disease and obesity.

Loss to follow-up

In the Copenhagen City Heart Study and in the Diet, Cancer and Health Study, less than one percent of participants were lost during follow-up. Such minor loss cannot have had significant effect on any of our results.

CONCLUSIONS AND PERSPECTIVES

Findings from the study of genetic variation in genes coding for alcohol degrading enzymes suggest that this partly predicts the individual's drinking pattern and risk of alcoholism.

- Slow versus fast alcohol degradation is associated with a higher alcohol intake, with daily, heavy and excessive drinking and with risk of alcoholism.

Findings from studies on drinking pattern and coronary heart disease, obesity and all-cause mortality consistently point toward important and independent effects of drinking pattern.

- For coronary heart disease, drinking frequency may be the primary determinant of the inverse association between alcohol intake and coronary heart disease among men. For women, however, amount of alcohol may be more important than frequency.
- Drinking frequency and obesity was inversely associated, so that for a given amount of alcohol intake, the most frequent drinkers had the lowest probability of being obese and of having large waist circumferences. If this finding represent causal association, it is likely that obesity can explain part of the association between drinking frequency and coronary heart disease.
- For the same weekly amount of alcohol intake, a non-frequent intake implies a higher risk of all-cause mortality than a frequent one.

Examining various sources of bias (confounding, misclassifications and selection bias) gave no reason to believe that each single bias is likely to considerably have affected our results.

More needs to be learned about effects of alcohol dehydrogenase genes on specific measures of drinking patterns, as for instance binge drinking, and on context-dependent effects (gene-gene and gene-environment interactions).

The association between drinking pattern and coronary heart disease should be investigated in other studies, with emphasis on studying differences between men and women, including if alcohol pharmacokinetics and sex hormones can explain a potential sex-specific associations.

The association between drinking pattern and obesity should be examined in a prospective design with the possibility of deciding the temporality. Causal inference, followed by public health implications based on isolated findings like these should not be drawn. Given the rise of obesity prevalence in Western societies, a possible causal association between drinking pattern and obesity could be of considerable public health relevance.

Future cohorts with purposes of addressing alcohol exposure and health endpoints should include measures of drinking pattern, preferable characterising amount,

frequency and episodes of heavy drinking (binge drinking). Also, future studies should take social factors such as socio-economic status and social network into account.

The increased risk of mortality among non-frequent drinkers should be examined in more detail, with emphasis on studying causes of deaths. If, for instance the excess mortality mainly is due to external causes, such as traffic accidents, preventive strategies could with advantage focus on high-risk groups. Also, associations between drinking pattern and mortality should be examined in other age groups, especially in the young, among whom health effects of alcohol are predominantly detrimental, and hazardous implications of different drinking patterns may be considerably greater than among the middle-aged.

In the light of results of this thesis, it may seem that regular compared with episodic drinking is less health damaging. However, the 'optimal' drinking pattern is likely to depend on the nature of the endpoint: the risk of alcohol related conditions such as liver cirrhosis may be lower among episodic drinkers than among regular drinkers, because the liver is allowed to reconstitute between drinking sessions. Furthermore, habitual heavy alcohol drinking is expectedly associated with many adverse social circumstances and diseases, regardless of the drinking pattern.

SUMMARY IN ENGLISH

This thesis is based on studies conducted in the period from 2003 to 2006 at Center for Alcohol Research, the National Institute of Public Health in cooperation with the Department of Clinical Biochemistry, Herlev University Hospital. Obtained results are presented in four scientific papers three of which are published, and one is submitted.

Main purposes of the thesis were to examine genetic predictors of alcohol-drinking patterns and of alcoholism, and to examine associations between drinking pattern and coronary heart disease, obesity and all-cause mortality.

Alcohol is degraded in the liver by alcohol dehydrogenase (ADH), which is a class of isoenzymes. Genetic variation with functional implications exists in *ADH2* and *ADH3*, resulting in different alcohol degradation rates. By genotyping 9080 participants in the Copenhagen City Heart Study, we found that participants with *ADH2* slow versus fast alcohol degradation had an approximately 30 percent higher alcohol intake, more often drank alcohol every day and more often were heavy and excessive drinkers. Also, there was a tendency that participants with *ADH2* slow versus fast alcohol degradation had higher risks of alcoholism. For *ADH3*, we found that participants with slow versus fast degradation more often were heavy and excessive drinkers.

Among more than 50.000 participants in the Diet, Cancer and Health Study, we found an inverse association between drinking frequency and risk of coronary heart disease for men and this association seemed to be independent of the amount of alcohol intake. Among women, we found an inverse association between amount of alcohol and risk of coronary heart disease, which seemed to be independent of drinking frequency.

Within the same cohort, we found among both men and women an inverse association between drinking frequency and prevalence of obesity, so that participants, who were drinking alcohol frequently were less likely to be obese than participants, who drank less frequently. Results were similar for men and women and were independent of the amount of alcohol intake.

Also in the Diet, Cancer and Health Study, we compared mortality risk among participants with different drinking frequencies. We found that the risk was higher among women drinking 7 drinks/week and among men drinking 14 drinks/week if this amount was taken on one day of the week compared with distributing the same amount on more days of the week.

In conclusion, genetic variation in alcohol degrading enzymes is partly predicting drinking patterns and alcoholism. Drinking patterns is independently associated with risk of coronary heart disease (among men) and all-cause mortality, and with prevalence of obesity. These results are important for future studies of the biological effects of alcohol on health and for public guidelines on alcohol.

SUMMARY IN DANISH

Denne afhandling bygger på studier, som er gennemført i perioden 2003 til 2006 på Center for Alkoholforskning, Statens Institut for Folkesundhed i samarbejde med Klinisk Biokemisk Afdeling på Herlev Universitetshospital. Afhandlingen er baseret på fire videnskabelige artikler, hvoraf de tre er publiceret, mens en er indsendt.

Afhandlingens overordnede formål er at undersøge genetiske prædiktorer for alkohol-drikkemønstre og for alkoholisme, samt at undersøge drikkemønstrets betydning for koronar hjertesygdom, fedme og dødelighed.

Alkohol nedbrydes i leveren af alkoholdehydrogenase (ADH), som er en klasse af isoenzymer. Der findes genetisk variation i *ADH2* og *ADH3*, hvilket forårsager forskellig alkoholnedbrydningshastighed. Ved genotypebestemmelse af 9080 deltagere i Østerbroundersøgelsen fandt vi, at personer med *ADH2* langsom versus hurtig alkoholnedbrydning drak cirka 30 procent mere alkohol, oftere drak alkohol hver dag og oftere overskred Sundhedsstyrelsens genstandsgrænser (14 genstande om ugen for kvinder og 21 genstande om ugen for mænd). Der var desuden en tendens til at deltagere med *ADH2* langsom versus hurtig alkoholnedbrydning havde en højere risiko for alkoholisme. For *ADH3* fandt vi at deltagere med langsom versus hurtig alkoholnedbrydning oftere overskred Sundhedsstyrelsens genstandsgrænser.

Blandt de mere end 50.000 deltagere i Kost, Kræft og Helbreds kohorten fandt vi blandt mænd en invers sammenhæng mellem alkoholdrikkefrekvens og risiko for koronar hjertesygdom. Denne sammenhæng syntes at være uafhængig af det samlede ugentlige alkoholforbrug. Blandt kvinder fandt vi en invers sammenhæng mellem det ugentlige alkoholforbrug og risiko for koronar hjertesygdom, som syntes at være uafhængig af drikkefrekvensen.

I samme kohorte fandt vi blandt både mænd og kvinder en invers sammenhæng mellem drikkefrekvens og forekomst af fedme, således at deltagere, der drak alkohol hyppigt var slankere end deltagere, som drak mindre hyppigt. Denne sammenhæng syntes at være uafhængig af det samlede ugentlige alkoholforbrug.

Ligeledes i Kost, Kræft og Helbreds kohorten sammenlignede vi risikoen for at dø mellem deltagere med forskellig drikkefrekvens. Vi fandt at risikoen for at dø var større hvis et ugentligt forbrug på over 7 genstande for kvinder og over 14 genstande for mænd blev indtaget på en dag sammenlignet med at sprede et tilsvarende forbrug på flere af ugens dage.

Konkluderende kan siges, at genetisk variation i alkoholnedbrydende enzymer til en vis grad prædikterer drikkemønstre og alkoholisme. Alkohol-drikkemønstre har, uafhængigt af det samlede alkoholforbrug, indflydelse på risiko for koronar hjertesygdom (for mænd) og for død, og måske på fedmeudvikling. Disse resultater har betydning for såvel fremtidige studier af biologiske effekter af alkohols betydning for helbred som for folkesundhedsmæssige budskaber om alkohol.

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