Original Article

Alcohol Consumption and Type 2 Diabetes

Influence of Genetic Variation in Alcohol Dehydrogenase

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OBJECTIVE—We sought to investigate whether a polymorphism in the alcohol dehydrogenase 1c (ADH1C) gene modifies the association between alcohol consumption and type 2 diabetes.

RESEARCH DESIGN AND METHODS—In nested case-control studies of 640 women with incident diabetes and 1,000 control subjects from the Nurses' Health Study and 383 men with incident diabetes and 382 control subjects from the Health Professionals Follow-Up Study, we determined associations between the ADH1C polymorphism, alcohol consumption, and diabetes risk.

RESULTS—Moderate to heavy alcohol consumption (>5 g/day for women and >10 g/day for men) was associated with a decreased risk of diabetes among women (odds ratio [OR] 0.45 [95% CI 0.33–0.63]) but not men (1.08 [0.67–1.75]). ADH1C genotype modified the relation between alcohol consumption and diabetes for women ($P_{\text{interaction}}$ = 0.02). The number of ADH1C*2 alleles, related to a slower rate of ethanol oxidation, attenuated the lower risk of diabetes among women consuming ≥5 g alcohol/day (P_{trend} = 0.002). These results were not significant among men. Results were similar in pooled analyses ($P_{\text{interaction}}$ = 0.02) with ORs for diabetes among moderate drinkers of 0.44 (95% CI 0.21–0.94) in ADH1C*1 homozygotes, 0.65 (0.39–1.06) for heterozygotes, and 0.78 (0.50–1.22) for ADH1C*2 homozygotes compared with those for ADH1C*1 homozygote abstainers (P_{trend} = 0.02).

CONCLUSIONS—ADH1C genotype modifies the association between alcohol consumption and diabetes. The ADH1C*2 allele, related to a slower oxidation rate, attenuates the lower diabetes risk among moderate to heavy drinkers. This suggests that the association between alcohol consumption and diabetes may be causal but mediated by downstream metabolites such as acetate rather than ethanol itself. ***Diabetes*** **56:2388–2394, 2007**

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ADH, alcohol dehydrogenase; HPFS, Health Professionals Follow-Up Study; NHS, Nurses' Health Study.

Moderate alcohol consumption is associated with a decreased risk of type 2 diabetes compared with abstention (1,2). Moreover, randomized controlled trials have shown that moderate drinking improves insulin sensitivity (3,4), suggesting that this relation may be causal and due to the effect of ethanol or its metabolites.

Ethanol, when consumed in moderation, is oxidized to acetaldehyde and acetate by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (5). Four genes of the ADH family, coding for class I (ADH1A-C) and class II (ADH2) enzymes, are involved in hepatic metabolism of ethanol (6). At the ADH1B locus, a functional polymorphism occurs exhibiting a 30- to 40-fold difference in $V_{\max}$ for ethanol oxidation, but the variant allele is rare among Caucasians (<5%) (7).

At the ADH1C locus, two polymorphisms occur at amino acids 271 and 349 that are in nearly complete linkage disequilibrium (8,9). The 271Arg/349Ile allele is frequently designated as γ1 or ADH1C*1 and the 271Gln-349Val as γ2 or ADH1C*2 (10). The γ1 subunit has a 2.5-fold higher $V_{\max}$ for ethanol oxidation than that for γ2 and has a prevalence of 50–60% among Caucasian populations (7,10). Although the extent to which this polymorphism affects blood alcohol concentrations remains unclear (11), slower ethanol oxidation is thought to increase hepatic exposure to ethanol. Thus, this polymorphism could modify the association of alcohol consumption with disease risk but with an effect anticipated only among drinkers. Indeed, such an interaction between alcohol consumption and ADH1C genotype has been observed in some, but not all (8,12), studies of coronary heart disease and levels of HDL cholesterol (13–15).

Whether this interaction is also present for type 2 diabetes has not been studied to date. The finding that ADH1C genotype modifies the association of alcohol intake with diabetes would support the hypothesis that the association is causal because genotypes are distributed randomly and hence mimic the random assignment of alcohol exposure that would occur in a randomized trial (sometimes referred to as Mendelian randomization)

TABLE 1
Flow chart of selection of case and control subjects in the NHS and HPFS

	NHS	HPFS
1990		
Blood draw	32,826	—
1994		
Blood draw	—	18,225
1996		
Incident diabetes cases identified	397*	—
Matching to two controls	793	—
1998		
Incident diabetes cases identified	189†	—
Matching to one control	189	—
Two controls in top decile BMI	17	—
2000		
Incident diabetes cases identified	138‡	427§
Matching to one control	138	431
Two controls in top decile BMI	3	—
Exclusions		
Missing ADH1C (n = 116)	116	32
Missing alcohol consumption (n = 15)	15	9
Non-Caucasians (n = 93)	93	52
Case subjects (n = 640)	640	383
Control subjects (n = 1,000)	1,000	382

Data are n. *Between 1990 and 1996; †between 1996 and 1998; ‡between 1998 and 2000; §between 1994 and 2000.

(16,17). Therefore, we investigated the interaction between ADH1C and alcohol consumption on risk for type 2 diabetes in nested case-control studies from the Nurses' Health Study (NHS) and the Health Professionals Follow-Up Study (HPFS). Moderate drinking has previously been associated with lower risk of diabetes in the overall populations of both cohorts (18–21).

RESEARCH DESIGN AND METHODS

The NHS began in 1976, when 121,700 female nurses aged 30–55 years responded to a questionnaire of health-related information. Questionnaires have been administered biennially to update health information and identify new cases of disease. During 1989–1990, 32,826 women free of diagnosed diabetes, coronary heart disease, stroke, or cancer provided blood samples. Women providing blood samples had higher prevalence of obesity and family history of diabetes and a lower prevalence of current smoking but were otherwise similar to women not providing blood. By 2000, 678 of these women had a confirmed diagnosis of type 2 diabetes. Control subjects providing blood samples were matched to diabetes cases by year of birth, date of blood draw, race, and fasting status at blood draw. From 1990 to 1996, two control subjects were matched to each case subject based on the above factors. One of the two control subjects was also matched according to BMI within 1 kg/m^2. After 1996, one control subject was matched to each case subject based on the same characteristics, and another control subject was matched on these characteristics and BMI to each of the case subjects in the top decile of the BMI distribution. Non-Caucasian women were excluded, leaving 640 case and 1,000 control subjects for analysis. The selection of case and control subjects for both studies is depicted in Table 1.

The HPFS began in 1986, when 51,529 male health professionals aged 40–75 years completed the initial questionnaire. Biennial follow-up has mirrored the NHS. During 1993–1994, 18,225 participants of the HPFS provided a blood sample. Characteristics of participants providing blood samples were similar to those of other HPFS participants. By 2002, 431 incident cases of type 2 diabetes, free from cardiovascular disease or cancer (except nonmelanoma skin cancer) at baseline, were confirmed. One control subject was matched per case subject by year of birth, month of blood collection, and fasting status at blood draw using the risk-set sampling strategy (22). We excluded non-Caucasian men, leaving 383 case and 382 control subjects for analysis.

Subjects provided written informed consent, and the studies were approved by the institutional review board of Partners HealthCare System, Boston, Massachusetts.

Ascertainment of diabetes. Incident cases of type 2 diabetes were identified by self-report and confirmed by a validated supplementary questionnaire detailing symptoms, diagnostic laboratory test results, and diabetes treatment. The diagnosis was confirmed if participants reported at least one of the following on the questionnaire: treatment with either insulin or an oral hypoglycemic agent, at least one classic symptom of diabetes (e.g., polyuria, polydipsia, or weight loss) plus elevated plasma glucose level, or an elevated plasma glucose level on at least two occasions in the absence of symptoms. Elevated plasma glucose was defined as at least 140 mg/dl (≥7.8 mmol/l) fasting, at least 200 mg/dl (≥11.1 mmol/l) nonfasting, or at least 200 mg/dl (≥11.1 mmol/l) at ≥2 h after an oral glucose tolerance test for cases diagnosed before 1998; for cases diagnosed in 1998 and later, the fasting plasma glucose threshold was lowered to ≥126 mg/dl (≥7.0 mmol/l) (23). The validity of self-reported diabetes has been confirmed with medical record review in a sample (18,24).

Assessment of alcohol consumption. We assessed average alcohol consumption within a semiquantitative food frequency questionnaire (25) including separate items for beer, white wine, red wine, and liquor. We specified standard portions as a glass, bottle, or can of beer; a 4-oz glass of wine; and a shot of liquor. For each beverage, participants were asked to estimate their average consumption over the past year. We calculated ethanol intake by multiplying the frequency of consumption of each beverage by the alcohol content of the specified portion size (12.8 g for beer, 11.0 g for wine, and 14.0 g for liquor) and summing across beverages (26). We used alcohol consumption reported on the food frequency questionnaire in 1990 for women and 1994 for men and replaced information with data from 1986 onward in case of missing data for alcohol consumption. In 1988, men and women also reported the number of days per week that they typically drank any form of alcohol.

We previously assessed the validity of alcohol consumption estimated with the food frequency questionnaire against intake from two 1-week dietary records collected ~6 months apart among 173 women and 136 men residing in eastern Massachusetts; the Spearman correlation coefficient between these two measures was 0.90 for women and 0.86 for men (27).

Assessment of lifestyle factors. Lifestyle factors were assessed using questionnaires, including smoking, body weight, physical activity, family history of diabetes, menopausal status, and use or nonuse of postmenopausal hormone therapy. Reported weights have been shown to correlate well with measured weights (r = 0.96) (28), and the assessment of physical activity was previously validated (29). We obtained energy intake, glycemic load, coffee consumption, and energy-adjusted intakes of saturated fat, *trans* fatty acids, polyunsaturated fatty acids, and dietary fiber from the semiquantitative food frequency questionnaire (30).

Laboratory procedures. All samples were genotyped using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) in 384-well format. The PCR amplifications were carried out on a minimum of 5–20 ng DNA using 1 X TaqMan universal PCR master mix (No Amp-erase UNG). Amplification conditions on an AB 9700 dual-plate thermal cycle (Applied Biosystems) were as follows: one cycle of 95°C for 10 min, followed by 50 cycles of 92°C for 15 s and 60°C for 1 min. TaqMan primers and probes were designed using the Primer Express Oligo Design software (version 2.0; ABI PRISM) and are available on request. Genotyping was successful in 94% of women and 96% of men (Table 1). For both the NHS and HPFS, 21 quality control sets were genotyped, with no discordant pairs detected.

Biomarkers related to diabetes were assessed only among women. Insulin levels were measured using a double-antibody system with <0.2% cross-reactivity between insulin and its precursors (Linco Research, St. Louis, MO). A1C was measured by immunoassay (Roche Diagnostics, Indianapolis, IN). The coefficients of variation were 3.5–11.7% for fasting insulin and 1.9–3.0% for A1C.

Statistical analysis. We used a χ^2 test to determine whether ADH1C genotypes were in Hardy-Weinberg equilibrium. Logistic regression adjusted for matching factors was used to estimate odds ratios (ORs) (95% CI) for type 2 diabetes based on alcohol consumption and ADH1C genotypes (ADH1C*1/*1, ADH1C*1/*2, and ADH1C*2/*2). We simultaneously adjusted for BMI (quintiles) and smoking (never, former, or current smokers of 1–14, 15–34, or ≥35 cigarettes/day), family history of diabetes in a first-degree relative (present or not), physical activity (five categories), postmenopausal hormone therapy (premenopausal or never, past, or current user), energy intake, coffee consumption, and energy-adjusted intakes of saturated fat, *trans* fatty acids, polyunsaturated fatty acids, glycemic load, and dietary fiber (each in quintiles for women and tertiles for men).

We first examined independent associations of alcohol consumption and ADH1C genotype with type 2 diabetes risk. Second, their interaction on type 2 diabetes risk (the primary test of our hypothesis) was assessed, and we examined the main effect of ADH1C genotype within strata of alcohol consumption (0, <5, and ≥5 g/day for women and 0, <10, and ≥10 g/day for men). Interaction terms of alcohol consumption (modeled on the log scale to

TABLE 2
Characteristics of incident cases of type 2 diabetes and matched control subjects

	Women		Men	
	Control subjects	Case subjects	Control subjects	Case subjects
n	1,000	640	382	383
Age (years)	57.0	57.0	60.4	60.6
BMI (kg/m^2)*	27.3†	30.5	25.8†	29.0
Physical activity (METs/week)	15.1†	12.4	36.6†	27.0
Alcohol consumption (g/day)	5.2†	2.9	11.5	10.7
Smoking (%)				
Never	46.7	42.2	49.2†	38.4
Past	42.0	44.2	46.1†	56.4
Current	10.8	13.6	3.7	4.7
Family history of diabetes (%)	21.2†	44.8	16.5†	26.6
Menopausal status (%)	79.5	82.0	NA	NA
Nutrients‡				
Energy (kcal)	1,780	1,828	2,135	2,130
Saturated fatty acids (g)	21.3†	22.5	22.3†	23.3
Trans fatty acids (g)	3.1	3.2	3.0	3.2
Polyunsaturated fatty acids (g)	11.9	12.2	12.2	12.5
Fiber (g)	19.9	20.2	22.3	21.4
Glycemic load	117.8	121.1	134.6†	130.6
Coffee consumption (cups/day)	2.2	2.0	2.1	1.9
Biomarkers				
A1C (%)	5.6†	6.4	—	—
Insulin (μU/ml)	9.6†	13.5	—	—

*Cases in the top decile of the BMI distribution were matched to an additional control on BMI. †$P < 0.05$ for case vs. control subjects. ‡All nutrients are energy-adjusted except energy.

maximize model fit) multiplied by the ADH1C genotype (modeled as the number of variant alleles) were tested. Finally, we examined the joint association of alcohol consumption and ADH1C genotype with risk of type 2 diabetes. To combine men and women, β-coefficients of the interaction term were pooled using the DerSimonian and Laird random effects model (31).

We also assessed whether ADH1C genotype modified the relation between alcohol consumption, fasting insulin, and A1C among control subjects, using ANOVA adjusted for covariates, described for conditional logistic regression above. For these analyses, fasting insulin was log transformed because of deviation from homogeneity of variance.

We performed sensitivity analyses using cutoffs of 7 g/day for women and 14 g/day for men, drinking frequency (0, 1–4, and ≥5 days/week) rather than average quantity, linear modeling instead of categorical analysis, and a restricted subset of women with A1C concentrations ≤6.5%; excluding heavier drinkers (>30 g/day for women and >40 g/day for men); and using conditional logistic regression—all of which gave similar results. Analyses were performed using SAS statistical package (version 8.2; SAS Institute, Cary, NC) and Intercooled STATA (version 9.0; STATA, College Station, TX).

RESULTS

Baseline characteristics and allele frequencies. Baseline characteristics of the case and control subjects from the NHS and HPFS are shown in Table 2. Women who developed type 2 diabetes tended to have higher BMI, fasting insulin, and A1C than control subjects. Alcohol consumption and physical activity were lower among women developing type 2 diabetes compared with that among matched control subjects. Men who developed type 2 diabetes had higher BMI and lower physical activity than control subjects.

The frequencies of the ADH1C*2 allele among control subjects were 41% among women and 40% among men. The distribution of ADH1C genotype was in Hardy-Weinberg equilibrium in the total population of the NHS ($P = 0.09$) and HPFS ($P = 0.72$) and among control subjects from the NHS ($P = 0.41$) and HPFS ($P = 0.38$). We assessed differences in alcohol consumption between genotypes within strata of alcohol consumption. Among women, ADH1C*1 homozygotes consumed 2.2, heterozygotes 2.1, and ADH1C*2 homozygotes 2.1 g/day ($P = 0.78$) in the stratum of light drinkers. In the stratum of moderate drinkers, ADH1C*1 homozygotes consumed 16.2, heterozygotes 14.1, and ADH1C*2 homozygotes 14.4 g/day ($P = 0.32$). Among men, ADH1C*1 homozygotes consumed 4.5, heterozygotes 4.5, and ADH1C*2 homozygotes 4.0 g/day ($P = 0.35$) among light drinkers. In the stratum of moderate drinkers, ADH1C*1 homozygotes consumed 25.9, heterozygotes 26.3, and ADH1C*2 homozygotes 25.7 g/day ($P = 0.97$). The lifestyle factors included in our multivariate models were not associated with ADH1C genotype among women ($P = 0.13$ to $P = 0.95$) or men ($P = 0.06$ to $P = 0.92$) (see supplementary Table in the online appendix [available at http://dx.doi.org/10.2337/db07-0181]).

Main effects of alcohol consumption and ADH1C genotype. We first examined the main effects of alcohol consumption and ADH1C genotype. Alcohol consumption was inversely associated ($P < 0.001$) with risk of type 2 diabetes within this nested case-control population from the NHS, with a multivariate-adjusted OR of 0.45 (95% CI 0.33–0.63) among women consuming ≥5 g alcohol/day compared with abstainers, as previously reported for the entire NHS (19,20). Despite the previously reported inverse association between alcohol consumption and risk of type 2 diabetes in the entire HPFS cohort (18), alcohol consumption was not associated with risk of type 2 diabetes in this nested case-control study (OR 1.08 [95% CI 0.67–1.75]) for intake of ≥10 g alcohol/day versus abstention).

ADH1C genotype per se was not associated with risk of type 2 diabetes among men or women (Table 3). Adjusting for alcohol consumption did not alter these results.

TABLE 3
ADH1C polymorphism and risk of type 2 diabetes among 640 case and 1,000 control subjects from the NHS and 383 case and 382 control subjects from the HPFS†

	ADH1C*1/*1	ADH1C*1/*2	ADH1C*2/*2	*P* for linear trend
Case subjects	376 (36.8)	469 (45.8)	178 (17.4)	—
Control subjects (*n*/%)	479 (34.7)	665 (48.1)	238 (17.2)	—
Matched, BMI adjusted	1.0	0.93 (0.77–1.12)	1.00 (0.78–1.29)	0.83
Matched, multivariate adjusted‡	1.0	0.96 (0.79–1.18)	1.05 (0.80–1.36)	0.87
Matched, alcohol and multivariate adjusted‡	1.0	0.97 (0.79–1.19)	1.11 (0.85–1.45)	0.59

Data are *n* (%) or ORs (95% CI) unless otherwise indicated. †Results obtained from pooling the β-coefficient and SE estimates for men and women using the DerSimonian and Laird random effects model. ‡Adjusted for BMI, physical activity, smoking, family history of diabetes, postmenopausal hormone replacement therapy, energy intake, and energy-adjusted intake of saturated fat, *trans* fatty acids, polyunsaturated fat, dietary fiber, glycemic load, and coffee consumption.

Interaction between alcohol consumption and ADH1C genotype. We next assessed the main effect of ADH1C genotype within strata of alcohol consumption. ADH1C genotype significantly modified the association between alcohol consumption and type 2 diabetes among women ($P_{interaction}$ = 0.02) (Table 4). The ADH1C*2 allele attenuated the decreased risk of type 2 diabetes in a dose-dependent manner among consumers of ≥5 g alcohol/day, while ADH1C genotype was not associated with risk of type 2 diabetes among abstainers and light drinkers.

Among men, the overall interaction between alcohol consumption and ADH1C genotype was not significant ($P_{interaction}$ = 0.41). As among women, we observed a higher type 2 diabetes risk associated with the ADH1C*2 allele among men consuming ≥10 g/day, but this trend was not significant.

Because we found no heterogeneity in the interaction between alcohol consumption and genotype among women and men ($P_{homogeneity}$ = 0.46), estimates for women and men were pooled. We observed a significant ($P_{interaction}$ = 0.02) interaction between alcohol consumption and ADH1C genotype in the pooled analysis (Fig. 1). Moderate to heavy alcohol consumption was associated with a decreased risk of type 2 diabetes. However, because the ADH1C*2 allele tended to attenuate the lower risk associated with moderate drinking, the risk among moderate drinkers homozygous for the ADH1C*2 allele was nearly identical to that among abstainers homozygous for the ADH1C*1 allele.

Mediating biomarkers. We also assessed whether ADH1C genotype modified the relation between alcohol consumption and markers of type 2 diabetes among control participants. Alcohol consumption was inversely associated with fasting insulin (β-coefficient ± SE −0.017 ± 0.010), with concentrations of 8.2 ± 0.41, 8.0 ± 0.41, and 6.9 ± 0.39 among nondrinkers and consumers of 0.1–4.9 and ≥5.0 g/day, respectively. ADH1C genotype was not associated with fasting insulin among abstainers (P_{trend} = 0.81) and light drinkers (P_{trend} = 0.89). Among consumers of ≥5 g alcohol/day, homozygotes for ADH1C*2/*2 (8.3 ± 1.4 μU/ml) tended to have higher concentrations of fasting insulin than homozygotes ADH1C*1/*1 (7.0 ± 0.7 μU/ml) and heterozygotes (5.8 ± 0.6 μU/ml), but the interaction between alcohol consumption and ADH1C genotype was not significant ($P_{interaction}$ = 0.86).

No association between alcohol consumption and A1C

TABLE 4
Alcohol consumption, ADH1C polymorphism, and risk of type 2 diabetes among 640 case and 1,000 control subjects from the NHS and 383 case and 382 control subjects from the HPFS

	ADH1C*1/*1	ADH1C*1/*2	ADH1C*2/*2	P_{trend}
Women				
0 g/day				
Cases/controls	139/132	144/192	40/53	
Multivariate adjusted†	1.0	0.76 (0.53–1.09)	0.78 (0.46–1.33)	0.20
0–4.9 g/day				
Cases/controls	75/109	95/140	44/71	
Multivariate adjusted†	0.60 (0.39–0.91)	0.74 (0.50–1.10)	0.62 (0.38–1.02)	0.76
≥5 g/day				
Cases/controls	24/106	49/141	30/56	
Multivariate adjusted†	0.24 (0.14–0.43)	0.41 (0.26–0.66)	0.60 (0.34–1.07)	0.009
Men				
0 g/day				
Cases/controls	34/32	46/48	19/18	
Multivariate adjusted†	1.0	0.64 (0.31–1.30)	0.96 (0.40–2.35)	0.72
0–9.9 g/day				
Cases/controls	65/50	68/69	23/15	
Multivariate adjusted†	1.16 (0.58–2.30)	0.86 (0.44–1.67)	1.22 (0.49–3.04)	0.63
≥10 g/day				
Cases/controls	39/50	67/75	22/25	
Multivariate adjusted†	0.73 (0.35–1.54)	0.96 (0.48–1.91)	0.93 (0.39–2.25)	0.29

†Adjusted for BMI, physical activity, smoking, family history of diabetes, postmenopausal hormone replacement therapy, energy intake, and energy-adjusted intake of saturated fat, *trans* fatty acids, polyunsaturated fat, dietary fiber, glycemic load, and coffee consumption.

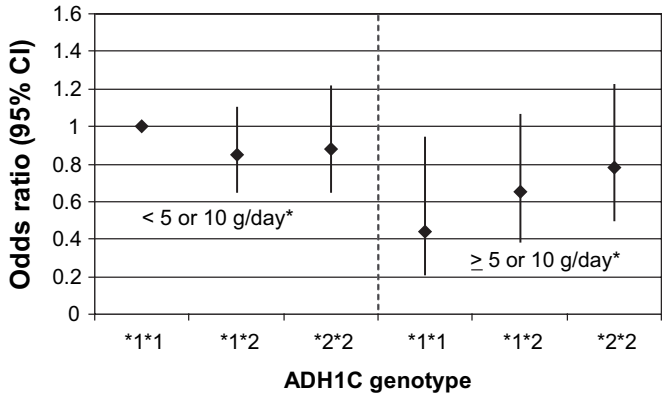

FIG. 1. Joint association of alcohol consumption and the ADH1C genotype with risk of type 2 diabetes among 1,023 case and 1,382 control subjects from the NHS and HPFS. ORs are adjusted for BMI, physical activity, smoking, family history of diabetes, postmenopausal hormone replacement therapy, energy intake, and energy-adjusted intake of saturated fat, *trans* fatty acids, polyunsaturated fat, dietary fiber, glycemic load, and coffee consumption and pooled for men and women using the DerSimonian and Laird random effects model. *Cutoff for alcohol consumption of 5 g/day for women and 10 g/day for men.

was observed, nor was an interaction between alcohol consumption and ADH1C genotype (data not shown).

DISCUSSION

The primary function of ADH1C is ethanol oxidation. A common polymorphism of this gene is associated with a slower rate of oxidation and may increase exposure to ethanol. Therefore, we hypothesized that the ADH1C genotype would modify the association between alcohol consumption and type 2 diabetes risk. In support of this hypothesis and of a causal relationship between alcohol consumption and type 2 diabetes, an interaction between ADH1C genotype and alcohol consumption for type 2 diabetes was observed among women and in the pooled analysis of both studies.

A key finding of this study was that the ADH1C*2 allele, related to slower ethanol oxidation, was associated with a dose-dependent attenuation in the lower diabetes risk among moderate to heavy drinkers, while no associations between the ADH1C genotype and diabetes were observed for those consuming less or no alcohol. This interaction is in the opposite direction from previous observations for cardiovascular disease (13), where the benefit from moderate alcohol consumption is mainly due to increased HDL cholesterol concentrations. Ethanol itself directly increases HDL cholesterol in a dose-dependent manner (32). Thus, slower gastric and hepatic metabolism of ethanol would lead to increased HDL cholesterol concentrations and reduced cardiovascular risk, as observed (13). In contrast, we observed a lower risk of diabetes with moderate drinking in this study, but this was attenuated (rather than accentuated) by alleles conferring slower oxidation (Fig. 1).

This disparity between our findings for type 2 diabetes and those for cardiovascular disease suggests different mechanisms underlying the associations with moderate drinking. Ethanol oxidation produces measurable downstream metabolites such as acetaldehyde and acetate (33), which could affect risk of type 2 diabetes themselves instead of ethanol. Slower ethanol oxidation would tend to produce lower concentrations of these metabolites.

Perhaps the strongest data relating ethanol metabolites to type 2 diabetes risk involve acetate and its effects on peripheral tissue (34). Acetate, the end product of ethanol oxidation, is oxidized to acetyl-CoA by acetyl-CoA syntethase (35,36) primarily in peripheral tissues such as muscle (37). Acetyl-CoA enters the Krebs cycle, stimulating oxidative phosphorylation (38). Several studies have shown that impaired mitochondrial oxidative phosphorylation is associated with insulin resistance (39) and type 2 diabetes (40,41). In addition, alcohol consumption via acetate acutely affects energy expenditure and fat oxidation (7,42). Moreover, acetate itself decreases lipolysis and thereby free fatty acid concentrations (7,43). Indeed, in human studies, acetic acid and acetate acutely decrease free fatty acid concentrations (44) and improve insulin sensitivity (45).

Studies directly manipulating ADH activity confirm decreased acetate concentrations, which may support our hypothesis. Sarkola et al. (33) showed that 4-methylpyrazole, an ADH inhibitor, decreased production of acetate in men and women after ingestion of 0.4 g/kg alcohol. Although the effect of genotype on blood alcohol and acetate concentrations remains uncertain, the ADH1C*2 genotype has been associated with decreased production of salivary acetaldehyde as expected (46).

The association of ADH1C genotype per se with risk of type 2 diabetes was not significant. This reflects the fact that abstainers or light drinkers, in whom we hypothesized and observed no association of genotype with risk, comprised a high proportion of participants in both studies (75% of women and 64% of men). In other populations with larger proportions of frequent drinkers, we hypothesize that genotype itself would be associated with risk.

Strengths of our study include its prospective design and detailed assessment of alcohol consumption, lifestyle factors, and diet. Nonetheless, several limitations of this study need to be addressed. First, our study population consists of relatively light drinkers, with limited variation in alcohol consumption. We therefore could not explore the effect of ADH1C genotype on the association between alcohol consumption and type 2 diabetes among heavier drinkers. However, it is possible that genetic variation in ADH1C would have less impact among heavier drinkers because at higher concentrations, ethanol is also metabolized through ADH4, CYP2E4, and other enzymes with higher K_m values for alcohol.

Second, we could not replicate the inverse association between alcohol consumption and type 2 diabetes observed in the entire HPFS cohort in the smaller nested case-control study. We have previously shown a strong inverse association in the full HPFS cohort of men. When we examined the entire cohort of men from 1994 (as opposed to 1986, the original baseline of the study) onward, we observed an inverse association between alcohol intake and risk of diabetes similar to that in our previous studies, suggesting that results in this smaller case-control study, which also dates from 1994, may be due to chance. The interaction, however, tended to be qualitatively similar among both men and women.

Third, the ADH1C *Arg271Gln* polymorphism was not assessed in this study, but since linkage disequilibrium is nearly complete, similar associations for both polymorphisms with coronary heart disease have been shown (8). Finally, the ADH1C polymorphisms are in linkage disequilibrium with the ADH1B*47His polymorphism (47), although the latter single nucleotide polymorphism is quite rare in Caucasian populations. However, regardless of

which ADH single nucleotide polymorphism is truly responsible for this interaction, the finding that genetic variation in alcohol dehydrogenase enzymes significantly modifies the alcohol-diabetes relationship provides important evidence that this relationship may be causal.

In conclusion, this study indicates that ADH1C genotype modifies the association between alcohol consumption and type 2 diabetes. Among moderate to heavy drinkers, the ADH1C*2 allele, related to a slower rate of ethanol oxidation, was associated with a dose-dependent attenuation in the lower risk of type 2 diabetes among moderate to heavy drinkers. This suggests that the association between alcohol consumption and type 2 diabetes may be causal but due to downstream metabolites such as acetate rather than ethanol itself.

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