

Relationships of alcohol dehydrogenase 1B (*ADH1B*) and aldehyde dehydrogenase 2 (*ALDH2*) genotypes with alcohol sensitivity, drinking behavior and problem drinking in Japanese older men

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Received: 26 November 2015 / Accepted: 11 January 2016
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Abstract

Objectives Many East Asians have the genetic polymorphisms rs1229984 in alcohol dehydrogenase 1B (*ADH1B*) and rs671 in aldehyde dehydrogenase 2 (*ALDH2*). Here we analyzed the relationships of the two genotypes with alcohol sensitivity, drinking behavior and problem drinking among older and younger men living in rural areas of Japan.

Methods The subjects were 718 Japanese men aged 63.3 ± 10.8 (mean \pm SD), categorized into the older (≥ 65 years, $n = 357$) and younger (< 65 years, $n = 361$) groups. Facial flushing frequency, drinking behavior and positive CAGE results were compared among the genotypes using Bonferroni-corrected χ^2 test and a multivariate logistic regression analysis adjusting for age, BMI and lifestyle factors.

Results The frequency of ‘always’ facial flushing among the *ADH1B**1/*2 carriers was significantly lower than that among the *ADH1B**2/*2 carriers in the older group ($P < 0.01$). The alcohol consumption (unit/day) in the *ADH1B**1/*2 carriers tended to be higher compared with that in the *ADH1B**2/*2 carriers among the older group ($P = 0.050$). In the younger group, no significant differences in alcohol sensitivity and drinking habits were

generally found among the *ADH1B* genotypes. The *ADH1B**1/*1 genotype tended to be positively associated with problem drinking in the older group ($P = 0.080$) but not in the younger group. The *ALDH2* genotypes consistently and strongly affected the alcohol sensitivity, drinking behavior and problem drinking in both the younger and older group.

Conclusions We for the first time observed a significant difference in alcohol sensitivity between *ADH1B**1/*2 and *ADH1B**2/*2 in older men aged 65 and above.

Keywords Alcohol dehydrogenase 1B · Aldehyde dehydrogenase 2 · Aging · Alcohol sensitivity · Problem drinking behavior

Introduction

The Global Burden of Disease Study 2010 revealed that alcohol use was the third- and eighth-ranked risk factor for disability-adjusted life-years in men and women, respectively [1]. In Japan in 2007, alcohol use was the third and 11th risk factor for deaths in men and women, respectively [2]. The alcohol consumption (liters) per capita in Japan is as much as that in the US and several European countries [3], and according to a 2005 nationwide study in Japan, a high proportion of men in their 60s and 70s drink alcohol every day [4].

Several studies have reported that the amount of alcohol consumption decreases to some extent in the older compared to younger people [4–8]. Other studies have indicated that because the peak blood ethanol concentration of older people was higher than that of younger people, the prevalence of alcohol sensitivity may be increased in older people [9–11]. Older people

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may thus be particularly vulnerable to harmful effects of alcohol consumption [12, 13].

Because the percentage of older people (i.e., those aged ≥ 65 years) in Japan has reached 25 % of the population [14] and is projected to increase to 40 % in the near future, the prevention of alcohol abuse among older people is of great importance, as it is for younger people.

Among East Asians, high frequencies have been reported for the genetic polymorphisms rs1229984 in alcohol dehydrogenase 1B (*ADH1B*) and rs671 in aldehyde dehydrogenase 2 (*ALDH2*), which affect alcohol sensitivity and alcohol drinking behavior [15–18]. The slow-metabolizing *ADH1B**1/*1 carriers are less sensitive to alcohol [16] and have a higher risk for both alcoholism and esophageal cancer [19–21]. *ALDH2* genotypes have been strongly associated with alcohol sensitivity because the *ALDH2**2 enzyme encoded by the mutant *ALDH2**2 allele has no enzymatic activity [22]. Therefore, *ALDH2**2 allele carriers are likely to experience the ‘flushing response’ quickly after consuming a small amount of alcohol [23], and as a result, they tend to drink less alcohol [24]. The *ALDH2**1/*1, *1/*2 and *2/*2 genotypes have been associated with higher risks for alcoholism, esophageal cancer and myocardial infarction, respectively [19, 20, 25–27].

The relationships between the *ADH1B* and *ALDH2* genotypes and alcohol sensitivity and drinking behavior among older Asian people have not been clarified. In this study, we analyzed the relationships between these two genotypes with the flushing response, drinking behavior and problem drinking among older and younger people living in a rural area of Japan.

Materials and methods

Study subjects

The study population was male residents of two rural towns in western Japan who participated in annual health checkups conducted in 2012 and 2013. A total of 718 subjects aged 35–88 years were evaluated. The subjects were only the respondents who answered all of the items on the questionnaire described below. Written informed consent to participate in this study was obtained from each subject. This study was approved by the ethical committee for analytical research on the human genome of Wakayama Medical University (Approval No. 92).

Data collection

A self-administered questionnaire including items on alcohol sensitivity, drinking behavior, problem drinking, cigarette smoking, walking status and dietary habits was

used for data collection. The questionnaire was given to the subjects at the annual health checkup in either 2012 or 2013.

For the evaluation of the subjects’ alcohol sensitivity, the questionnaire included an item asking how often the subject experienced facial flushing after consuming a common dose of alcohol, with the following possible answers: always, sometimes, never, or unsure due to non-drinker.

The subjects’ drinking frequency was identified by his choice of the following: everyday, sometimes, or scarcely. The subjects’ usual alcohol consumption per day was examined in the questionnaire using a traditional Japanese drink unit called *gou*; one *gou* is equal to 180 ml of Japanese *sake* [Japanese rice wine, 15 % (v/v)], a medium bottle (500 ml) of beer [5.0 % (v/v)], half a glass (110 ml) of *shochu* [25 % (v/v)], which is Japanese distilled spirits made from barley, sweet potato, rice or any combination of these; double shots (60 ml) of whiskey [43 % (v/v)] and a quarter bottle (180 ml) of wine [14 % (v/v)]. All of these alcoholic beverages have roughly equivalent ethanol content (approx. 20–23 g). One unit of alcohol is equal to one *gou*, and in this study the amounts of alcohol consumption are described using the term ‘unit.’

We used the CAGE questionnaire to identify problem drinking [28]. The acronym “CAGE” stands for the following: Cutting down, Annoyance due to criticism, Guilty feeling, and Eye-openers. The CAGE questionnaire is comprised of the following four yes/no questions: ‘Have you ever felt you should cut down on your drinking?’; ‘Have people annoyed you by criticizing your drinking?’; ‘Have you ever felt bad or guilty about your drinking?’ and ‘Have you ever had a drink first thing in the morning to steady your nerves or get rid of a hangover (eye-opener)?’. We used the Japanese version of the CAGE questionnaire, which has been shown to have satisfactory reliability and validity. Each question is scored 0 for ‘no’ and 1 for ‘yes,’ and a total score of 2 or greater was considered a positive CAGE result, identifying the subject as a problem drinker.

Our questionnaire’s other items asked the subject to report his lifestyle habits: smoking (current, never or ex-smoker), walking status (≥ 1 h/day or < 1 h/day) and skipping breakfast (≥ 3 days/week or < 3 days/week). Current smoker is a person who has smoked a total of ≥ 100 cigarettes or smoked for ≥ 6 months and has been smoking till the last month.

Determinations of the *ADH1B* and *ALDH2* genotypes

The subjects’ dried whole blood samples were directly genotyped without DNA extraction by the TaqMan assay on an ABI 7300 Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA) [29, 30]. TaqMan[®] SNP

Genotyping Assays (Applied Biosystems) were used for the following (gene, SNP, assay ID): *ADH1B*, rs1229984, C_2688467_20; *ALDH2*, rs671, C_11703892_10.

Statistical analysis

Study subjects were categorized by their median age into two groups: the older (≥ 65 years old) and the younger (< 65 years old). We used the χ^2 test to compare the frequency of positive CAGE results among the lifestyle variables. The frequencies of facial flushing, drinking habits and positive CAGE results were compared among the carriers of *ADH1B*, *ALDH2* and a combination of these genotypes, using the Bonferroni-corrected χ^2 test. Then, to analyze the effects of these genotypes on ‘always’ facial flushing response, multivariate logistic regression analyses adjusted for body mass index (BMI), smoking, walking, skipping breakfast and usual alcohol consumption were used. In addition, interactions between the age group and the *ADH1B* genotypes were also tested using the interaction term of the age group \times the *ADH1B* genotypes adjusted for the variables mentioned above including the *ALDH2* and/or *ADH1B* genotypes. We used the Kruskal–Wallis test with Bonferroni correction to compare the alcohol consumption among the groups of subjects with the *ADH1B*, *ALDH2* and combination genotypes. Univariate and multivariate logistic regression analyses were used to examine the effects of age, BMI, smoking, walking, skipping breakfast, alcohol consumption, the *ADH1B* genotypes and the *ALDH2* genotypes on the risk for a positive CAGE result. All statistical analyses were carried out using SPSS software, version 20 (IBM, Chicago, IL). *P* values < 0.05 were considered significant.

Results

The characteristics of the study subjects according to their age bracket are presented in Table 1. The total subject population was 718 men aged 63.3 ± 10.8 years (mean \pm SD). Median (interquartile range) age was 64.0 (57.0, 70.0) years. The younger group was the 361 men aged 55.1 ± 8.2 years, and the older group was the 357 men aged 71.6 ± 5.4 years. A significant relationship between usual alcohol consumption and positive CAGE result (i.e., problem drinker) was seen in both groups. In the younger group only, a significant relationship between positive CAGE result and skipping breakfast was observed, as was a significant relationship between positive CAGE result and alcohol drinking frequency.

Table 2 summarizes the relationships of *ADH1B* and *ALDH2* genotypes with facial flushing frequency. The *ADH1B* and *ALDH2* genotype frequencies were within the

Hardy–Weinberg equilibrium ($\chi^2 = 1.21$, $P \geq 0.05$ and $\chi^2 = 0.047$, $P \geq 0.05$, respectively). In the older group, the frequency of ‘always’ facial flushing among the *ADH1B**1/*2 carriers ($n = 39$) was significantly lower than that among the *ADH1B**2/*2 carriers ($n = 92$). Adjusted odds ratio (OR) for this *ADH1B**1/*2 was 0.42 (95 % CI 0.24–0.72). Furthermore, the interaction between the age group and these *ADH1B* genotypes was significant ($P = 0.004$).

In the younger and older groups, the frequency of ‘always’ facial flushing in the *ALDH2**1/*1 carriers was significantly lower than those in the *ALDH2**1/*2 and *2/*2 carriers. In the younger group, the ORs for these *ALDH2**1/*2 and *2/*2 were 19.7 (95 % CI 10.8–35.7) and 10.1 (95 % CI 4.0–25.8), respectively. In the older group, the ORs for these *ALDH2**1/*2 and *2/*2 were 14.9 (95 % CI 7.8–28.7) and 14.1 (95 % CI 5.4–36.7), respectively.

Table 3 presents the relationships of the combination of *ADH1B* and *ALDH2* genotypes with facial flushing frequency. In the younger group with *ALDH2**1/*1, the frequency of ‘always’ facial flushing in the *ADH1B**1/*2 carriers tended to be higher compared with that in the *ADH1B**2/*2 carriers of the younger group ($P < 0.1$). Adjusted OR for this *ADH1B**1/*2 was 2.9 (95 % CI 1.2–7.0). The interaction between the age group and these *ADH1B* genotypes was significant ($P = 0.002$). In the younger group with *ALDH2**1/*2, the frequency of ‘always’ facial flushing in the *ADH1B**1/*1 carriers tended to be lower compared with that in *ADH1B**2/*2 carriers ($P < 0.1$). Adjusted OR for this *ADH1B**1/*1 was 0.19 (95 % CI 0.041–0.84).

In the older group, no significant differences in the frequency of ‘always’ facial flushing among the *ADH1B* genotypes were seen in each *ALDH2* genotype subgroup. In the older group with *ALDH2**1/*1, adjusted OR for *ADH1B**1/*2 was 0.30 (95 % CI 0.11–0.86, $P < 0.05$). In the older group with *ALDH2**1/*2, adjusted OR for *ADH1B**1/*2 was 0.49 (95 % CI 0.23–1.03, $P < 0.1$).

Table 4 presents the relationships of the *ADH1B* and *ALDH2* genotypes with usual alcohol consumption (units/day), frequency of drinking (≥ 1.0 unit/day and drink everyday) and frequency of positive CAGE result (i.e., score ≥ 2). No significant differences in these variables among the *ADH1B* genotypes were seen in the younger group, whereas in the older group, the frequency of drinking (i.e., everyday) in the *ADH1B**1/*2 carriers were significantly higher than those in the *ADH1B**2/*2 carriers. Adjusted OR for this *ADH1B**1/*2 was 1.7 (95 % CI 1.0–2.9, $P < 0.05$). However, the interaction between the age group and these *ADH1B* genotypes was not significant. The alcohol consumption (units/day) in the *ADH1B**1/*2 carrier tended to be higher compared with that in the

Table 1 Characteristics of the study subjects

Variables	All		Age <65 years		Age ≥65 years	
		CAGE ≥2 (%)		CAGE ≥2 (%)		CAGE ≥2 (%)
<i>n</i>	718	79 (11.0)	361	44 (12.2)	357	35 (9.8)
Age (years)	63.3 ± 10.8	–	55.1 ± 8.2	–	71.6 ± 5.4	–
BMI (kg/m ²)	23.0 ± 3.1	–	23.4 ± 3.3	–	22.7 ± 2.9	–
Smoking status (%)						
Never or ex-smoker	552 (76.9)	56 (10.1)	245 (67.9)	25 (10.2)	307 (86.0)	31 (10.1)
Current smoker	166 (23.1)	23 (13.9)	116 (32.1)	19 (16.4)	50 (14.0)	4 (8.0)
Walking status (%)						
≥1 h/day	340 (47.4)	42 (12.4)	184 (51.0)	19 (10.3)	194 (54.3)	17 (10.4)
<1 h/day	378 (52.6)	37 (9.8)	177 (49.0)	25 (14.1)	163 (45.7)	18 (9.3)
Skipping breakfast (%)						
<3 days/week	656 (91.4)	64 (9.8)	321 (88.9)	34 (10.6)	335 (93.8)	30 (9.0)
≥3 days/week	62 (8.6)	15 (24.2) ^c	40 (11.1)	10 (25.0) ^d	22 (6.2)	5 (22.7) ^e
Alcohol drinking frequency (%)						
Not every day ^a	312 (43.5)	15 (4.8)	165 (45.7)	4 (2.4)	147 (41.2)	11 (7.5)
Every day	406 (56.5)	64 (15.8) ^b	196 (54.3)	40 (20.4) ^b	210 (58.8)	24 (11.4)
Usual alcohol consumption (%)						
<1 unit/day	413 (57.5)	22 (5.3)	201 (55.7)	7 (3.5)	212 (59.4)	15 (7.1)
≥1 unit/day	305 (42.5)	57 (18.7) ^b	160 (44.3)	37 (23.1) ^b	145 (40.6)	20 (13.8) ^d

Values are mean ± SD, number of the subjects

^a Including non-drinkers, ^b $P < 0.001$, ^c $P < 0.01$, ^d $P < 0.05$, and ^e $P < 0.1$ vs. the other group

*ADH1B**2/*2 in the older group ($P < 0.1$). There were no significant differences in the frequency of drinking (≥1.0 unit/day) and positive CAGE results between the *ADH1B**1/*2 and *ADH1B**2/*2 carriers in the older group.

In both younger and older groups, the alcohol consumption (units/day) and frequency of drinking (≥1.0 unit/day and drink everyday) in the *ALDH2**1/*1 carriers were significantly larger and higher, respectively, than those of the *ALDH2**1/*2 and *2/*2 carriers. In addition, in both younger and older groups, the alcohol consumption and the frequency of drinking among the *ALDH2**1/*2 carriers were significantly larger and higher, respectively, than those in the *ALDH2**2/*2 carriers. In both younger and older groups, the frequency of positive CAGE results among the *ALDH2**1/*1 carriers was significantly higher than that in the *ALDH2**1/*2 carriers. In the younger group, the frequency of positive CAGE results among the *ALDH2**1/*1 carriers tended to be higher compared with that in the *ALDH2**2/*2 ($P < 0.1$).

Table 5 presents the relationships of the combination of *ADH1B* and *ALDH2* genotypes with the variables related to alcohol drinking habit and positive CAGE result frequency. In both the younger and older group, no significant differences in these variables among the *ADH1B* genotypes were seen in each *ALDH2* genotype subgroup.

Table 6 presents the relationships of the genetic and environmental factors with positive CAGE result. The

*ALDH2**1/*1 genotype, alcohol consumption (≥1.0 unit/day) and skipping breakfast (≥3 days/week) were positively associated with problem drinking in the multivariate analysis in all subjects. In the younger group, alcohol consumption (≥1.0 unit/day) and skipping breakfast (≥3 days/week) were positively associated with problem drinking in the multivariate analysis. In the older group, only the *ALDH2**1/*1 genotype was positively associated with problem drinking in the multivariate analysis. The *ADH1B**1/*1 genotype also tended to be positively associated with problem drinking in the older group in the multivariate analysis ($P = 0.080$).

Discussion

To our knowledge, our study is the first to analyze the relationships of *ADH1B* and *ALDH2* genotypes with alcohol sensitivity, drinking behavior and problem drinking among Asian older men aged 65 and over.

We compared alcohol sensitivity evaluated by an ‘always’ facial flushing response after drinking alcohol among *ADH1B* and *ALDH2* genotypes and the combination of these genotypes in this study. Our findings revealed that in the older men, the alcohol sensitivity of the *ADH1B**1/*2 carriers was significantly lower than that in the

Table 2 Relationships between the *ADH1B* and *ALDH2* genotypes with self-reported facial flushing with usual dose of alcohol

Gene	Genotype	Facial flushing							
		Age <65 years				Age ≥65 years			
		Total n (%)	Always (%)	Sometimes/never/ unsure ^a (%)	OR (95 % CI)	Total n (%)	Always (%)	Sometimes/never/ unsure ^a (%)	OR (95 % CI)
<i>ADH1B</i>	<i>*1/*1</i>	23 (6.4)	26.1	26.1/39.1/8.7	0.29 (0.10–0.90) ^g	18 (5.0)	33.3	22.2/38.9/5.6	0.34 (0.11–1.11)
	<i>*1/*2</i>	98 (27.1)	48.0	18.4/26.5/7.1	1.46 (0.79–2.71)	141 (39.5)	27.7 ^b	23.4/37.6/11.3	0.42 (0.24–0.72) ^{h, j}
	<i>*2/*2</i>	240 (66.5)	45.8	19.6/27.1/7.5	1.00 (Reference)	198 (55.5)	46.5	17.2/22.7/13.6	1.00 (Reference)
	<i>P</i> value		0.16				0.002		
<i>ALDH2</i>	<i>*1/*1</i>	179 (49.6)	14.5 ^{c, d}	27.4/53.6/4.5	1.00 (Reference)	176 (49.3)	13.1 ^{e, f}	28.4/56.2/2.3	1.0 (Reference)
	<i>*1/*2</i>	151 (41.8)	77.5	14.6/2.0/6.0	19.7 (10.8–35.7) ⁱ	147 (41.2)	63.3	14.3/3.4/19.0	14.9 (7.8–28.7) ⁱ
	<i>*2/*2</i>	31 (8.6)	64.5	0.0/3.2/32.3	10.1 (4.0–25.8) ⁱ	34 (9.5)	61.8	0.0/2.9/35.3	14.1 (5.4–36.7) ⁱ
	<i>P</i> value		<0.001				<0.001		

OR was adjusted for BMI, smoking, walking, skipping breakfast, alcohol consumption and *ALDH2* or *ADH1B* genotypes

^a Due to non-drinker

^b *P* < 0.01 vs. *ADH1B**2/*2

^c *P* < 0.001 vs. *ALDH2**1/*2

^d *P* < 0.001 vs. *ALDH2**2/*2

^e *P* < 0.001 vs. *ALDH2**1/*2

^f *P* < 0.001 vs. *ALDH2**2/*2

^g *P* < 0.05

^h *P* < 0.01

ⁱ *P* < 0.001

^j *P* < 0.01 (for interaction term of the age group × the *ADH1B* genotypes (*1/*2 and *2/*2))

*ADH1B**2/*2 carriers. The interaction between the age group and these *ADH1B* genotypes was also significant.

It has been reported that the frequency of flushing response due to drinking a small amount of alcohol was lower in *ADH1B**1/*1 carriers than in *ADH1B**2 allele carriers, and this result was remarkable in *ALDH2**1/*2 carriers [16]. A similar result was reported by Yokoyama et al. [31]. In an in vitro study, the *ADH1B**1/*2 and *2/*2 enzyme activity exhibited 100 and 200 times higher, respectively, than the *ADH1B**1/*1 enzyme activity [32]. The results of an ethanol patch test also suggested that the *ADH1B**1/*1 enzyme prevents the flushing response in the skin regardless of *ALDH2* genotypes [33, 34]. However, there have been no reports describing the difference in alcohol sensitivity between *ADH1B**1/*2 and *2/*2 carriers.

In Japanese men, Mizoi et al. reported that the *ADH1B* genotypes did not affect alcohol metabolism after intake of 0.4 g of ethanol per kg of body weight [35]. Kang et al.

recently reported a similar result [36]. In addition, Kang et al. also reported that the blood ethanol levels of *ADH1B**1/*2 carriers were lower than those of *ADH1B**2/*2 carriers after intake of 0.5 g of ethanol per kg of body weight regardless of the *ALDH2* genotype [36]. In older people, there is tendency to an elevation of blood ethanol levels with increasing age [37]. Thus, higher blood ethanol levels of older men might result in the difference in blood ethanol levels between *ADH1B**1/*2 and *2/*2 carriers as well as Kang's results. This difference in blood ethanol levels might partly account for the difference in alcohol sensitivity between *ADH1B**1/*2 and *2/*2 carriers in the older group in our present investigation.

In the younger group with *ALDH2**1/*1, the frequency of 'always' facial flushing in *ADH1B**1/*2 carriers tended to be higher compared with that in the *ADH1B**2/*2 carriers (*P* < 0.1). The interaction between the age group and these *ADH1B* genotypes was significant. However, a similar result was not seen in those with *ALDH2**1/*2. We

Table 3 Relationships between the combination of *ADH1B* and *ALDH2* genotypes with self-reported facial flushing with usual dose of alcohol

<i>ALDH2</i>	<i>ADH1B</i>	Facial flushing							
		Age <65 years				Age ≥65 years			
		Total <i>n</i>	Always (%)	Sometimes/never/ unsure ^a (%)	OR (95 % CI)	Total <i>n</i>	Always (%)	Sometimes/never/ unsure ^a (%)	OR (95 % CI)
*1/*1	*1/*1	11	0.0	18.2/81.2/0.0	–	7	0.0	0.0/100.0/0.0	–
	*1/*2	52	25.0 ^b	23.1/48.1/3.8	2.9 (1.19–6.97) ^d	78	7.7	26.9/62.8/2.6	0.30 (0.11–0.86) ^{d, f}
	*2/*2	116	11.2	30.2/53.4/5.2	1.00 (Reference)	91	18.7	31.9/47.3/2.2	1.00 (Reference)
	<i>P</i> value		0.024				0.062		
*1/*2	*1/*1	9	44.4 ^c	44.4/0.0/11.1	0.19 (0.04–0.84) ^d	9	44.4	44.4/0.0/11.1	0.26 (0.06–1.14)
	*1/*2	41	78.0	14.6/0.0/7.3	0.82 (0.33–2.04)	54	55.6	22.2/7.4/14.8	0.49 (0.23–1.03) ^c
	*2/*2	101	80.2	11.9/3.0/5.0	1.00 (Reference)	84	70.2	6.0/1.2/22.6	1.00 (Reference)
	<i>P</i> value		0.048				0.11		
*2/*2	*1/*1	3	66.7	0.0/0.0/33.3	0.45 (0.02–8.32)	2	100.0	0.0/0.0/0.0	–
	*1/*2	5	40.0	0.0/20.0/40.0	0.26 (0.03–2.63)	9	33.3	0.0/0.0/66.7	0.12 (0.02–0.92) ^d
	*2/*2	23	69.6	0.0/0.0/30.4	1.00 (Reference)	23	69.6	0.0/4.3/26.1	1.00 (Reference)
	<i>P</i> value		0.46				0.086		

OR was adjusted for BMI, smoking, walking, skipping breakfast and alcohol consumption

^a Due to non-drinker

^b $P < 0.1$ vs. *ALDH2**1/*1 & *ADH1B**2/*2

^c $P < 0.1$ vs. *ALDH2**1/*2 & *ADH1B**2/*2

^d $P < 0.05$

^e $P < 0.1$

^f $P < 0.01$ (for interaction term of the age group × the *ADH1B* genotypes (*1/*2 and *2/*2))

need to consider a possibility that this result is simply due to chance because of the small sample size. Thus, we need to interpret this result cautiously.

With regard to the effects of *ADH1B* genotypes on drinking behavior, we observed that the frequency of drinking (i.e., everyday) in the *ADH1B**1/*2 carriers was significantly higher than those in the *ADH1B**2/*2 carriers. However, the interaction between the age group and these *ADH1B* genotypes was not significant. The alcohol consumption in *ADH1B**1/*2 carriers also tended to be higher compared with that in the *ADH1B**2/*2. Though these results might reflect the difference in alcohol sensitivity between *ADH1B**1/*2 and *2/*2 carriers in the older group, further analyses are needed between the different age and *ADH1B* genotype groups. In this study, on the other hand, the effects of alcohol dehydrogenase 1C (*ADH1C*) polymorphism on drinking behavior were not investigated in our analyses. Several reports have indicated

that the *ADH1B* and *ADH1C* polymorphisms are in tight linkage disequilibrium [21, 38]. Therefore, the effects of *ADH1C* genotypes on drinking behavior have been obscure. In Japanese nonalcoholic men, however, the *ADH1C* genotypes significantly affected drinking behavior regardless of *ADH1B* and *ALDH2* genotypes [39]. The effects of *ADH1B* and *ALDH2* genotypes considering *ADH1C* genotypes on drinking behavior may also need to be further examined in future research.

Alcohol consumption decreases with advancing age [8], and the alcohol consumption in Japanese men ≥70 years old was shown to be decreased compared to that of Japanese men under 70 years old [4]. In our study, however, alcohol consumption and drinking frequency in the entire older group were not decreased remarkably compared with those in the entire younger group. This may be because the younger and older groups were not far apart in their mean age. The effects of aging on alcohol drinking behavior

Table 4 Relationships between the *ADH1B* and *ALDH2* genotypes with alcohol consumption, drinking habits and positive CAGE result

Gene	Genotype	Age <65 years				Age ≥65 years					
		Alcohol consumption (units/day)	Drinking habit <i>n</i> (%)		Total (<i>n</i>)	CAGE ≥2 <i>n</i> (%)	Alcohol consumption (units/day)	Drinking habit <i>n</i> (%)		CAGE ≥2 <i>n</i> (%)	Total (<i>n</i>)
			≥1.0 unit/day	Everyday				≥1.0 unit/day	Everyday		
<i>ADH1B</i>	*1/*1	0.50 (0.0–1.0)	9 (39.1)	12 (52.2)	23	3 (13.0)	0.75 (0.0–1.25)	9 (50.0)	11 (61.1)	4 (22.2)	18
	*1/*2	0.50 (0.25–1.0)	45 (45.9)	57 (58.2)	98	13 (13.3)	0.50 (0.25–1.0) ^a	64 (45.4)	95 (67.4) ^b	13 (9.2)	141
	*2/*2	0.50 (0.0–1.0)	106 (44.2)	127 (52.9)	240	28 (11.7)	0.50 (0.0–1.0)	72 (36.4)	104 (52.5)	18 (9.1)	198
	<i>P</i> value	0.69	0.84	0.67		0.91	0.040	0.18	0.023	0.19	
<i>ALDH2</i>	*1/*1	1.0 (0.50–2.0) ^{c, d}	113 (63.1) ^{c, d}	133 (74.3) ^{c, d}	179	34 (19.0) ^{e, f}	1.0 (0.50–1.0) ^{c, d}	111 (63.1) ^{c, d}	145 (82.4) ^{c, d}	27 (15.3) ^e	176
	*1/*2	0.25 (0.0–1.0) ^d	47 (31.1) ^d	63 (41.7) ^d	151	9 (6.0)	0.25 (0.0–0.50) ^d	34 (23.1) ^e	64 (43.5) ^d	7 (4.8)	147
	*2/*2	0.0 (0.0–0.0)	0 (0.0)	0 (0.0)	31	1 (3.2)	0.0 (0.0–0.0)	0 (0.0)	1 (2.9)	1 (2.9)	34
	<i>P</i> value	<0.001	<0.001	<0.001		<0.001	<0.001	<0.001	<0.001	<0.001	0.002

Values are median (interquartile range), number of the subjects

^a *P* < 0.1 vs. *ADH1B**2/*2

^b *P* < 0.05 vs. *ADH1B**2/*2

^c *P* < 0.001 vs. *ALDH2**1/*2

^d *P* < 0.001 vs. *ALDH2**2/*2

^e *P* < 0.01 vs. *ALDH2**1/*2

^f *P* < 0.1 vs. *ALDH2**2/*2

^g *P* < 0.01 vs. *ALDH2**2/*2

Table 5 Relationships between the combination of *ADH1B* and *ALDH2* genotypes with alcohol consumption, drinking habits and positive CAGE result

<i>ALDH2</i>	<i>ADH1B</i>	Age <65 years				Age ≥65 years			
		Alcohol consumption (units/day)		Drinking habit (%)		Alcohol consumption (units/day)		Drinking habit (%)	
				≥1.0 unit/day	Everyday			≥1.0 unit/day	Everyday
*1/*1	*1/*1	1.0 (0.25–1.0)	6 (54.5)	7 (63.6)	2 (18.2)	2.0 (0.5–3.0)	5 (71.4)	6 (85.7)	3 (42.9)
	*1/*2	1.0 (0.5–2.0)	34 (65.4)	38 (73.1)	10 (19.2)	1.0 (0.5–1.0)	50 (64.1)	66 (84.6)	12 (15.4)
	*2/*2	1.0 (0.5–2.0)	73 (62.9)	88 (75.9)	22 (19.0)	1.0 (0.5–1.0)	56 (61.5)	73 (80.2)	12 (13.2)
	<i>P</i> value	0.55	0.79	0.66	1.0	0.19	0.85	0.74	0.11
*1/*2	*1/*1	0.5 (0.0–1.0)	3 (33.3)	5 (55.6)	1 (11.1)	0.5 (0.0–1.0)	4 (44.4)	5 (55.6)	1 (11.1)
	*1/*2	0.25 (0.0–1.0)	11 (26.8)	19 (46.3)	3 (7.3)	0.5 (0.0–1.0)	14 (25.9)	28 (51.9)	1 (1.9)
	*2/*2	0.25 (0.0–1.0)	33 (32.7)	39 (38.6)	5 (5.0)	0.25 (0.0–0.5)	16 (19.0)	31 (36.9)	5 (6.0)
	<i>P</i> value	0.90	0.78	0.48	0.69	0.10	0.19	0.17	0.36
*2/*2	*1/*1	0.0 (0.0–0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0.0 (0.0–0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	*1/*2	0.0 (0.0–0.125)	0 (0.0)	0 (0.0)	0 (0.0)	0.0 (0.0–0.0)	0 (0.0)	1 (11.1)	0 (0.0)
	*2/*2	0.0 (0.0–0.0)	0 (0.0)	0 (0.0)	1 (4.3)	0.0 (0.0–0.0)	0 (0.0)	0 (0.0)	1 (4.3)
	<i>P</i> value	0.40	–	–	0.84	0.70	–	0.24	0.78

Values are median (interquartile range), number of the subjects

depending on the different genotypes should also be further examined in future research.

In this study, the alcohol sensitivity in the *ALDH2**1/*1 carriers was significantly lower than that in the *ALDH2**1/*2 and *2/*2 carriers in both the older and younger groups. The amount of alcohol consumption (units/day) and the frequency of drinking habits (≥1.0 unit/day and drink everyday) in the *ALDH2**1/*1 carriers were also significantly higher than those in the *ALDH2**1/*2 and *2/*2 carriers in both age groups. These results are in good agreement with those of several previous studies [40–42]. Thus, it seems that the *ALDH2* genotypes consistently and strongly affect alcohol sensitivity and drinking behavior regardless of age.

We observed that the *ADH1B**1/*1 genotype tended to be positively associated with problem drinking in the older group. The CAGE questionnaire, used as an index of problem drinking in our study, was developed by Ewing [28] and is one of the most widely used alcohol screening questionnaires as an easy-to-use tool to identify severe alcoholism [43, 44]. The *ADH1B**1/*1 genotype has been positively associated with problem drinking as assessed by the Kurihama Alcohol Screening Test (KAST) [42], and individuals with a high KAST score have a higher risk for alcohol dependence [25, 45]. The present results in the older people are in line with these previous reports.

The reason why the tendency for an association between *ADH1B**1/*1 and problem drinking was observed only in the older people remains to be elucidated. We observed no significant difference in the frequency of problem drinking among the *ADH1B* genotypes in the present study. On the other hand, the *ALDH2**1/*1 genotype was significantly associated with problem drinking in the older subjects of our study and tended to be associated in the younger subjects (*P* = 0.061), which is in agreement with previous reports [41, 42].

In older people, life-changing events such as loss or reduction of income, retirement, and family deaths affect drinking behavior [46, 47]. Additional studies of older people are thus needed to analyze the effects of both the *ALDH2* and *ADH1B* genotypes on problem drinking, because few such studies have been performed in Asian countries.

In all of our subjects and in the younger group, skipping breakfast was positively associated with problem drinking. Skipping breakfast tended to be positively associated with problem drinking in the older group. Alcohol consumption was associated with a positive CAGE result (Table 1), and thus symptoms such as a hangover caused by alcohol intake may affect the habit of having breakfast and result in skipping breakfast among individuals who drink large amounts of alcohol. Indeed, those who skip breakfast tend to drink larger amounts of alcohol [48]. Both alcohol

Table 6 Relationships between the *ADH1B* and *ALDH2* genotypes and environmental factors with positive CAGE result

Variable (high-risk group)	All				Age <65 years				Age ≥65 years			
	Univariate		Multivariate ^a		Univariate		Multivariate ^a		Univariate		Multivariate ^a	
	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI	OR	95 % CI
Age (≥65 years)	0.78	0.49–1.3	0.90	0.54–1.5	–	–	–	–	–	–	–	–
BMI (>23.0 kg/m ²)	0.90	0.56–1.4	1.0	0.62–1.7	0.74	0.39–1.4	1.1	0.57–2.3	1.1	0.55–2.2	0.90	0.43–1.9
Smoking (current smoker)	1.4	0.85–2.4	1.2	0.67–2.1	1.7	0.91–3.3	1.6	0.80–3.2	0.77	0.26–2.3	0.58	0.18–1.9
Walk (<1 h/day)	1.3	0.81–2.1	1.3	0.77–2.1	1.4	0.76–2.7	1.4	0.72–2.8	1.1	0.57–2.3	1.2	0.56–2.4
Skipping breakfast (≥3 days/week)	3.0	1.6–5.6	2.7	1.3–5.3	2.8	1.3–6.3	2.4	1.0–5.9	3.0	1.0–8.7	3.0	0.93–9.9
Alcohol consumption (≥1.0 unit/day)	4.1	2.4–6.9	2.9	1.6–5.0	8.3	3.6–19.3	6.6	2.7–16.1	2.1	1.0–4.3	1.3	0.58–2.8
<i>ADH1B</i> genotype (*1/*1)	1.7	0.74–4.0	1.8	0.73–4.5	1.1	0.31–3.8	1.3	0.32–5.1	2.8	0.88–9.2	3.2	0.87–12.1
<i>ALDH2</i> genotype (*1/*1)	4.0	2.3–6.9	2.6	1.5–4.8	4.0	1.9–8.4	2.2	0.97–4.8	3.9	1.7–8.9	3.8	1.5–9.3

^a Adjusted for all variables

consumption and drinking frequency were significantly associated with skipping breakfast [49]. Further prospective studies would be useful to clarify the relationship between having breakfast and problem drinking.

This study has several limitations. First, this was a cross-sectional study. However, the *ADH1B* and *ALDH2* genotypes would have affected the subjects' drinking behavior since the subjects started drinking. It is thus possible that the relationships between these genotypes and drinking behavior are a causal association. A second limitation is the small numbers of subjects with the *ADH1B**1/*1 or *ALDH2**2/*2 allele. Studies with larger numbers of subjects are needed to evaluate the effects of *ADH1B**1/*1 or *ALDH2**2/*2 on alcohol sensitivity, drinking behavior and problem drinking. A third limitation concerns the subjects whose questionnaire response with regard to facial flushing was 'unsure due to non-drinker.' These subjects were not considered in our analysis of the relationships between the genotypes and facial flushing. Judging from their response that they are been non-drinkers, it is possible or even likely that most of them are always-flushers, but it is difficult to confirm the presence of facial flushing in these subjects.

In conclusion, this is the first report of a significant difference in alcohol sensitivity between *ADH1B**1/*2 and *ADH1B**2/*2 carriers among Asian older men. Further research to evaluate the effect of age, drinking behavior and the genotypes on individuals' health is greatly desired, especially in Asian countries.

Acknowledgments This study was conducted with a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan (25460811 and 25350894) and with a Grant-in-Aid from the Japan Agency for Medical Research and Development, AMED (15ck0106102h0102).

Compliance with ethical standards

Conflict of interest The authors have declared that no conflicts of interest exist.

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