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ORIGINAL ARTICLE

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Association of alcohol dehydrogenase 2*1 allele with liver damage and insulin concentration in the Japanese

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Abstract The Japanese have a polymorphism in the alcohol dehydrogenase 2 gene (*ADH2*). The alleles of *ADH2* (*ADH2*1* and *ADH2*2*) encode more active and less active forms for ethanol metabolism, respectively. We examined whether liver damage and the insulin–glucose axis vary according to *ADH2* genotype in the Japanese. The 2,232 subjects (1,126 men and 1,106 women) were recruited from a population-based prospective cohort study. Clinical evaluations including alcohol consumption, percentage of alcohol drinkers, plasma glucose, HbA1c, insulin, AST, ALT, γ-GTP, and prevalence of diabetes were compared among the *ADH2* genotypes. The percentage of drinkers, alcohol consumption, AST, ALT, and γ-GTP were higher in group *ADH2*1/1* than in group *ADH2*1/2* or *ADH2*2/2* (all $P<0.05$). Hence, *ADH2*1/1* is associated with excess alcohol intake and liver disorders. However, the prevalence of diabetes did not differ among the three groups. For the glucose–insulin axis, we examined subjects who did not receive insulin therapy or oral anti-diabetes medication. While amounts of alcohol consumed and glucose levels were nearly the same between *ADH*1/2* and *ADH2*2/2*, insulin concentrations were lower in *ADH2*2/1* than in *ADH2*2/2* ($P<0.05$ in men). This finding suggests that the *ADH2*1* allele is associated with a lower insulin concentration when alcohol intake is light or moderate. It also suggests that the genetic effect of *ADH2*1* plays an important role in alcohol drinking behavior and in the occurrence of liver injury, but the effect is so mild that it does not influence the glucose–insulin axis or prevalence of diabetes.

Keywords Alcohol dehydrogenase 2 · ADH2 · Diabetes · Insulin resistance · Liver dysfunction · Alcohol · Prospective cohort study

Abbreviations: ALDH: Aldehyde dehydrogenase · ADH: Alcohol dehydrogenase · PCR: Polymerase chain reaction

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Introduction

A reduced incidence of type 2 diabetes has been observed among drinkers in several large prospective studies. Conigrave et al (2001) reported a 12-year prospective study in a cohort of 46,892 US male health professionals, in which 1,571 new cases of type 2 diabetes were reported. The frequency of alcohol consumption was inversely associated with diabetes. Hu et al (2001) reported a large cohort study of 84,941 female nurses from 1980 to 1996, in which abstinence from alcohol use was associated with a significantly increased risk of diabetes. In contrast, other studies (Holbrook et al 1990) have shown an increased risk of diabetes among a proportion of subjects in the top alcohol consumption category. In Japanese men, Tsumura et al (1999) reported that heavy drinking is associated with an increased risk of type 2 diabetes, while moderate drinking is associated with a decreased risk of type 2 diabetes, showing a U-shaped relationship.

The genotypes involved in ethanol metabolism are now known to be associated not only with drinking, but also with longevity and oxidative stress parameters (Ohsawa et al 2003). In Japanese, the pharmacokinetics of alcohol metabolism have been well studied. Alcohol dehydrogenase (ADH) is one of the key enzymes in alcohol metabolism. Class I ADH isoenzymes, encoded

by *ADH1*, *ADH2* and *ADH3*, form dimers among the isoenzymes and oxidize ethanol and other small aliphatic alcohols (Borson et al 1988). About 85% of the Japanese population are carriers of the β2-subunit encoded by the *ADH2*2* allele, while isoenzymes with the β2-subunit have been found in only 5% or less of Europeans and white Americans. The β1- and β2-subunits differ by only one amino acid residue: Arg-47 in the NAD(H) pyrophosphate-binding site is substituted with His-47 in the β2-subunit. ADH2 functions as a dimer and the $\beta2\beta2$ dimer exhibits about 100 times more catalytic activity for ethanol oxidation than the $\beta1\beta1$ dimer at physiological pH (Borson et al 1988), whereas the $\beta1\beta2$ heterodimer exhibits nearly the same activity as the $\beta1\beta1$ homodimer. Thus, relative enzymatic activities of *ADH2*1/1*:*ADH2*1/2*:*ADH2*2/2* can be estimated as 1:26:100 if a dimer were to form between the subunits of ADH2*1 and ADH2*2 (Borson et al 1988; Yoshida et al 1981).

Several studies (Higuchi et al 1996; Yamauchi et al 2001) have reported that the *ADH2* genotype is associated with excess alcohol intake and alcohol-related disorders in the Japanese population. We have previously reported that the *ADH2* genotype affected LDL-cholesterol levels and the occurrence of cerebral infarction in a community-dwelling Japanese population (Suzuki et al 2004). We therefore examined whether the glucose–insulin axis or prevalence of diabetes is associated with the *ADH2* genotype in the same Japanese population.

Research design and methods

The National Institute for Longevity Sciences–Longitudinal Study of Aging (NILS–LSA), a population-based prospective cohort study of aging and age-related diseases, was begun in 1997 (Ohsawa et al 2003; Shimokata et al 2000; Yamada et al 2002). All participants were independent residents of the Aichi prefecture in Japan. Residents aged 40–79 years old were randomly selected from the register in co-operation with the local government.

The area of study is located in the south of Nagoya City. It is a commuter town and contains an industrial area belonging to the Toyota group, but it has many orchards and farms, so it has both urban and rural characteristics. This area is geographically located in the center of Japan, and its climate is average for Japan. We examined a representative sample of the area's population via a national postal questionnaire of prefecture-stratified random samples of 3,000 households from all prefectures in Japan, and previously showed that the lifestyle of people in this area was the most typical of all areas in Japan.

The sample consisted of 2,232 subjects (1,126 men and 1,106 women) who were randomly recruited. We refer to them as "subjects-1." Subjects-1 was stratified by both age and sex. Randomly selected men and women were invited, by mail, to attend an explanatory meeting. At the meeting, the procedures for each examination and follow-up schedule were fully explained. Written informed consent to the entire procedure was obtained from each participant. Participants in the present study were recruited from subjects examined in 1997–1999. The study protocol was approved by the Committee on the Ethics of Human Research of National Chubu Hospital and the National Institute for Longevity Sciences.

Descriptions of the physical examinations performed have been published before (Ohsawa et al 2003; Shimokata et al 2000; Yamada et al 2002). In brief, lifestyle, medical history and prescribed drugs were examined by questionnaire. Anthropometric measurements were taken by a physician. A drinker is defined as a subject who has drunk more than 5 g of alcohol on average per day during the past year. Amounts of alcohol consumed were carefully examined by taking pictures before and after drinking as well as with questionnaires. The percentage of non-smokers to smokers was also noted.

Venous blood was collected early in the morning after at least 12 h fasting. The mean of two determinations of blood chemistry data was obtained for each participant. Clinical evaluations included gender, age, height, body-mass index, smoker status, alcohol consumption, percentage of alcohol drinkers, and blood chemistry (fasting plasma glucose (FPG), HbA1c, insulin, AST, ALT, and γ-GTP levels). Diagnosis of diabetes was based on medical records, or it was defined as a FPG concentration greater than 126 mg/dl or an HbA1c of more than 6.5%, and/or if medication was taken to lower the blood glucose level. Namely, not all subjects whose FPG level was greater than 110 mg/dl did not receive the 75 g oral glucose tolerance test according to the criteria of the Japan Diabetes Society. In the analysis of glucose–insulin associated parameters, to exclude the effect of medications, the diabetic patients who received insulin therapy or oral medications for diabetes were excluded from subjects-1, and the remaining subjects were defined as the "subjects-2" group.

Genotyping of *ADH2*

Samples of DNA were isolated from peripheral blood cells. Genotypes were determined with a fluorescence-based allele-specific DNA primer-probe assay system (Toyobo Gene Analysis, Tsuruga, Japan). To determine the genotype with the G214A substitution (Arg-47-His), the polymorphic region of *ADH2* was amplified by polymerase chain reaction (PCR) with an antisense primer labeled at the 5′ end with biotin (5′-GATGGTGGCTGTAGGAATCTG-3′) and a G allele-specific sense primer labeled with FITC (5′-CCACGTGGTCATCTGTNCG-3′) or A allele-specific sense primer labeled with Texas red (5′-AACCACGTGGTCATCTGTNTG-3′).

Table 1 Comparison of parameters among three groups of men (subjects-1), divided according to *ADH2* genotype. Right columns indicate *P*-values of statistical differences between two groups

Variables	Men			*P*-value		
ADH genotype	2/2	1/2	1/1	2/2 vs. 1/2	2/2 vs. 1/1	1/2 vs. 1/1
Subjects-1 $n=$	689	378	59			
Age (years)	59.5 ± 0.4	58.9 ± 0.6	58.0 ± 1.4	n.s.	n.s.	n.s.
Height (cm)	164.4 ± 0.2	164.7 ± 0.3	164.6 ± 0.8	n.s.	n.s.	n.s.
BMI	23.0 ± 0.1	22.8 ± 0.1	22.9 ± 0.4	n.s.	n.s.	n.s.
Smoking (%)	61/39	63/37	63/37	n.s.	n.s.	n.s.
Alcohol (g/day)	28.8 ± 1.4	29.5 ± 1.9	44.5 ± 4.8	n.s.	0.0049**	0.0102**
Drinkers (%)	67.0	67.1	85.5	($P < 0.0175$)		
AST (IU/l)	26.6 ± 0.7	26.6 ± 0.9	33.6 ± 2.3	n.s.	0.0038**	0.0049**
ALT (IU/l)	27.1 ± 0.9	26.8 ± 1.2	34.3 ± 3.0	n.s.	0.02*	0.02*
γ-GTP (IU/l)	58.2 ± 3.1	57.3 ± 4.1	80.3 ± 10.5	n.s.	0.04*	0.04*
Diabetics (%)	13.3	13.3	13.6	n.s.	n.s.	n.s.

AST 2/2 ± 1/2 vs. 1/1, $P < 0.0033$; ALT 2/2 ± 1/2 vs. 1/1, $P < 0.02$; γ-GTP 2/2 ± 1/2 vs. 1/1, $P < 0.04$; drinkers 2/2 ± 1/2 vs. 1/1, $P < 0.005$; alcohol 2/2 ± 1/2 vs. 1/1, $P < 0.005$
*$P < 0.05$
**$P < 0.01$

Statistical analysis

Data are presented as means ± SE. The statistical significance of any difference in mean values and frequencies was determined with the Student's *t*-test or the Tukey–Kramer test. We used a one-way analysis of variance to test for overall differences among multiple groups, and the Fisher LSD post hoc test to identify which group differences accounted for the significant *P*-value. The significance of deviation from Hardy–Weinberg equilibrium was analyzed using the chi-square test. A *P*-value of < 0.05 was considered statistically significant.

Results

Influence of *ADH2* genotypes on drinking behavior and liver function

Among the 2,232 subjects, 1,355 (men 689, women 666) had the *ADH2*2/2* genotype, 759 (men 378, women 381) had the *ADH2*2/1* genotype, and 118 (men 59, women 59) had the *ADH2*1/1* genotype. The *ADH2*2/2*, *ADH2*2/1*, and *ADH2*1/1* genotypes were in Hardy–Weinberg equilibrium. There was no gender difference.

First, we compared the percentage of drinkers dependent upon *ADH2* genotype. The percentage of drinkers was significantly higher in both men and women in the *ADH2*1/1* group, showing overall differences among the groups (Table 1 and Fig. 1a). The difference was statistically significant according to the Fisher LSD post hoc test in men ($P < 0.0175$), women ($P < 0.0166$), and total subjects-1 ($P < 0.0033$) (Table 1). Moreover, amounts of alcohol consumed were much higher in the *ADH2*1/1* group than the other *ADH2* groups in men and total subjects-1 ($P < 0.01$ in *ADH2*2/2* vs. *ADH2*1/1* and $P < 0.05$ in *ADH2*1/2* vs. *ADH2*1/1*) (Tables 1, 3 and Fig. 1b). On the other hand, no significant difference in alcohol consumption among *ADH2*1/1* and the other groups was found in women, probably because much less alcohol was consumed by women than men (Table 2 and Fig. 1b). For smoking (percentage of non-smokers to smokers), there was no difference according *ADH2* genotype in men and in women.

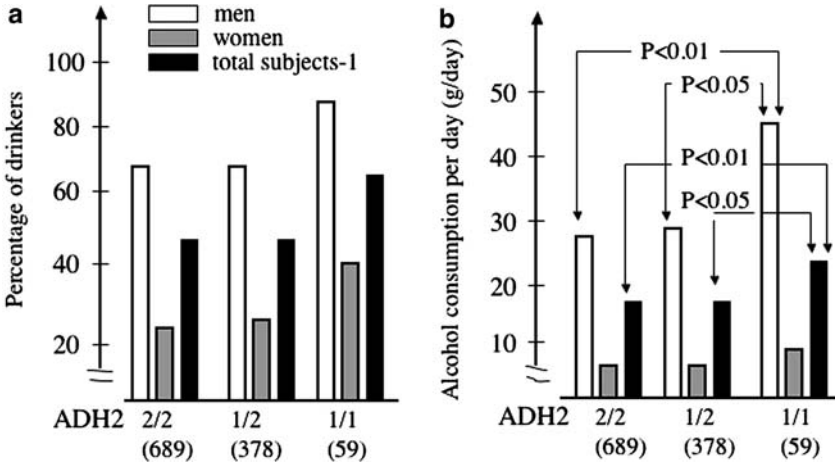

Fig. 1a, b Correlation of *ADH2* genotype with alcohol drinking behavior. **a** Percentage of drinkers in three groups based on *ADH2* genotype. Values in parentheses indicate the total number of subjects (*white bars* men, *gray bars* women, and *black bars* total subjects). **b** Average amounts of alcohol consumed per day. Subjects in the *ADH2*1/1* group drink more alcohol than those in the *ADH2*2/2* and *ADH2*1/2* groups

Table 2 Comparison of parameters among three groups of women (in subjects-1), divided according to the three *ADH2* genotypes. Right columns indicate *P*-value of statistical difference between each two group

Variables	Women			*P*-value		
ADH genotype	2/2	1/2	1/1	2/2 vs. 1/2	2/2 vs. 1/1	1/2 vs. 1/1
Subjects-1 *n* =	666	381	59			
Age (years)	59.4 ± 0.4	59.1 ± 0.6	60.0 ± 1.4	n.s.	n.s.	n.s.
Height (cm)	151.3 ± 0.2	151.1 ± 0.3	151.1 ± 0.8	n.s.	n.s.	n.s.
BMI	23.0 ± 0.1	22.7 ± 0.2	23.1 ± 0.4	n.s.	n.s.	n.s.
Smoking (%)	93/7	93/7	92/8	n.s.	n.s.	n.s.
Alcohol (g/day)	5.2 ± 0.6	5.4 ± 0.8	6.4 ± 2.0	n.s.	n.s.	n.s.
Drinkers (%)	22.9	25.5	39.7	< 0.0166		
AST (IU/l)	24.5 ± 0.6	23.5 ± 0.7	23.3 ± 1.8	n.s.	n.s.	n.s.
ALT (IU/l)	21.2 ± 0.8	20.1 ± 1.0	18.9 ± 2.5	n.s.	n.s.	n.s.
γ-GTP (IU/l)	27.9 ± 1.1	28.5 ± 1.4	29.4 ± 3.6	n.s.	n.s.	n.s.
Diabetics (%)	9.16	10.5	6.78	n.s.	n.s.	n.s.

Drinkers 2/2 ± 1/2 vs. 1/1, $P < 0.01$

Next, we compared blood parameters of liver function, namely AST, ALT, and γ-GTP activities. In men, levels were significantly higher in the *ADH2*1/1* group than the other two *ADH2* groups (Table 1, AST; $P < 0.01$ in *ADH2*2/2* vs. *ADH2*1/1* and $P < 0.01$ in *ADH2*1/2* vs. *ADH2*1/1*. ALT; $P < 0.05$ in *ADH2*2/2* vs. *ADH2*1/1* and $P < 0.05$ in *ADH2*1/2* vs. *ADH2*1/1*. γ-GTP; $P < 0.05$ in *ADH2*2/2* vs. *ADH2*1/1* and $P < 0.05$ in *ADH2*1/2* vs. *ADH2*1/1*), indicating that more alcohol intake in the *ADH2*1/1* group causes damage to the liver. On the other hand, no significant difference was found in women (Table 2); nevertheless the *ADH2*1/1* group consumed more alcohol than the other groups, probably because women drink less than men.

In subjects-1, the percentage of those with diabetes was compared among the three ADH2 genotypic groups. However, there was no statistical difference in the prevalence of diabetes among the three groups (men; *ADH2*2/2*:13.3%, *ADH2*1/2*:13.3%, and *ADH2*1/1*:13.6%, women; *ADH2*2/2*:9.2%, *ADH2*1/2*:10.5%, and *ADH2*1/1*:6.8%, total subjects-1; *ADH2*2/2*:11.2%, *ADH2*1/2*:11.9%, and *ADH2*1/1*:10.2%) (Tables 1, 2, 3).

Influence of *ADH2* genotype on fasting insulin concentration

We tried to clarify the correlation of insulin concentration with *ADH2* genotype. To exclude the effect of medication, subjects were limited to those (subjects-2) not treated with insulin therapy and/or with oral medications for diabetes. Although habits or behaviors generally depend upon genetic factors, we would like to distinguish the genetic effects from the secondary results of alcohol consumption. Since the frequency of drinking and the amountsof alcohol consumed were the same in the *ADH2*1/2* and *ADH2*2/2* groups (Fig. 1 and Tables 1, 2, 3), we compared fasting insulin concentrations between these two groups. Insulin levels were lower in the *ADH2*1/2* than *ADH2*2/2* group in total subjects-2 ($P < 0.02$). In men, insulin levels were lower in the *ADH2*1/2* than *ADH2*2/2* group ($P < 0.05$), while in

Table 3 Comparison of parameters among three groups of total subjects-1 divided according to *ADH2* genotype. Right columns indicate *P*-values of statistical differences between two groups

Variables	Total (men + women)			*P*-value		
ADH genotype	2/2	1/2	1/1	2/2 vs. 1/2	2/2 vs. 1/1	1/2 vs. 1/1
Subjects-1 *n* =	1,352	756	118			
Age (years)	59.4 ± 0.3	59.0 ± 0.4	59.0 ± 1.0	n.s.	n.s.	n.s.
Height (cm)	158.2 ± 0.2	158.1 ± 0.3	156.8 ± 0.8	n.s.	n.s.	n.s.
BMI	23.0 ± 0.1	22.7 ± 0.1	23.1 ± 0.3	n.s.	n.s.	n.s.
Smoking (%)	77/23	78/22	78/22	n.s.	n.s.	n.s.
Alcohol (g/day)	17.2 ± 0.9	17.6 ± 1.1	24.9 ± 2.8	n.s.	0.0089**	0.0158**
Drinkers (%)	45.4	45.6	62.0	< 0.0033		
AST (IU/l)	25.6 ± 0.4	25.0 ± 0.6	28.3 ± 1.4	n.s.	n.s.	0.0383**
ALT (IU/l)	24.2 ± 0.6	23.4 ± 0.8	26.5 ± 2.0	n.s.	n.s.	n.s.
γ-GTP (IU/l)	43.3 ± 1.7	42.9 ± 2.3	54.4 ± 5.7	n.s.	n.s.	n.s.
Diabetics (%)	11.2%	11.9%	10.2%	n.s.	n.s.	n.s.

Drinkers 2/2 ± 1/2 vs. 1/1, $P < 0.001$; alcohol 2/2 ± 1/2 vs. 1/1, $P < 0.01$
* $P < 0.05$
** $P < 0.01$

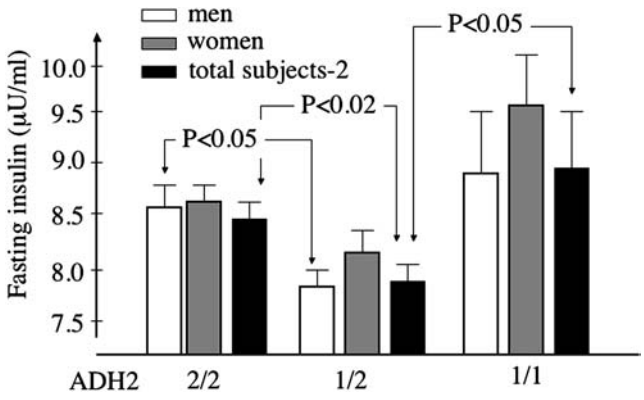

Fig. 2 Correlation of *ADH2* genotype with fasting insulin concentration in subject-2 group. Fasting insulin concentration (μU/ml): a significant difference was found between *ADH2*2/2* and *ADH2*1/2* in men (8.56 ± 0.24 vs. 7.77 ± 0.32, $P < 0.05$), and between *ADH2*2/2* and *ADH2*1/2* in total subjects-2 (8.44 ± 0.15 vs. 7.84 ± 0.20, $P < 0.02$). A significant difference was found between *ADH2*1/2* and *ADH2*1/1* in total subjects-2 (7.84 ± 0.20 vs. 8.92 ± 0.50, $P < 0.05$)

women, the *ADH2*1/2* group tended to have lower insulin concentrations (Fig. 2 and Table 4). This suggests that the *ADH2*1* allele has a lowering effect on the concentration of insulin.

Next, we compared the concentration of insulin between *ADH2*1/2* and *ADH2*1/1*. The concentration tended to be higher in the *ADH2*1/1* group than the *ADH2*1/2* group in men, women and total subjects-2, but a significant difference was only found in total subjects-2 (insulin, *ADH2*1/2*: 7.84 ± 0.20 μU/ml, *ADH2*1/1*: 8.92 ± 0.50 μU/ml, $P < 0.05$, Table 3 and Fig. 2). Because the *ADH2*1/1* group is small, the difference may have become statistically insignificant in men or in women.

In subjects-2, while the difference was statistically insignificant, the average level of HbA1c tended to be lower in the *ADH2*1/2* group than the *ADH2*1/1* or *ADH2*2/2* group (Fig. 3 and Table 4). For instance, in total subjects-2, HbA1c was $5.20 \pm 0.02\%$, $5.17 \pm 0.02\%$, and $5.23 \pm 0.05\%$, respectively, in the *ADH2*2/2*, *ADH2*1/2*, and *ADH2*1/1* groups. Therefore, low insulin levels in the *ADH2*1/2* group seem to parallel low HbA1c levels, showing a U-shaped relationship with *ADH2* genotype as in Figs. 2 and 3.

Discussion

By examining the correlation between *ADH2* genotype and drinking behavior, we confirmed the previous observation that *ADH2* genotype influences the amount of alcohol consumed in a Japanese population (Higuchi et al 1996). In addition to alcohol consumption and percentage of drinkers, men from the *ADH2*1/1* group had the highest levels of AST, ALT, and γ-GTP, suggesting that they drink so much alcohol that their livers become damaged. This coincides with the observation of Tanaka et al (1996), supporting the idea that *ADH2* polymorphisms play an important role in alcoholic liver diseases.

In terms of the mechanism involved, since carriers of *ADH2*1/1* have less enzymatic activity for ethanol than carriers of *ADH2*2/1* or *ADH2*2/2*, the slow rate of ethanol clearance could damage the liver, but this is unlikely because ethanol is less toxic than acetaldehyde. Alternatively, it is possible that the slow rate of ethanol clearance protects the subjects from the uncomfortable feeling caused by acetaldehyde, thereby causing them to drink too much alcohol and leading to liver damage.

Interestingly, concentrations of insulin were higher in the *ADH2*1/1* than the *ADH2*1/2* group. Onishi et al (2003) reported that excess alcohol intake can induce insulin resistance with enhanced PI3-kinase activation. Therefore, in the *ADH2*1/1* group, excess alcohol intake may cause insulin resistance, resulting in hyperinsulinemia. Otherwise, some liver dysfunction caused by excess alcohol intake may cause a high glucose output from liver, thereby inducing hyperinsulinemia.

Table 4 Comparison of glucose–insulin axis parameters among three groups of subjects-2 divided according to the three *ADH2* genotypes

Variables				*P*-value		
ADH genotype	2/2	1/2	1/1	2/2 vs. 1/2	2/2 vs. 1/1	1/2 vs. 1/1
Men $n=$	640	346	57			
FPG (mg/dl)	103.3 ± 0.7	102.6 ± 0.9	103.3 ± 2.2	n.s.	n.s.	n.s.
HbA1c (%)	5.24 ± 0.02	5.22 ± 0.03	5.27 ± 0.08	n.s.	n.s.	n.s.
Insulin (μU/ml)	8.46 ± 0.22	7.69 ± 0.31	8.47 ± 0.75	0.0452*	n.s.	n.s.
Women $n=$	623	354	57			
FPG (mg/dl)	98.6 ± 0.6	99.3 ± 0.8	99.2 ± 2.1	n.s.	n.s.	n.s.
HbA1c (%)	5.15 ± 0.02	5.11 ± 0.03	5.17 ± 0.06	n.s.	n.s.	n.s.
Insulin (μU/ml)	8.42 ± 0.19	8.00 ± 0.26	9.36 ± 0.65	n.s.	n.s.	n.s.
Total $n=$	1,263	700	114			
FPG (mg/dl)	101.0 ± 0.46	101.0 ± 0.6	101.2 ± 1.5	n.s.	n.s.	n.s.
HbA1c (%)	5.20 ± 0.02	5.17 ± 0.02	5.23 ± 0.05	n.s.	n.s.	n.s.
Insulin (μU/ml)	8.44 ± 0.15	7.84 ± 0.20	8.92 ± 0.50	0.018*	n.s.	0.045*

*$P < 0.05$
**$P < 0.01$

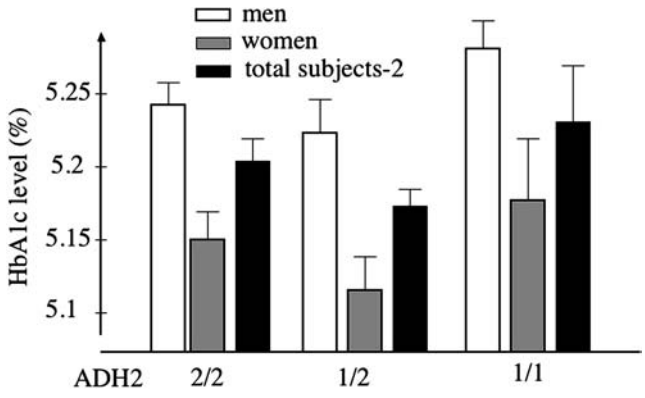

Fig. 3 Correlation of *ADH2* genotype with HbA1c level in subject-2. A significant difference was not found between the three groups. However, the HbA1c level showed a U-shaped relationship as if correlated to the insulin level

Next, we tried to focus on the *ADH2*'s genetic effects on the insulin–glucose axis. Because alcohol produces complicated effects, it is generally difficult to distinguish the genetic effects from the influence of alcohol drinking behavior. Interestingly, alcohol consumption or percentage of drinkers did not differ between the *ADH2*1/2* and *ADH2*2/2* groups (Tables 1, 2, 3 and Fig. 1a, b). This enabled us to compare the insulin concentration, dependent upon the difference in ADH2 activity itself, based on the *ADH2* polymorphism, almost independently from alcohol intake. Among subjects-2, we found that fasting insulin concentrations were significantly lower in the men and total subjects-2 with the *ADH2*1/2* genotype than those with the *ADH2*2/2* genotype (Table 4 and Fig. 2). A similar trend was seen in women, suggesting that this trend is reproducible irrespective of gender.

Thus, this study suggests that *ADH2*1* has a biphasic effect on the insulin concentration, a lowering effect with *ADH2*1/2*, and a raising effect with *ADH2*1/1* on excess alcohol intake. Interestingly, the average levels of HbA1c in subjects-2 tended to be lower in the *ADH2*1/2* group than the *ADH2*1/1* or *ADH2*2/2* groups. These two parameters seem to exhibit a U-shaped relationship (Figs. 2, 3). In nondiabetic subjects, a low insulin concentration together with a low HbA1c level usually coincides with low insulin resistance. Therefore, the above relationship suggests that light-to-moderate drinkers with the *ADH2*1* allele are likely to have reduced insulin resistance. Interestingly, this coincides with numerous other observations (Conigrave et al 2001; Hu et al 2001; Tsumura et al 1999) in terms of the notion that light drinking could benefit glucose tolerance.

Alcohol dehydrogenase catalyzed the first step in the metabolism of ethanol but has a wide range of substrates, including both aliphatic and aromatic alcohols, aldehydes, sterols, and ω-hydroxy fatty acids. We previously reported that, in the same population study, the *ADH2*1* allele is associated with increased levels of LDL-cholesterol and high blood pressure, and an increased risk of cerebral infarction (Suzuki et al 2004). The concentration of insulin or resistance to insulin could be affected by sex hormones, sex hormone-binding globulin or obesity (Falkner et al 1999; Collison et al 2000). Therefore, as another possibility, the interaction of the *ADH2*1* allele with several hormones associated with sex or lipids may decrease the insulin resistance in target tissues (Harada et al 1998).

However, in this study, the prevalence of diabetes did not differ among the three *ADH2* genotypes in subjects-1. Therefore, the effect of *ADH2* genotype on insulin resistance may be so mild or complex that it did not influence the prevalence of diabetes in the community-dwelling Japanese population. Alternatively, since all of the subjects whose FPG levels were higher than 110 mg/dl were not confirmed by the oral glucose tolerance test, if the subjects who had postprandial hyperglycemia had been included in subject-1, the result could have been different. To clarify this, a further study will be needed.

It is well known that drinking behavior is influenced more by *ALDH2* (aldehyde dehydrogenase 2) genotype than *ADH2* genotype (Higuchi et al 1996). However, although a similar investigation was performed on the correlation between *ALDH2* genotypes and their phenotype, no genetic effect of *ALDH2* was found in insulin–glucose axis and liver dysfunction (Ohsawa et al 2003). Thus, amounts of alcohol consumed would not simply depend upon insulin level.

In conclusion, this is the first paper to propose an effect of *ADH2* genotype on insulin concentrations in the Japanese. The effect seems small, although it was statistically significant due to the large number of subjects. The effect is possibly too small to have a significant bearing on the prevalence of diabetes. However, this finding provides several insights into the complex relationship between alcohol metabolism, genetic background, change in alcohol drinking behavior, the insulin–glucose axis, and the prevalence of diabetes and liver dysfunction.

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