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ALDH2 polymorphism is associated with fasting blood glucose through alcohol consumption in Japanese men

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ABSTRACT

Associations between alcohol consumption and type 2 diabetes risk are inconsistent in epidemiologic studies. This study investigated the associations of *ADH1B* and *ALDH2* polymorphisms with fasting blood glucose levels, and the impact of the associations of alcohol consumption with fasting blood glucose levels in Japanese individuals. This cross-sectional study included 907 men and 912 women, aged 35–69 years. The subjects were selected from among the Japan Multi-institutional Collaborative Cohort study across six areas of Japan. The *ADH1B* and *ALDH2* polymorphisms were genotyped by Invader Assays. The *ALDH2* Glu504Lys genotypes were associated with different levels of fasting blood glucose in men (P = 0.04). Mean fasting glucose level was positively associated with alcohol consumption in men with the *ALDH2 504 Lys* allele (P_{trend} = 0.02), but not in men with the *ALDH2 504Glu/Glu* genotype (P_{trend} = 0.45), resulting in no statistically significant interaction (P = 0.38). Alcohol consumption was associated with elevated fasting blood glucose levels compared with non-consumers in men (P_{trend} = 0.002). The *ADH1B* Arg48His polymorphism was not associated with FBG levels overall or after stratification for alcohol consumption. These findings suggest that the *ALDH2* polymorphism is associated with different levels of fasting blood glucose through alcohol consumption in Japanese men. The interaction of *ALDH2* polymorphisms in the association between alcohol consumption and fasting blood glucose warrants further investigation.

Key Words: *ADH1B* and *ALDH2* polymorphisms, type 2 diabetes, fasting blood glucose, alcohol consumption

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INTRODUCTION

Associations between alcohol consumption and type 2 diabetes risk are inconsistent in epidemiologic studies. In a meta-analysis of 15 prospective cohort studies performed worldwide, moderate alcohol consumption was associated with a decreased risk of type 2 diabetes, whereas high alcohol consumption was not[1]. In contrast, a systematic review of five prospective cohort studies in Japan reported that moderate alcohol consumption was associated with an increased risk of type 2 diabetes in subjects with a low body mass index (BMI)[2]. These inconsistent results may be due to different genetic susceptibilities to alcohol exposure between different populations. Therefore, it is necessary to investigate the possible associations between fasting blood glucose (FBG) levels and polymorphisms in *ADH1B* or *ALDH2*, taking into account alcohol consumption.

Alcohol is primarily oxidized to acetaldehyde by alcohol dehydrogenase (ADH) enzymes. Acetaldehyde is further oxidized to acetate by aldehyde dehydrogenase (ALDH) enzymes. Functional polymorphisms are found in the genes encoding *ADH1B*, affecting alcohol consumption in humans[3, 4]. The *ADH1B* Arg48His polymorphism (rs1229984) greatly affects enzyme activity, as the *48His* allele is associated with faster oxidation of ethanol. The *ADH1B 48His* allele is fairly common in Asian populations and rare in Caucasian populations. *ALDH2* is the gene encoding mitochondrial ALDH, which contributes the majority of acetaldehyde oxidation in the human liver, and contains a functional polymorphism of Glu504Lys (rs671), with the minor *504Lys* allele resulting in an inactive form. The minor allele of *ALDH2 504Lys* is mainly found in Asian populations[5, 6].

Several studies in Japan have investigated the associations between these alcohol-related genetic polymorphisms and glucose metabolism and type 2 diabetes. A study has found elevated fasting plasma glucose levels in men with the *ADH1B 48Arg/Arg* genotype who consumed ≥ 10 g of alcohol per day[7]. Another study reported that fasting plasma insulin levels are lower in subjects with the *ADH1B 48Arg* allele than in subjects with the *48His/His* genotype[8]. Our previous study showed that the *ADH1B* His48Arg polymorphism modified the association between alcohol consumption and type 2 diabetes in middle-aged Japanese men[9], but the sample size was small. In terms of the *ALDH2* Glu504Lys polymorphism, the *ALDH2 504Lys* allele was associated with impaired glycemic control, as assessed by hemoglobin A_{1c} concentrations, in Japanese patients with type 2 diabetes and habitual light-to-moderate alcohol consumption[10]. Another study reported that the *ALDH2 504Lys* allele was associated with elevated levels of fasting plasma glucose in Japanese women who consumed ≥ 5 g of alcohol per day[7]. Our previous study reported that the *ALDH2 504Lys* allele is associated with a decreased risk of type 2 diabetes[9]. However, the study did not have enough statistical power to assess the interactions.

Two studies mentioned that the association of some gene polymorphisms with type 2 diabetes mellitus was sex-specific in Japanese individuals[7, 11]. The present study therefore examined the association of the *ADH1B* and *ALDH2* polymorphisms to FBG levels in a cross-sectional study in Japanese by sex, focusing on effect modifications by gene-environment interactions.

MATERIAL AND METHODS

Study population

The study subjects were among participants in the Japan Multi-institutional Collaborative Cohort (J-MICC) study. The design of the J-MICC study and the characteristics of the participants in the present cross-sectional study are described in more detail elsewhere[12, 13]. In brief, the subjects were enrolled across 10 study areas (Chiba, Shizuoka, Okazaki, Aichi Cancer Center,

Takashima, Kyoto, Tokushima, Fukuoka, Saga, and Amami) in Japan between 2004 and 2008. The subjects for the cross-sectional study comprised about 500 participants enrolled consecutively and arbitrarily in each area of the J-MICC study, except in two areas, where fewer participants had been recruited. Of them, individuals aged 35–69 years enrolled across six areas (Shizuoka, Okazaki, Takashima, Kyoto, Tokushima, and Amami) that had collected baseline blood glucose data were included in this analysis.

The present study included 2415 subjects. Of these, 596 subjects were excluded for the following reasons: non-fasting blood samples (individuals had a meal within 3 h of blood sampling, n =180), history of diabetes (n = 83), chronic hepatitis or liver cirrhosis (n = 49), fatty liver (n =186), history of cardiovascular or cerebrovascular disease (n = 78), history of cancer (n = 15), and missing measurements of blood glucose levels (n = 5). Therefore, a total of 1819 subjects (907 men and 912 women) were included in the present analysis.

The protocol for the J-MICC Study was approved by the ethics committees of Nagoya University School of Medicine and at the other participating institutions. All of the study subjects gave written informed consent before participating in this study.

Lifestyle questionnaire and clinical characteristics

Subjects completed a questionnaire to record alcohol consumption, smoking habits, physical activity, diseases under current or previous treatment, family history of selected diseases, and other lifestyle habits. Current alcohol consumers were defined as subjects who reported consuming alcoholic beverages at least once a month. Past and current drinkers were asked to state the age at which they began drinking habitually. Current alcohol consumers also stated the frequency and amount of consumption for six alcoholic beverages (sake, shochu, chuhai, beer, whisky, and wine). The frequency of consumption options was recorded as almost never, 1–3 times/month, 1–2 times/week, 3–4 times/week, 5–6 times/week or daily. The subjects were also asked to state the amount of each beverage consumed. Daily ethanol intake was estimated for current alcohol consumers based on the frequencies and amount of each type of alcoholic beverage consumed over the past year. Regarding smoking status, participants were asked whether they had ever smoked, the age when they started smoking (for ever smokers), and the age when they quit smoking (for former smokers). Weekly and daily frequency of coffee consumption and the number of cups of coffee consumed per day were also recorded.

Regarding physical activity, participants were asked about work-related activity (including domestic housework) and leisure-time activity. For work-related activity, participants reported the amount of time spent per day in sedentary activity, standing, walking, and performing strenuous labor using one of eight options: never, < 1, 1–2.9, 3–4.9, 5–6.9, 7–8.9, 9–10.9, or ≥11 h. For leisure-time activity, the frequency and amount of time per occasion were ascertained for three categories of exercise intensity (light activity: resulting in no shortness of breath, moderate activity: causing shortness of breath but not preventing speaking, and heavy activity: causing shortness of breath and difficulty in speaking). The frequency was recorded as never, 1–3 times/month, 1–2 times/week, 3–4 times/week, and ≥ 5 times/week. The duration of each activity was recorded as < 30 min, 30–59 minutes, 1–1.9 h, 2–2.9 h, 3–3.9 h, and ≥ 4 h. The intensity of each physical activity was determined in terms of the metabolic equivalent (MET) value (work-related activity: sedentary activity, 0; standing, 0; walking, 3.0; and strenuous labor, 4.5 METs; leisure-time activity: light, 3.4; moderate, 7; and heavy, 10 METs). Work-related and leisure-time physical activities were each expressed as the total number of METs multiplied by the duration of each activity per week (MET-h/week). The subjects also stated whether they had parental history of diabetes mellitus (i.e., yes, no, or unknown).

Clinical data, including height, weight, and blood glucose were measured using routine

methods. BMI (kg/m^2) was calculated as weight (kg)/height (m^2). Because blood glucose returns to near fasting levels approximately 3 h after a meal[14], the samples that were collected ≥ 3 h after the last meal were defined as fasting samples in this study.

Genotyping

DNA was extracted from buffy coat fractions using a BioRobot M48 Workstation (Qiagen Group, Tokyo, Japan) at the central office of the J-MICC study. The *ADH1B* Arg48His and *ALDH2* Glu504Lys polymorphisms were genotyped using the multiplex polymerase chain reaction (PCR)-based Invader assay[15] (Third Wave Technologies, Madison, WI) at the Laboratory for Genotyping Development, Center for Genomic Medicine, RIKEN, Japan.

Statistical analysis

Departure of the genotype distribution from the Hardy–Weinberg equilibrium was tested using the χ^2 test with one degree of freedom. The characteristics of the study subjects according to *ADH1B* and *ALDH2* genotypes were statistically analyzed using χ^2 tests for proportions, analysis of covariance for means, and the Kruskal–Wallis test for medians. Associations of genetic polymorphisms with FBG levels were evaluated using analysis of variance or covariance. Analyses were adjusted for age, area (Shizuoka and Okazaki = 0, Kyoto and Takashima = 1, Tokushima = 2, Amami = 3), BMI, smoking, alcohol consumption, coffee consumption, physical activity, and parental history of diabetes mellitus (no = 0, unknown = 1, yes = 2). All of the factors included for adjustment were entered as continuous variables, except for area and parental history of diabetes mellitus. Trends for associations were tested by linear regression analysis using an ordinal score for the variable of interest. Alcohol consumption was stratified as never, past consumption, and current consumption. Because 1 go of Japanese sake is equal to about 23 g ethanol, the amount of alcohol consumed was classified as < 23.0, 23.0–45.9, and ≥ 46.0 g/day for men, or as < 11.5, 11.5–22.9, and ≥ 23.0 g/day for women. BMI was stratified for < 22.5, 22.5–24.9, 25.0–27.4, and ≥ 27.5 kg/m^2. Interactions were evaluated using linear regression analysis (for example: Multiplying the variable that summarizes the drinking and the variable that summarizes the *ALDH2* genotypes, put into a linear regression model). Statistical significance was declared if the two-sided *P*-value was < 0.05. All statistical analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC).

RESULTS

The *ADH1B* Arg48His and *ALDH2* Glu504Lys genotypes were not determined in three and one subjects, respectively. The genotype distributions of the *ADH1B* and *ALDH2* polymorphisms in individual subjects were in agreement with the Hardy–Weinberg equilibrium (*ADH1B*: P = 0.88 for men, P = 0.60 for women; *ALDH2*: P = 0.18 for men, P = 0.32 for women).

Selected characteristics of the study subjects stratified by *ADH1B* Arg48His and *ALDH2* Glu504Lys genotypes are summarized in Table 1. BMI and alcohol intake were greater in subjects with the *ADH1B 48Arg/Arg* or *ALDH2 504Glu/Glu* genotypes compared with each *ADH1B 48His* allele and *ALDH2 504Lys* allele, respectively, in men and women. Coffee consumption was higher in men with the *ALDH2 504Lys* allele, but lower in women with this allele. Parental history of diabetes mellitus was less frequent in women with the *ALDH2 504Glu/Lys* genotype. Age, smoking, and physical activity did not vary among subjects with these polymorphisms.

The *ADH1B* Arg48His polymorphism was not associated with FBG levels in either men or women. By contrast, the *ALDH2 504Lys* allele was associated with lower FBG levels compared

Table 1 Characteristics of the study subjects according to *ADH1B* and *ALDH2* genotype

Characteristics	*ADH1B* Arg48His [a]			*P*-value [b]	*ALDH2* Glu504Lys [a]			*P*-value [b]
	His/His	*His/Arg*	*Arg/Arg*		*Glu/Glu*	*Glu/Lys*	*Lys/Lys*	
Men	(n = 515)	(n = 338)	(n = 54)		(n = 499)	(n = 337)	(n = 71)	
Age, mean (SD)	54.7 (9.2)	55.4 (9.3)	55.5 (9.4)	0.57	54.6 (9.1)	55.4 (9.2)	56.3 (10.1)	0.21
BMI (kg/m^2), mean (SD)	23.2 (2.8)	23.6 (2.9)	24.4 (3.0)	0.004	23.6 (3.0)	23.3 (2.8)	22.8 (2.3)	0.06
Ever-smoking, N (%)	362 (70.3)	238 (70.4)	41 (75.9)	0.68	340 (68.1)	252 (74.8)	49 (69.0)	0.11
Cigarette-years, median (IQR) [c]	520 (280–810)	490 (280–780)	660 (340–880)	0.53	540 (270–820)	500 (290–780)	510 (272–800)	0.96
Current alcohol consumption, (%)	400 (77.8)	263 (77.8)	46 (85.2)	0.44	460 (92.2)	245 (72.9)	4 (5.6)	< 0.0001
Alcohol consumption (g/day), median (IQR) [d]	25 (12–46)	25 (12–51)	35 (13–55)	0.40	31 (15–55)	17 (7–34)	6 (2–9)	< 0.0001
Coffee consumption (cups/day), median (IQR)	1.5 (0.3–2.0)	1.5 (0.3–3.0)	1.5 (0.1–1.6)	0.70	1.0 (0.1–1.6)	1.5 (0.5–3.0)	1.5 (0.5–3.0)	0.01
MET-h/week, median (IQR)	10 (5–21)	9 (5–20)	12 (4–22)	0.50	10 (5–21)	9 (4–20.5)	12 (6–23)	0.19
Parental diabetes mellitus, N (%)	64 (12.4)	50 (14.8)	5 (9.3)	0.42	65 (13.0)	48 (14.2)	6 (8.8)	0.42
Women	(n = 514)	(n = 343)	(n = 52)		(n = 547)	(n = 311)	(n = 53)	
Age, mean (SD)	55.2 (9.2)	55.0 (8.6)	55.3 (8.4)	0.93	55.4 (8.9)	54.8 (9.0)	54.8 (9.4)	0.62
BMI (kg/m^2), mean (SD)	22.8 (3.3)	22.7 (3.2)	23.9 (3.5)	0.03	23.0 (3.3)	22.6 (3.3)	22.1 (2.7)	0.054
Ever-smoking, N (%)	40 (7.8)	22 (6.4)	4 (7.7)	0.75	35 (6.4)	28 (9.0)	3 (5.7)	0.33
Cigarette-years, median (IQR) [c]	340 (160–560)	227 (140–435)	800 (240–840)	0.17	332 (164–568)	340 (160–620)	105 (12–270)	0.24
Current alcohol consumption, N (%)	174 (33.9)	128 (37.3)	25 (48.1)	0.10	260 (47.5)	66 (21.2)	2 (3.8)	< 0.0001
Alcohol consumption (g/day), median (IQR) [d]	5 (3–14)	5 (2–10)	10 (5–14)	0.046	6 (3–14)	4 (2–7)	2 (0–5)	0.01
Coffee consumption (cups/day), median (IQR)	1.5 (0.3–1.6)	1.5 (0.3–1.6)	1.5 (0.3–3.0)	0.53	1.5 (0.5–1.6)	1.5 (0.3–2.3)	0.8 (0.1–2.0)	0.02
MET-h/week, median (IQR)	10 (5–21)	11 (6–19)	8 (5–18)	0.50	10 (5–20)	9 (6–19)	12.5 (6–22)	0.55
Parental diabetes mellitus, N (%)	79 (15.4)	59 (17.2)	5 (9.6)	0.35	100 (18.3)	33 (10.6)	11 (20.8)	0.007

BMI, body mass index; IQR, interquartile range; SD, standard deviation; N, number; TC, total cholesterol; HDL, high-density lipoprotein.

a) The genotype was not determined for three subjects for *ADH1B* and one subject for *ALDH2*.

b) χ^2 tests for proportions, analysis of variance for means, and the Kruskal–Wallis test for medians.

c) Among ever-smokers.

d) Among current alcohol consumers (current alcohol consumers were defined as subjects who reported consuming alcoholic beverages at least once a month).

Table 2 Associations of *ADH1B* and *ALDH2* polymorphisms with fasting blood glucose levels in men and women

Genotype	Men				Women			
	N	Crude mean (SE)	Adjusted mean (SE) [b]	Adjusted mean (SE) [c]	N	Crude mean (SE)	Adjusted mean (SE) [b]	Adjusted mean (SE) [c]
ADH1B Arg48His [a]								
His/His	515	98.2 (0.5)	98.3 (0.5)	98.2 (0.5)	514	92.4 (0.5)	92.7 (0.4)	92.6 (0.4)
Arg/His	338	99.0 (0.6)	98.3 (0.6)	98.2 (0.6)	343	94.0 (0.6)	93.2 (0.5)	93.2 (0.5)
Arg/Arg	54	99.6 (1.6)	99.0 (1.5)	99.4 (1.7)	52	95.2 (1.5)	93.1 (1.2)	92.9 (1.3)
P-value [d]		0.51	0.89	0.75		0.04	0.69	0.66
ALDH2 Glu504Lys [a]								
Glu/Glu	499	99.9 (0.5)	99.2 (0.5)	99.1 (0.5)	547	93.6 (0.5)	92.9 (0.4)	92.8 (0.4)
Glu/Lys	337	97.7 (0.6)	97.8 (0.6)	97.8 (0.6)	311	92.9 (0.6)	93.2 (0.5)	93.3 (0.5)
Lys/Lys	71	93.9 (1.4)	95.4 (1.3)	95.6 (1.3)	53	90.5 (1.5)	90.9 (1.2)	91.1 (1.2)
P-value [d]		< 0.0001	0.01	0.04		0.23	0.13	0.21

N, number; SE, standard error.

a) The genotype was not determined for three subjects for *ADH1B* and one subject for *ALDH2*.

b) Adjusted for age, area, body mass index, smoking, coffee consumption, physical activity, and parental diabetes mellitus.

c) Adjusted for age, area, body mass index, smoking, alcohol consumption, coffee consumption, physical activity, and parental diabetes mellitus.

d) Analysis of variance or covariance for means.

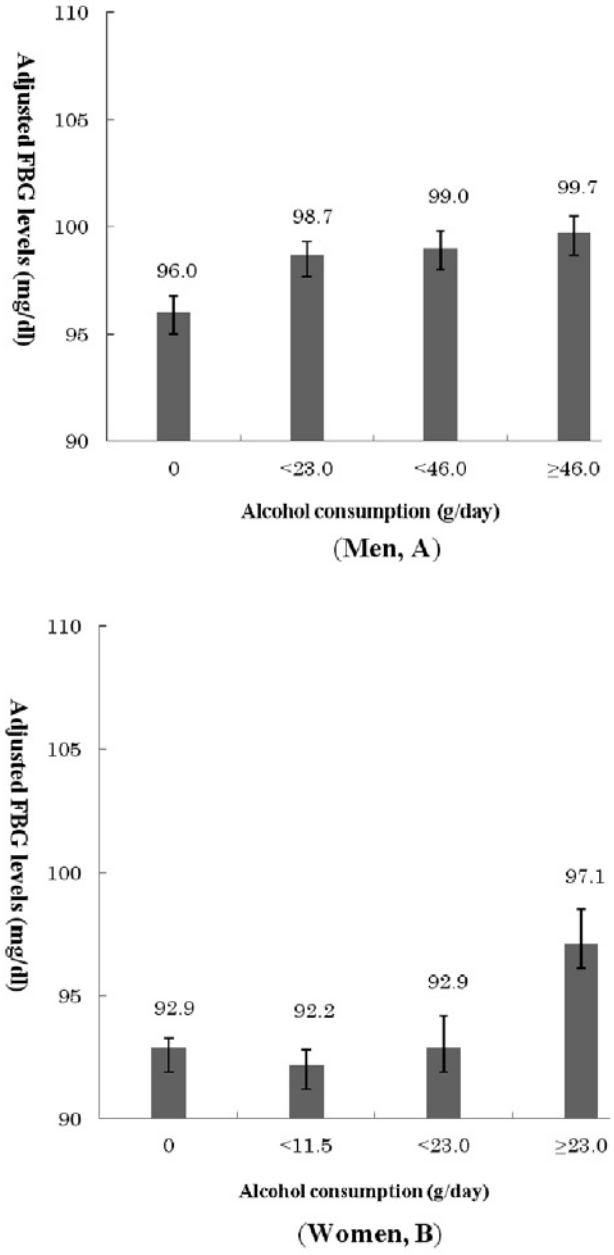

Fig. 1 Fasting blood glucose (FBG) levels in men (panel A) and women (panel B) according to alcohol consumption. FBG levels were adjusted for age, area, BMI, smoking, coffee consumption, physical activity, and parental diabetes mellitus. The values shown at the top of each bar are FBG levels (mean). The error bar shows the standard error (SE). The *P* for trend was 0.002 in men and 0.11 in women. The *P* for trend was determined by linear regression analysis, with ordinal numbers assigned to each category of alcohol consumption (0 = never, 1 = light, 2 = moderate, and 3 = heavy).

Table 3 Associations of *ADH1B* and *ALDH2* polymorphisms with fasting blood glucose levels according to alcohol consumption status in men and women

Alcohol consumption (g/day)	*ADH1B* Arg48His [a]						*P* for trend [c]	*ALDH2* Glu504Lys [a]				*P* for differences [c]
	His/His		*Arg/His*		*Arg/Arg*			*Glu/Glu*		*Glu/Lys+Lys/Lys*		
	N	Adjusted mean (SE) [b]	N	Adjusted mean (SE) [b]	N	Adjusted mean (SE) [b]		N	Adjusted mean (SE) [b]	N	Adjusted mean (SE) [b]	
Men												
Never use	105	96.3 (1.0)	70	95.5 (1.2)	7	97.5 (3.8)	0.83	28	97.2 (2.0)	154	95.8 (0.8)	0.45
< 23.0	172	98.6 (0.8)	109	98.6 (1.0)	16	99.9 (2.8)	0.96	155	99.1 (0.8)	142	98.2 (0.9)	0.60
23.0–45.9	108	99.7 (1.0)	60	97.7 (1.3)	11	99.8 (3.2)	0.49	122	99.6 (1.0)	57	97.9 (1.4)	0.18
≥ 46.0	94	98.3 (1.1)	70	101.2 (1.3)	15	101.1 (2.8)	0.28	142	99.7 (0.9)	37	99.6 (1.8)	0.63
P for trend		*P* = 0.06		*P* = 0.03		*P* = 0.62			*P* = 0.45		*P* =0.02	
		Interaction *P* = 0.35							Interaction *P* = 0.38			
Women												
Never use	334	92.8 (0.5)	211	92.9 (0.6)	27	93.9 (1.7)	0.77	279	93.0 (0.5)	294	92.9 (0.5)	0.93
<11.5	104	91.1 (0.9)	82	93.4 (1.0)	15	92.1 (2.3)	0.10	155	92.3 (0.7)	47	91.3 (1.3)	0.30
11.5–22.9	20	94.4 (2.0)	15	92.0 (2.2)	4	92.3 (4.2)	0.42	36	93.5 (1.5)	3	90.5 (4.9)	0.57
≥ 23.0	25	96.6 (1.8)	11	99.8 (2.7)	5	92.7 (4.3)	0.65	34	96.8 (1.5)	7	98.6 (3.5)	0.16
P for trend		*P* = 0.21		*P* = 0.16		*P* = 0.25			*P* = 0.13		*P* = 0.47	
		Interaction *P* = 0.66							Interaction *P* = 0.46			

N, number; SE, standard error.

a) The genotype was not determined for three subjects for *ADH1B* and one subject for *ALDH2*.

b) Adjusted for age, area, body mass index, smoking, coffee consumption, physical activity, and parental diabetes mellitus.

c) Linear regression analysis with ordinal numbers (0, 1, and 2) assigned to each genotype.

with the *504Glu/Glu* genotype in men, but not in women (Table 2). FBG levels were also lower in individuals with the *ALDH2 504Lys* allele, even after adjusting for covariates including alcohol consumption.

Alcohol consumption was associated with elevated FBG levels compared with non-consumers in men (Fig. 1A). By contrast, light to moderate alcohol consumption was not associated with increased FBG levels compared with non-consumers in women (Fig. 1B). Former alcohol drinkers (n =25) were excluded from this analysis. The distribution of alcohol consumption in men was 182 (21.7%), 297 (35.5%), 179 (21.4%), and 179 (21.4%) for non-consumers, < 23.0, 23.0–45.9, and ≥ 46.0 g/day, respectively. That in women was 574 (67.1%), 198 (23.1%), 43 (5.0%), and 41 (4.8%) for non-consumers, < 11.5, 11.5–22.9, and ≥ 23.0 g/day, respectively.

In analyses stratified by alcohol consumption status, the homozygous *ALDH2 504Lys* allele was combined with the heterozygous *ALDH2 504Lys* allele, because the rates of alcohol consumption were very low in these subjects. Also, former alcohol drinkers (n =25) were excluded from this analysis. Alcohol consumption was associated with a dose-dependent increase in FBG levels in men with the *ALDH2 504Lys* allele (P_{trend} = 0.02), but not in men with the *ALDH2 504Glu/Glu* genotype (P_{trend} = 0.45). However, the greater decreases in FBG with increasing alcohol consumption identified in men with the *ALDH2 504Lys* allele than in men with the *ALDH2 Glu/Glu* genotype were not apparent, as the interaction was not statistically significant (*P* = 0.38). There was no measurable interaction between *ADH1B* polymorphisms and alcohol consumption in either men or women (Table 3).

DISCUSSION

The present study showed that FBG levels were lower in men, but not in women, in those with the *ALDH2 504Lys* allele than in those without. Alcohol consumption was associated with

a dose-dependent increase in FBG levels in men with the *ALDH2 504Lys* allele but not in men with the *ALDH2 504Glu/Glu* genotype. The *ADH1B* Arg48His polymorphism was not associated with FBG levels in either men or women.

The *ALDH2 504Lys* allele was associated with higher fasting plasma glucose levels in women with high alcohol consumption (≥ 5 g/day)[7]. However, unlike this earlier observation, the present study showed that men with the *ALDH2 504Lys* allele may have lower FBG levels, whereas no change was observed in women. It is possible that individuals with the *ALDH2 504Lys* allele may follow more favorable lifestyle factors, such as abstaining from alcohol consumption. For example, in the present study, men with the *ALDH2 504Lys* allele reported greater coffee consumption, which is associated with decreased risk of type 2 diabetes[16]. It was reported that BMI is a risk factor for type 2 diabetes[17], and individuals with the *ALDH2 504Lys* allele had lower BMI compared with individuals with the *ALDH2 504Glu/Glu* genotype in this study. Nevertheless, even when adjusting the potential confounders including alcohol consumption, the level of FBG was lower in the *ALDH2 504Lys/Lys* genotype. However, we also found that alcohol consumption was associated with a dose-dependent increase in FBG levels in men with the *ALDH2 504Lys* allele. This finding supports the results of a previous study in Japan in which the *ALDH2 504Lys* allele was associated with impaired glycemic control in patients with type 2 diabetes reporting habitual light-to-moderate alcohol consumption[10]. This result is particularly important in the context of diabetes prevention, because individuals with the *ALDH2 504Lys* allele should be encouraged to abstain from alcohol.

Alcohol consumption was associated with a dose-dependent increase in FBG levels in men. In contrast, light-to-moderate alcohol consumption was associated with lower FBG levels in women. Therefore, the association between alcohol consumption and risk of type 2 diabetes may differ between men and women in Japan. Several studies in Japanese men also reported that alcohol consumption was associated with an increased risk of type 2 diabetes[9, 18, 19], but not in women.

The mechanisms for an association between *ALDH2* polymorphism and FBG levels, as well as an association between alcohol consumption and FBG levels, are still unknown. Because type 2 diabetes is associated with both insufficient insulin secretion and high insulin resistance, it appears likely that the alcohol-induced increase in blood glucose levels has adverse effects on one or both of those variables. Several studies have shown that ethanol causes insulin resistance in the liver and skeletal muscle by interfering with insulin signaling[20-22]. In addition, several experimental studies have reported that chronic ethanol feeding in rodents causes pancreatic β-cell apoptosis[23, 24] and decreases β-cell mass[20, 25]. One recent study reported that ethanol causes endoplasmic reticulum stress and impairment of insulin secretion in pancreatic β-cells[26]. However, one study reported that moderate alcohol consumption is associated with improved insulin sensitivity, reduced basal insulin secretion rate and a lower fasting glucagon concentration in women, but not men[27]. Clearly, further studies in this area are needed.

The lack of an association between *ADH1B* Arg48His polymorphisms and FBG in the present study is consistent with an earlier cross-sectional study in Japan[8]. That study found no differences in fasting plasma glucose or hemoglobin A_{1c} concentrations among *ADH1B* genotypes. However, fasting plasma insulin concentrations were lower in men and women with the *ADH1B 48His/Arg* genotype than in those with the *48His/His* genotype, although there was no difference in alcohol consumption between the *48His/His* and *48His/Arg* genotypes. In the present study, we could not analyze the possible associations of these polymorphisms with hemoglobin A_{1c} or plasma insulin concentrations, because these parameters were not measured in a sufficient number of subjects. Another study reported that fasting plasma glucose concentrations were higher in men with the *ADH1B 48Arg/Arg* genotype than in those with the *His/His* or *His/Arg* genotype, when alcohol consumption was high (≥ 10 g/day)[7]. However, the sample size of that study was small.

The *ADH1C 349Ile* allele, which is associated with fast oxidation of ethanol, was reported to decrease the risk of type 2 diabetes associated with alcohol consumption in the United States[28]. Those results suggest that acetate, the end product of alcohol oxidation, is involved in the protective association between alcohol and type 2 diabetes. The *ADH1C* Ile349Val polymorphism was not assessed in the present study because the *ADH1C 349Val* allele is extremely rare in Japanese individuals[5]. The *ADH1B* Arg48His polymorphism is in linkage disequilibrium with the *ADH1C* Ile349val polymorphism in Asian and Caucasian individuals[29, 30]. Therefore, the present results do not fully support the hypothesis that fast alcohol oxidation confers protection against the risk of type 2 diabetes associated with alcohol consumption.

There are several advantages and limitations of this study. The study subjects were representative of Japanese men and women in the general population. The frequencies of *ADH1B 48Arg* (25%) and *ALDH2 504Lys* (25%) alleles were similar to those reported in other general Japanese populations[31, 32]. However, an attrition bias is possible in cross-sectional studies. It is desirable that we should evaluate the association of these polymorphisms with hemoglobin A_{1c} or C-peptide, because those biomarkers are more stable than either FBG levels or insulin. However, the study only assessed the association between FBG levels and alcohol-related polymorphisms, because those other biomarkers were not measured in a sufficient number of subjects.

Blood samples obtained equal to and more than 3 h after the last meal were defined as fasting samples in this study. Nevertheless, similar results were obtained when we restricted the analysis to subjects (1546 total; men 794, women 752) with blood samples obtained 8 h after a meal and subjects (1405 total; men 726, women 679) with blood samples obtained 12 h after a meal. For the 8 h after a meal group, FBG levels (mg/dL) (SE) were 99.5 (0.6), 97.8 (0.6), and 95.1 (1.4) for the *ALDH2* genotypes of Glu/GLu, Glu/Lys and Lys/Lys, respectively (*P*-value was 0.02). For the 12 h after a meal group, FBG levels (SE) were 99.4 (0.6), 97.8 (0.7), and 95.1 (1.4) mg/dL for the *ALDH2* genotypes of Glu/GLu, Glu/Lys, and Lys/Lys, respectively. It was also statistically significant ($P = 0.01$).

Taken together, the present findings suggest that *ALDH2* genotypes are associated with different FBG levels through alcohol consumption in Japanese men. The interaction of *ALDH2* polymorphisms in the association between alcohol consumption and FBG levels warrants further investigation.

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CONFLICT OF INTEREST

The authors declare that there is no duality of interest.

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