Original Article

Epidemiologic Study of the Association of Low- K_m Mitochondrial Acetaldehyde Dehydrogenase Genotypes with Blood Pressure Level and the Prevalence of Hypertension in a General Population

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In Japanese and other Asians, the prevalence of genetically decreased mitochondrial aldehyde dehydrogenase (ALDH2) activity is higher than in Caucasians. The aim of this study was to elucidate the relation between ALDH2 genotypes and blood pressure levels or hypertension in Japanese. After obtaining informed consent for genetic analysis from 917 men and 1,478 women who lived in a mountainous farming region near Kyoto and who were free from cardiovascular disease and liver dysfunction, the authors identified the ALDH2 genotype in all subjects. Differences in blood pressure level among genotypes were then compared by analysis of covariance, and the relation between genotypes and hypertension was also analyzed by logistic regression analysis. The frequencies of genotypes $*1/*1$, $*1/*2$, and $*2/*2$ were 44.7%, 46.9% and 8.4% in men, and 50.1%, 43.2% and 6.8% in women, respectively. In men, systolic and diastolic blood pressures tended to decrease in the order of $*1/*1 > *1/*2 > *2/*2$. However, adjustment for confounding factors including alcohol consumption resulted in the disappearance of significance. Logistic regression analysis adjusted for the same confounding factors for men showed that the odds ratios (OR) of being hypertensive in the $*2$ allele to not having $*2$ allele were 0.67 (95% confidence interval (CI): 0.47–0.96). However, in the subgroup analyses, this relation was not observed in the group having a below-median level of alcohol consumption (OR = 0.92; 95% CI: 0.53–1.62) or in the group not taking antihypertensive agents (OR = 0.77; 95% CI: 0.52–1.15). Furthermore, we did not observe any relation between the ALDH2/ $*2$ allele and hypertension in women (OR = 1.07; 95% CI: 0.80–1.42). The results suggest that there may be no causal relation between hypertension and the ALDH2 genotype *per se*, after excluding for some confounding factors, especially for alcohol drinking. (*Hypertens Res* 2002; 25: 857–864)

Key Words: aldehyde dehydrogenase 2, alcohol, blood pressure, hypertension, genetic

Introduction

The influence of drinking, particularly moderate to excessive

drinking, is known to affect the development of hypertension (1–12) and the morning rise in blood pressure (13). However, the physiologic mechanism of blood pressure elevation due to drinking remains to be elucidated. While drinking,

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many Asians, including Japanese, present a characteristic symptom of facial flushing called "oriental flushing" accompanied by palpitation, headache, nausea, vomiting, and perspiration. This excessive sensitivity to alcohol intake results from an elevation in the blood acetaldehyde level due to the genetically determined low metabolic function of low- K_m (Michaelis Constant) human mitochondrial aldehyde dehydrogenase (ALDH2), an acetaldehyde-metabolizing enzyme (14–17). The gene for ALDH2 is located on the long arm of chromosome 12, and the low metabolic function of the isozyme is due to a point mutation at position 1510 with a G to A transition in exon 12, changing the codon GAA encoding glutamic acid (Glu) at position 487 into a lysine codon (AAA) (14). Harada *et al.* found polymorphisms at -357 with a G to A substitution in the promoter region of the ALDH2 gene, and reported that the ALDH2*2 allele was linked to the G promoter allele and the ALDH2*1 allele was linked to the A promoter allele (18).

Several epidemiologic studies have investigated the association between hypertension and the ALDH2 genotype of Japanese and other Orientals. However, with the exception of a single study on urban residents (19), all of these studies have dealt with relatively small numbers of men in their workplaces (20–23). Furthermore, there has been no study employing a large number of subjects who live in rural communities. Accordingly, the purpose of this study was to elucidate and confirm the relation between ALDH2 genotypes and hypertension in Japanese by genotyping residents in a rural community.

Materials and Methods

Subjects

We conducted this study in S town, a rural farming mountain area located near Kyoto in the western part of Japan and having a population of about 15,000. Among the 2,892 residents who underwent medical check-ups in 1999, a total of 2,395 residents—*i.e.*, 917 men (mean age $\pm$ SD, 58.0 $\pm$ 15.7) and 1,478 women (mean age $\pm$ SD, 56.3 $\pm$ 15.6)—were enrolled in this study after providing their full and informed consent. Of these subjects, a total of 360 were excluded for the following reasons: genotypes undetermined ($n = 100$), serum GOT or GPT levels over 100 IU/l ($n = 13$), and a history of transient ischemic attack, stroke, angina pectoris, myocardial infarction, or diabetes mellitus ($n = 247$). The protocol of this study was approved by the Institutional Review Board of the Shiga University of Medical Science (No. 11–15, 1999).

Genetic Analysis

DNA was extracted from the buffy coats of whole blood cells using a DNA extraction kit (Wako Pure Chemical Industries, Osaka, Japan). ALDH2 genotypes were determined by the polymerase chain reaction-restriction fragment length

polymorphisms (PCR-RFLP) method developed by Takeshita *et al.* (24). Briefly, CAAATTACAGGGTCAACTGCT and CCACACTCACAGTTTCTCTT were used as 5 and 3 primers, respectively. The corresponding DNA segment of ALDH2 was amplified by PCR on a Gene Amp PCR System 9700 (Perkin Elmer, Norwalk, USA). The amplification conditions consisted of an initial denaturation at 94°C for 60 s, followed by 33 cycles of denaturing at 94°C for 60 s, annealing at 52°C for 30 s, and extension at 72°C for 30 s, with a final incubation step at 72°C for 60 s.

The amplified DNA fragment was cleaved by the restriction endonuclease KSP6321 (Nippon Roche, Tokyo, Japan), electrophoresed on a 3.5% Nusieve agarose gel (BioWhittaker Molecular Applications, Rockland, USA), and observed for a 112 base pair (bp) fragment (ALDH2*2/*2 genotype), a 23 bp fragment (ALDH2*1/*1 genotype), and examined for the presence of a 112 bp fragment (ALDH2*2/*2 genotype), a 23 bp fragment (ALDH2*1/*1 genotype), or both (ALDH2*1/*2 genotype). Genotypes were determined by the PCR-RFLP method on a total of 75 random samples consisting of 25 PCR products of each genotype, and confirmed by direct sequencing. Briefly, after fractionation of PCR-RFLP products on 1% agarose gels (Nippon Gene, Tokyo, Japan), the desired DNA bands were cut out, and the DNA was purified using a QIAquick Gel Extraction Kit (QIAGEN, Valencia, USA), amplified with the above 5 primer, and analyzed on an ABI PRISM 310 Genetic Analyzer (Perkin Elmer).

Physical Examinations and Assessment of Alcohol Intake

Systolic (SBP) and diastolic blood pressures (DBP) were measured twice using a standard sphygmomanometer on the right arm of seated subjects after an at least 5-min rest. Korotkov's first and fifth points were regarded as the SBP and DBP, respectively, and the blood pressure was measured by trained nurses. The mean of 2 measurements of each pressure was used for data analysis. Hypertensive subjects were defined as those with an SBP ≥ 140 mmHg and/or a DBP ≥ 90 mmHg and those taking hypertensive agents.

Serum γ -glutamyl-transpeptidase activity (γ -GTP) was measured using an enzymatic method. Body mass index (BMI) was calculated as weight (kg)/height² (m²).

Daily alcohol intakes were assessed by an interviewer. The frequency of drinking during a typical week and the alcohol intake on each occasion were determined and used to calculate the alcohol intake per week, which was then divided by 7 to obtain the average alcohol intake per day. Subjects were asked to estimate their alcohol intake based on *gou*, a traditional Japanese drinking unit corresponding to 23 g of ethanol. One *gou* is equivalent to 180 ml of sake, and its ethanol content is roughly equivalent to that of a bottle of beer (663 ml), 2 single whiskeys (70 ml), or 240 ml of wine. Drinkers were defined as those consuming more than 0.3 *gou* a week, as in the previous well-designed cohort studies in

Table 1. Means (SD) and Prevalence of Risk Characteristics for Surveyed Subjects

Characteristics	Men	Women	<i>p</i> values [†]
Number	749	1,286	
Age (years)	57.0 (15.8)	55.2 (15.6)	0.011
Body mass index (kg/m ²)	22.6 (2.9)	22.5 (3.2)	0.560
γ -GTP	28.4 (34.5)	12.0 (8.5)	< 0.001
SBP (mmHg)	132.4 (18.4)	127.3 (20.0)	< 0.001
DBP (mmHg)	78.7 (11.5)	75.4 (11.6)	< 0.001
Antihypertensive agents (%)	16.5	18.5	0.264
Hypertension (%)	41.8	37.1	0.036
Cigarette smoking (number/day) [‡]	10.7	0.9	< 0.001
Current smoker (%)	51.2	7.5	< 0.001
Alcohol consumption (g/day) [‡]	18.5	1.6	< 0.001
Drinker (%)	75.3	27.8	< 0.001
ALDH2(*1/*1, *1/*2, *2/*2) (%)	44.7/46.9/8.4	50.1/43.2/6.8	0.049

[†] Comparisons between means except for γ -GTP, number of cigarettes and alcohol consumption were analyzed using Student's or Welch's *t*-test, and the χ^2 test was used to compare proportions. [‡] Cigarette smoking and alcohol consumption are mean values for all subjects combined. The Wilcoxon rank sum test was used to compare these two values and γ -GTP.

Fig. 1. Gender-specific distribution of alcohol consumption according to ALDH2 genotype.

Japan (25, 26).

Statistical Analysis

The Statistical Package for Social Science (SPSS ver. 10.0J; SPSS Japan, Tokyo, Japan) was used for statistical analysis.

The Student's or Welch's *t*-test and the Wilcoxon rank sum test were used for comparisons of means between men and women. For comparisons of means among the 3 genotypes, one-way analysis of variance or the Kruskal-Wallis test was used according to the distributions. For comparison of means adjusted for confounding factors, analysis of covariance was performed twice: once by adjusting for age and BMI, and once by adjusting for age, BMI and alcohol intake. This analysis was also performed in two subgroups according to whether or not they were taking antihypertensive agents. The χ^2 test was used to compare proportions.

Logistic regression analysis was used to clarify the contribution of each independent variable to hypertension. In this analysis, hypertension was regarded as a dependent variable, and ALDH2 genotypes were regarded as independent variables; using the ALDH2*1/*1 genotype as the standard, two dummy variables were set for the *1/*2 and *2/*2 genotypes adjusting for age, BMI and smoking (Model 1). We used smoking as an independent variable because it was strongly correlated to alcohol consumption. In model 2, we added alcohol consumption as an independent variable. A further analysis that included a single dummy independent variable for the presence of the /2 mutant allele was performed; this final analysis included subgroup stratification by the median alcohol consumption and the taking of antihypertensive agents. All probability values were two-tailed and all confidence intervals were estimated at the 95% level.

Results

Direct sequencing of 25 randomly selected samples of each ALDH2 genotype identified by the PCR-RFLP method showed that there was no discrepancy between the PCR-RFLP-determined genotypes in exon 12 of ALDH2 and the

Table 2. Means (SD) and Prevalence of Risk Characteristics of Surveyed Subjects According to ALDH2 Genotype for Men

Risk characteristics	ALDH2 genotypes			<i>p</i> values [#]
	*1/*1	*1/*2	*2/*2	
Numbers	335	351	63	
Proportion of genotypes (%)	44.7	46.9	8.4	
Age (years)	56.8 (15.7)	58.1 (15.6)	52.8 (17.7)	0.048
Body mass index (BMI) (kg/m ²)	22.9 (3.0)	22.3 (2.8)	21.9 (2.7)	0.004
γ -GTP	36.8	22.8	15.2	< 0.001
Calculated alcohol consumption (g/day)	26.3	15.5	0.66	< 0.001
Frequency of drinking (time/week)	5.4	3.9	0.3	< 0.001
Current drinker (%)	89.0	73.8	11.1	< 0.001
Abstainer (%)	1.5	3.1	1.6	0.329
Number of cigarettes (number/day)	10.4	10.8	11.5	0.784
Current smoker (%)	52.5	49.3	56.5	0.489
Antihypertensive agents (%)	21	14.9	7.9	0.014
Hypertension (%)	48.2	38.3	27.0	0.001
Systolic blood pressure (mmHg)	134.2 (18.5)	131.8 (18.2)	125.8 (17.4)	0.003
Adjusted systolic blood pressure (mmHg) [†]	133.8	131.6	129.0	0.037
Adjusted systolic blood pressure (mmHg) ^{††}	133.5	131.8	130.0	0.212
Diastolic blood pressure (mmHg)	79.9 (11.7)	78.2 (11.2)	75.5 (11.2)	0.011
Adjusted diastolic blood pressure (mmHg) [†]	79.5	78.3	76.9	0.143
Adjusted diastolic blood pressure (mmHg) ^{††}	79.3	78.4	77.3	0.356
Without antihypertensive agents (<i>N</i> = 622)				
Adjusted systolic blood pressure (mmHg) ^{††}	130.5	129.5	127.9	0.513
Adjusted diastolic blood pressure (mmHg) ^{††}	78.9	78	77.4	0.516
With antihypertensive agents (<i>N</i> = 127)				
Adjusted systolic blood pressure (mmHg) ^{††}	146.8	143.7	141.9	0.614
Adjusted diastolic blood pressure (mmHg) ^{††}	81.3	80.2	76.0	0.535

[#] *p* values calculated by the analysis of variance for means or χ^2 test for proportions, and Kruskal-Wallis's test was used for γ -GTP, number of cigarettes and alcohol consumption. [†] *p* values calculated by the analysis of covariance adjusted for age and BMI. ^{††} *p* values calculated by the analysis of covariance adjusted for age, BMI and alcohol consumption.

1510G/A sequence variations. In addition, no sequence variations were found other than the reported 1464G/A, 1486G/A, and 1510G/A variations in exon 12 of ALDH2 (27).

Table 1 shows the characteristics of the subjects. Age, γ -GTP, SBP, DBP, the prevalence of hypertension, the prevalence of smoking, the number of cigarettes smoked per day, alcohol consumption and the prevalence of drinking were higher in men. No significant gender difference in BMI or the proportion of antihypertensive users was noted. The frequencies of the ALDH^{*1/*1}, ^{*1/*2} and ^{*2/*2} genotypes were 44.7%, 46.9% and 8.4% in men and 50.1%, 43.2% and 6.8% in women, respectively, suggesting a significant gender difference in distribution. Figure 1 shows the gender-specific distribution of alcohol consumption stratified by the ALDH2 genotype. There was a strong relation between the genotype and alcohol consumption. Compared with the ALDH2^{*1/*1} genotype, the distribution of alcohol consumption for the ^{*1/*2} type was shifted to a lower level, and there were no subjects with the ^{*2/*2} genotype who drank more than 11.5 g/day of ethanol.

In men (Table 2), a comparison among ALDH2 genotypes showed significant differences in age, γ -GTP, SBP, DBP, BMI, prevalence of drinking, frequency of drinking, alcohol consumption, proportion of antihypertensive agent users and prevalence of hypertension. SBP, DBP and the prevalence of drinking tended to decrease in the order of ^{*1/*1} > ^{*1/*2} > ^{*2/*2}. However, analysis of covariance involving adjustment for age, BMI and alcohol consumption resulted in the disappearance of the significant difference in SBP and DBP. When the groups were stratified according to whether or not subjects were taking antihypertensive agents, the results were similar for both groups. In women (Table 3), a comparison among ALDH2 genotypes showed significant differences in the proportion of drinkers, the prevalence of drinking and alcohol consumption, but not in the other factors. Analysis of covariance involving the same adjustment as in men also showed no differences in the other factors.

Table 4 shows the results of logistic regression analysis applying hypertension as a dependent variable. In model 1, age and BMI showed significant positive associations with hypertension in both genders, and both the ALDH2^{*1/*2}

Table 3. Means (SD) and Prevalence of Risk Characteristics of Surveyed Subjects According to ALDH2 Genotype for Women

Risk characteristics	*1/*1	*1/*2	*2/*2	<i>p</i> values [#]
Numbers	644	555	87	
Proportion of genotypes (%)	50.1	43.2	6.8	
Age (years)	54.8 (16.0)	55.1 (15.4)	58.5 (14.3)	0.121
Body mass index (BMI) (kg/m ²)	22.5 (3.1)	22.4 (3.3)	22.4 (3.1)	0.888
γ -GTP	12.3	11.6	12.0	0.725
Calculated alcohol consumption (g/day)	1.4	0.5	0.03	<0.001
Frequency of drinking (time/week)	2.7	0.8	0.06	<0.001
Current drinker (%)	40.7	16.9	2.3	<0.001
Abstainer (%)	0.9	0.2	0	0.164
Cigarette smoking (number/day)	0.69	1.04	1.02	0.167
Current smoker (%)	6.1	8.6	10.5	0.155
Antihypertensive agents (%)	17.4	18.2	24.1	0.316
Hypertension (%)	36.4	37.5	39.1	0.862
Systolic blood pressure (mmHg)	127.2 (20.4)	127.4 (19.9)	128.1 (18.7)	0.932
Adjusted systolic blood pressure (mmHg) [†]	127.5	127.5	125.7	0.607
Adjusted systolic blood pressure (mmHg) ^{††}	127.2	127.7	126.1	0.653
Diastolic blood pressure (mmHg)	75.1 (11.7)	75.5 (11.4)	76.7 (11.7)	0.473
Adjusted diastolic blood pressure (mmHg) [†]	75.2	75.5	75.9	0.747
Adjusted diastolic blood pressure (mmHg) ^{††}	75.0	75.7	76.2	0.431
Without antihypertensive agents (<i>N</i> = 1,052)				
Adjusted systolic blood pressure (mmHg) ^{††}	124.0	124.1	122.5	0.706
Adjusted diastolic blood pressure (mmHg) ^{††}	74.0	74.4	75.0	0.712
With antihypertensive agents (<i>N</i> = 234)				
Adjusted systolic blood pressure (mmHg) ^{††}	142.3	142.6	141.5	0.947
Adjusted diastolic blood pressure (mmHg) ^{††}	80.4	80.3	81.0	0.946

[#] *p* values calculated by the analysis of variance for means or χ^2 test for proportions, and Kruskal-Wallis's test was used for γ -GTP, number of cigarettes and alcohol consumption. [†] *p* values calculated by the analysis of covariance adjusted for age and BMI. ^{††} *p* values calculated by the analysis of covariance adjusted for age, BMI and alcohol consumption.

genotype and ALDH2*2/*2 genotype in men were significantly associated with lower prevalence of hypertension. In model 2, with further adjustment for alcohol consumption, the ALDH2*1/*2 genotype in men was still significantly associated with a lower prevalence of hypertension. The odds ratios of having hypertension in the ALDH2*1/*2 and *2/*2 to *1/*1 were 0.68 (95% confidence interval (CI): 0.47–0.98) and 0.59 (95% CI: 0.29–1.25), respectively.

Table 5 shows the odds ratios of having hypertension in the ALDH2/*2 allele to not having one. In all subjects, the ratio was 0.67 (95% CI: 0.47–0.96) for men. In the subgroup analyses, we only observed an inverse relation between the ALDH2/*2 allele and hypertension for men in the group with the median and above alcohol consumption (odds ratio: 0.57; 95% CI: 0.35–0.93), and we observed a similar inverse relation for men in the group taking antihypertensive agents (odds ratio: 0.42; 95% CI: 0.18–0.99). There was no significant relation between the ALDH2/*2 allele and hypertension for women.

Discussion

A previous study of 403 Japanese male workers reported that the frequencies of the ALDH2*1/*1, *1/*2 and *2/*2 genotypes were 60.3%, 33.5% and 6.2%, respectively (22), and they were 48.2%, 43.0% and 8.8% for men and 52.0%, 39.2% and 8.8% for women in a recent study of urban Japanese residents (19). Compared with these findings, the present study showed a lower frequency for *1/*1 and a higher frequency for *1/*2. Therefore, the present study had a relatively stronger statistical power for the ALDH2*1/*2 genotype than those reported in these previous studies.

There have been several studies on the association between blood pressure and individual differences—including ALDH2—in alcohol sensitivity. Most showed no relation between the ALDH2 genotype and hypertension after adjusting for drinking status. Okayama *et al.* reported that, after adjusting for the amount of alcohol consumption, no association existed between blood pressure and deficient activity of ALDH2 phenotypes detected by a simple patch test for alcohol (21). After genotyping ALDH2 in 403 men in the

Table 4. Multivariate Adjusted Relative Prevalence Odds Ratios and 95% CIs (Confidence Intervals) of ALDH2 Genotypes and Other Risk Factors for Hypertension[†]

Variables	Men		Women	
	Odds ratio (95% CI)	<i>p</i> values	Odds ratio (95% CI)	<i>p</i> values
Model 1 [‡]				
Age (years)	1.09 (1.07–1.10)	< 0.001	1.10 (1.09–1.12)	< 0.001
Body mass index (kg/m ²)	1.19 (1.12–1.27)	< 0.001	1.21 (1.16–1.27)	< 0.001
Cigarette smoking (number/day)	1.00 (0.98–1.01)	0.678	0.99 (0.95–1.03)	0.678
ALDH2 genotype				
ALDH2*1/*1	1.00		1.00	
ALDH2*1/*2	0.60 (0.42–0.85)	0.021	1.02 (0.77–1.37)	0.858
ALDH2*2/*2	0.44 (0.22–0.88)	< 0.001	0.83 (0.47–1.44)	0.506
Model 2 [‡]				
Age (years)	1.09 (1.07–1.10)	< 0.001	1.10 (1.09–1.12)	< 0.001
Body mass index (kg/m ²)	1.20 (1.13–1.27)	< 0.001	1.21 (1.16–1.27)	< 0.001
Cigarette smoking (number/day)	0.99 (0.98–1.01)	0.421	0.99 (0.95–1.03)	0.485
Alcohol consumption (1 <i>gou</i> = 23 g of ethanol)/day	1.30 (1.07–1.57)	0.007	2.07 (1.04–4.13)	0.039
ALDH2 genotype				
ALDH2*1/*1	1.00		1.00	
ALDH2*1/*2	0.68 (0.47–0.98)	0.039	1.10 (0.82–1.48)	0.539
ALDH2*2/*2	0.60 (0.29–1.25)	0.173	0.90 (0.51–1.59)	0.713

[†] Hypertension was defined as 140 mmHg in SBP and/or 90 mmHg in DBP and/or taking antihypertensive agents. [‡] Model 1 did not include alcohol consumption as an independent variable and Model 2 included alcohol consumption as an independent variable.

Table 5. Multivariate Adjusted Relative Prevalence Odds Ratios and 95% CIs (Confidence Intervals) of ALDH2*2 Allele for Hypertension

Groups	Odds ratio of ALDH2*2 allele for hypertension [†]			
	Men		Women	
	Odds ratio (95% CI)	<i>p</i> values	Odds ratio (95% CI)	<i>p</i> values
Whole subjects (<i>N</i> = 749 for men, <i>N</i> = 1,286 for women)	0.67 (0.47–0.96)	0.030	1.07 (0.80–1.42)	0.650
Subgroup analysis according to alcohol consumption				
Below the median [‡] of alcohol consumption (<i>N</i> = 384 for men)	0.92 (0.53–1.62)	0.778	— [§]	
Median [‡] and above of alcohol consumption (<i>N</i> = 365 for men)	0.57 (0.35–0.93)	0.023	—	
Subgroup analysis according to antihypertensive agents				
Without antihypertensive agents (<i>N</i> = 622 for men, <i>N</i> = 1,052 for women)	0.77 (0.52–1.15)	0.199	0.97 (0.70–1.35)	0.866
With antihypertensive agents (<i>N</i> = 127 for men, <i>N</i> = 234 for women)	0.42 (0.18–0.99)	0.047	0.80 (0.45–1.41)	0.442

[†] Hypertension (all) was defined as 140 mmHg in SBP and/or 90 mmHg in DBP and/or taking antihypertensive agents. [‡] Median of alcohol consumption for men: 11.2 g/day. [§] Odds ratio stratified by median of alcohol consumption was not calculated because of the small number of drinkers.

workplace, Tsuritani *et al.* reported that adjustment for the alcohol intake resulted in no difference in blood pressure elevation among genotypes (22). Takagi *et al.* (19) recently reported that no significant interaction was observed between the ALDH2 genotype and the level of alcohol consumption in determining blood pressure levels in an urban Japanese population, although they excluded subjects receiving antihypertensive agents from their interaction analysis. In con-

trast, Itoh *et al.* reported that, in a group of subjects who showed oriental flushing, increasing amounts of alcohol consumption were correlated with increasingly higher prevalence of hypertension (20); however, they did not investigate the ALDH2 genotypes in their subjects.

In the present study, adjustment for age, BMI and alcohol consumption resulted in no significant difference in the mean SBP or DBP among ALDH2 genotypes. In men, however,

logistic regression analysis applying hypertension as a dependent variable showed a significant inverse relation between the ALDH2/*2 allele and the prevalence of hypertension after adjusting for alcohol consumption. However, in the subgroup analyses, this relation was only observed in the group with a higher level of alcohol consumption or in the group taking antihypertensive agents, and we did not observe any relation between the ALDH2/*2 allele and hypertension in women. These results suggest that the statistical adjustment for alcohol consumption by logistic regression analysis was not adequate because the distribution of alcohol consumption was too different among genotypes and there may have been a confounding relation between genotypes, drinking and the taking of antihypertensive agents. In addition, Klag *et al.* investigated the relation between drinking and blood pressure in 946 Caucasians and 810 Japanese and concluded that, with comparable alcohol intake, the Japanese subjects had higher blood pressure than the Caucasians, and that there was no difference in the rate of elevation in blood pressure between the two populations (28). Based on this result, it is very unlikely that the ALDH2/*2 allele, which is rare in Caucasians, is preventive against hypertension.

Japanese citizens are entitled to almost free medical examinations on an annual basis. If there is an ALDH2*1/*1 genotype individual who has a transient nonstable elevation in blood pressure accompanying binge drinking, the examinee may acquire a prescription for antihypertensive agents. Furthermore, it may be highly difficult to control hypertension by medication in such binge drinkers. This may be a possible cause of the confounding relation between drinking and the taking of antihypertensive agents.

Furthermore, the interviewer may sometimes underestimate or overestimate alcohol intake in heavy drinkers, since it has been suggested that heavy drinkers are more likely to under- or overstate their alcohol intake than light to moderate drinkers (29, 30). If the heavy drinkers in the present study, most of whom had the ALDH2*1/*1 genotype, under-reported their true alcohol intake, then the statistical adjustment for alcohol consumption would have been weakened.

Another reason why the prevalence of hypertension was lower in subjects with the ALDH2*1/*2 and ALDH2*2/*2 genotypes after adjustment for alcohol intake may be the protracted effect of heavy drinking in the past. A survey conducted in Akita, which formerly showed the highest blood pressure levels in Japan and was one of the districts with high alcohol consumption, reported that blood pressure and the prevalence of hypertension were much higher in abstainers than in drinkers and nondrinkers (4), which suggests that the elevation in blood pressure after heavy drinking may persist for long periods even after abstention. However, in the present study, because we excluded subjects with liver dysfunction or past histories of cardiovascular diseases before analysis, the proportion of male abstainers was lowest in the group of *1/*1 individuals. So the influence of past-drinking

habits may have been small.

One drawback of the present study was the method used for blood pressure measurement, *i.e.*, casual blood pressure measurement using a standard sphygmomanometer. Further studies using the average of multiple blood pressure measurements or home blood pressure (31) or ambulatory blood pressure monitoring will be needed (32). Another drawback is that we did not estimate the number of subjects who reduced or increased their alcohol intake. The influence of alcohol intake on blood pressure might be better-assessed using lifetime drinking habits.

Finally, we concluded that there may be no causal relation between the ALDH2 genotype and hypertension provided certain confounding factors, such as alcohol consumption or the use of antihypertensive agents, are excluded. Further studies using detailed information on the lifetime history of drinking habits and more precise methods of blood pressure measurement will be required.

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