Genetic Polymorphisms in Alcohol Metabolizing Enzymes as Related to Sensitivity to Alcohol-induced Health Effects

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Abstract

Three hundred sixty-seven middle-aged Japanese men were analyzed for genotypes of low K_m aldehyde dehydrogenase (ALDH2) and cytochrome P450 2E1, and for the association with alcohol-induced health effects. Homozygotes for the normal ALDH2 gene (NN) and for the mutant gene (MM) and heterozygotes (NM) were found in 60, 6 and 33%, and homozygotes for the c1 gene (c1/1) and c2 gene (c2/2) of P450 2E1, and heterozygotes (c1/2) in 55, 5 and 40% of subjects, respectively. Mean alcohol consumption significantly differed in the three ALDH2 genotypes: 297 g per week in NN, 158 g in NM and 18 g in MM. It was not different in the three P450 2E1 genotypes, but tended to increase from c1/1 to c2/2 in the NN subjects while there was an inverse relationship in the subjects having the M gene. No difference in alcohol-induced health effects was observed in ALDH2 genotypes, but c2/2 genotype showed higher blood pressure and serum uric acid than the other P450 2E1 genotypes in the subjects consuming 200 g or more of alcohol per week. These results suggest an interactive effect of ALDH2 and P450 2E1 genes on alcohol consumption and a higher sensitivity to alcohol-induced health effects in c2/2 genotypes, although larger scale studies are required to confirm these findings.

Key words: Alcohol, ALDH2, Cytochrome P450 2E1, Genotype, Health effects

Introduction

Alcohol consumption is a potent agent affecting human health. Mild alcoholic liver damage (ALD) characterized by hepatic steatosis, dyslipidemia and hypertension are the well-known earliest adverse alcohol-induced health effects, and are often found even in mild to moderate regular alcohol consumers who are commonly found in a general population. However, a considerably wide variation is also observed in these alcohol-induced health effects. The exact reasons for the individual differences in sensitivity to alcohol remain unclear, and this may cause some obscurity or difficulty in promoting the prevention of alcohol-related health problems.

Alcohol is metabolized mainly by two pathways in humans. The alcohol dehydrogenase (ADH) system and a non-ADH pathway which is often called the microsomal ethanol oxidizing system (MEOS). Genetic polymorphisms in low- K_m aldehyde dehydrogenase (ALDH2) in the ADH system¹⁻³⁾ and in cytochrome P450 2E1⁴⁾ involved in the non-ADH pathway, are known and have been suggested to be related to the individual sensitivity to alcohol. ALDH2 catabolizes acetaldehyde, which is produced from ethanol by ADH, into acetic acid. Acetaldehyde is much more toxic than ethanol and has been suggested to play an important role in the development of ALD⁵⁻⁷⁾. A marked elevation of blood acetaldehyde has been observed in persons having a mutant ALDH2 gene after alcohol ingestion⁸⁻¹⁰⁾. On the other hand, higher activity of P450 2E1 in the liver may produce a higher amount of acetaldehyde after alcohol consumption, which may then be related to the development of ALD. Two types of P450 2E1 genes, c1 (wild) and c2 (variant), are known, and the c2 gene has been suggested to be related to higher enzyme activity in the liver^{4, 11, 12)}.

The association of genetic polymorphisms in these enzymes with the individual differences in sensitivity to alcohol, however, has been evaluated only in ALD patients¹³⁻²¹⁾, and the results were contradictory. Thus the implications of the genetic polymorphisms in the prevention of early and adverse alcohol-induced health effects in mild-to-moderate regular alcohol consumers in a general population remain unclear. The aim of the present study is to clarify the relevance of polymorphisms in ALDH2 and P450

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2E1 genes to alcohol-induced health effects in middle-aged Japanese men recruited from an occupational population.

Subjects and Methods

Three hundred sixty-seven men were selected as the study subjects among 387 unrelated male workers for a local government aged from 35 to 61 years, who participated in a health screening program provided by the Department of Health Care and Promotion of Kanazawa Medical University Hospital. Twenty of the workers were excluded from this study because of chronic illnesses that might affect the study results such as hypertension being treated with hypotensive agents, diabetes mellitus or chronic hepatitis. Written informed consent was obtained from each subject.

All the subjects came to the university hospital by 8 A.M. after a 12hr fast. Blood pressure (BP) was measured twice with a random zero sphygmomanometer in the sitting position after the subject rested on a chair for 15 minutes or longer. The cuff size was selected according to the recommendations of the American Heart Association, 1988. Diastolic BP (DBP) was defined as the disappearance of Korotkoff V sound. The mean of the two measurements was determined as the BP level.

Blood biochemical parameters possibly related to alcohol consumption, i.e. serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), choline esterase (ChE) and amylase (Amyl) activities, and serum total cholesterol (Tch), triglyceride (TG), high density lipoprotein cholesterol (HDLc) and glucose (Glc) concentrations, were analyzed using a Hitachi 7450 autoanalyzer (Hitachi, Tokyo), and the percent of hemoglobin A1c (HbA1c) was analyzed using an HLC-723 GHb II glycohemoglobin analyzer (Toso, Tokyo).

The determinations of genotypes of ALDH2 and P450 2E1 using genomic DNA extracted from white blood cells basically followed the methods described by Enomoto et al.²²⁾ and Hayashi et al.⁴⁾, respectively. The region of exon 12 of the ALDH2 gene in the sample DNA was amplified by the polymerase chain reaction (PCR) with 3'-site and 5'-site primers prepared following Enomoto et al.²²⁾. PCR products were slot blotted onto nitrocellulose filters and baked at 80 °C for 1h. The target sequence was determined by the enhanced chemiluminescence (ECL) reaction using the ECL-3' Oligolabelling and Detection System (Amersham International Plc., UK) as follows. The filters were hybridized with fluorescein-dUTP labeled probes for N and M genes of ALDH2, 5'-GTTTTCACTTCAGTGTATGCC-3' and 5'-GTTTTCACTTTAGTGTATGCC-3', at 50 °C for 2h, and were exposed to x-ray films for 5 to 10 min. The genotypes of ALDH2 were then divided into three types, normal homozygotes: NN, normal and mutant heterozygotes: NM, and mutant homozygotes: MM.

The sample DNA was also amplified by PCR with the primers for the P450 2E1 gene prepared following the methods reported by Hayashi et al.⁴⁾, and the genotypes were determined with the restriction fragment length polymorphism (RFLP) method using Rsa I and Pst I, i.e., the c1 gene was digested with Rsa I and detected as two fragment bands of 360 and 50 bps by 1.2% agarose gel electrophoresis while the c2 gene was digested into two fragment bands of 290 and 120 bps by Pst I. The analytical results using Rsa I were consistent with those using Pst I.

Genotypes of P450 2E1 were then categorized into three types, homozygous for the c1 gene: c1/1, heterozygous for c1 and c2 genes: c1/2, and homozygous for the c2 gene: c2/2.

The data on usual alcohol consumption by the subjects, that is, the frequency, kinds of alcoholic beverages and volume consumed at one time during the preceding one-year period, were obtained using a self-report questionnaire, and confirmed at interview by an experienced nurse. The subjects who had not drunk more than 10ml of alcohol at one time, and not more often than once a month were defined as non-drinkers. Alcohol consumption in the remaining drinkers was expressed as the average weight of absolute ethanol consumed per week (g/week). The subjects were then categorized into four levels of alcohol consumption; none (non-drinker), <200g/week (minimal drinker), 200-399 (mild), and 400g or more (moderate).

The differences in the means of age (y.o), body mass index (BMI: kg/m²) and alcohol consumption among the subjects showing the three different genotypes of ALDH2 and those of P450 2E1, and the contributions of alcohol consumption, the genotypes of ALDH2 and P450 2E1 to the values of possible alcohol-induced health effects, i.e. BP and blood biochemical parameters, adjusted for the effects of age and BMI, were evaluated by generalized linear model (GLM) analyses. The associations of alcohol consumption and P450 2E1 genotypes with the frequencies of subjects showing hypertension (BP ≥ 140/90mmHg) and hyperuricemia (serum UA ≥ 8.0mg/dl), being adjusted for age and BMI, were tested by multiple logistic regression analyses. All the statistical analyses were performed using the SAS program package (SAS Institute, USA). Statistical significance was defined as $p < 0.05$, and $0.05 \leq p < 0.10$ was regarded as borderline significance.

Results

The analysis for ALDH2 genotypes in the 367 subjects showed the NN type in 222 (60%), NM in 121 (33%), and MM in 24 (6%), and that for P450 2E1 genotypes showed the type c1/1 in 204 (55%), c1/2 in 146 (40%), and c2/2 in 17 (5%). These results were basically consistent with previous studies on the genotypes of ALDH2^{22, 23)} and P450 2E1^{17, 19)} in Japanese people. The frequencies of N and M genes and those of c1 and c2 genes in the subjects were also shown to be compatible with Hardy-Weinberg equilibrium²⁴⁾ when the frequencies of the dominant genes, N and c1, were assumed to present true frequencies ($\chi^2 = 2.28$ in ALDH2 and $\chi^2 = 2.39$ in P450 2E1, both < 3.84 for $p = 0.05$). As illustrated in Fig. 1, the frequencies of P450 2E1 genotypes were not different in the subjects showing the different ALDH2 genotypes, suggesting an independent distribution of the genotypes of the two genes.

The arithmetic means of age, BMI and usual alcohol consumption in the total group of 367 subjects and those in the subjects divided by ALDH2 and P450 2E1 genotypes are shown in Table 1. The mean age of the total group was 46.0 y.o, and no significant difference was detected in the different genotypes of ALDH2 and P450 2E1. The mean BMI, 24.5kg/m², in the total group was somewhat higher than that of general Japanese middle-aged men, around 22-23kg/m². However, the highest BMI in the subjects was 36.8kg/m², and thus no extremely obese subject was included. No significant difference in BMI was detected in the subjects having different genotypes.

Table 1 Numbers (N), means (M) and standard deviations (SD) of age, body mass index and alcohol consumption in middle-aged male workers having different genotypes of ALDH2 and P450 2E1.

	Genotype of ALDH2	Genotype of P450 2E1									Total		
		N	c1/1 M	SD	N	c1/2 M	SD	N	c2/2 M	SD	N	M	SD
Age (y.o)	NN	127	45.5	5.8	88	47.2	5.6	7	46.7	7.2	222	46.2	5.8
	NM	64	45.6	4.9	47	45.8	5.2	10	44.8	5.7	121	45.6	5.0
	MM	13	45.8	4.8	11	46.5	4.0	0	-	-	24	46.2	4.4
	Total	204	45.6	5.5	146	46.7	5.4	17	45.6	6.2	367	46.0	5.5
BMI (kg/m ²)	NN		24.7	2.8		24.7	2.9		23.1	3.0		24.7	2.9
	NM		24.4	3.2		24.1	2.5		24.0	2.3		24.3	2.9
	MM		25.3	2.8		24.4	2.1		-	-		24.9	2.5
	Total		24.7	2.9		24.5	2.8		23.6	2.6		24.5	2.8
Alcohol (g/week)	NN		269	188		332	221		368	172		297	203
	NM		154	148		174	214		101	133		158	176
	MM		25	32		9	14		-	-		18	26
	Total		218	185		257	233		211	198		233	206

Significant difference in the means was found only for alcohol consumption among the three genotypes of ALDH2 ($P < 0.001$).

Fig. 1 Frequencies of three genotypes of P450 2E1 (c1/1, c1/2, c2/2) in the subjects with three genotypes of ALDH2 (NN, NM, MM).

The mean weight of absolute ethanol consumed per week in the 367 subjects was 233g, but only 14 subjects (3.8%) drank 100g or more of alcohol per day, with the largest consumption being 975g/week. Thus, alcohol consumption in the present subjects was generally mild to moderate. The subjects showing the NN type of ALDH2 gene drank most; 297g/week as the average, followed by NM; 158g, while the MM subjects drank much less than the other two genotypes; 18g. These differences were highly significant ($p < 0.001$). On the other hand, no marked difference in the means was observed in the three P450 2E1 genotypes; 218 g/week in c1/1, 257g in c1/2 and 211g in c2/2. However, it was noted that mean alcohol consumption was largest in the subjects with the c2/2 genotype, followed by c1/2 and c1/1 in the normal homozygotes for ALDH2, while it was lowest for the c2/2 genotype in the heterozygous NM subjects and lower in the c1/2 genotype than in the c1/1 genotype among the subjects homozygous for the mutant ALDH2 gene.

Table 2 shows the numbers and frequencies of subjects showing the four levels of alcohol consumption; none, <200 g/week, 200-399, and 400g or more, in each of the different genotypes of ALDH2 and P450 2E1. Greater alcohol consump-

Table 2 Numbers and frequencies (%) of the subjects by four alcohol consumption levels and genotypes of ALDH2 and P450 2E1.

Genotype of ALDH2	Genotype of P450 2E1	Alcohol consumption (g/week)			
		none	<200	200-399	400-
NN	c1/1	7 (6%)	50 (39)	39 (31)	31 (24)
	c1/2	3 (3)	31 (35)	19 (22)	35 (40)
	c2/2	0 -	2 (29)	2 (29)	3 (43)
	Total	10 (5)	83 (37)	60 (27)	69 (31)
NM	c1/1	11 (17)	33 (52)	14 (22)	6 (9)
	c1/2	12 (26)	23 (49)	7 (15)	5 (11)
	c2/2	3 (30)	5 (50)	1 (10)	1 (10)
	Total	26 (21)	61 (50)	22 (18)	12 (10)
MM	c1/1	7 (54)	6 (46)	0 -	0 -
	c1/2	8 (73)	3 (27)	0 -	0 -
	c2/2	0 -	0 -	0 -	0 -
	Total	15 (63)	9 (38)	0 -	0 -
Total	c1/1	25 (12)	89 (44)	53 (26)	37 (18)
	c1/2	23 (16)	57 (39)	26 (18)	40 (27)
	c2/2	3 (18)	4 (41)	3 (18)	4 (24)
	Total	51 (14)	153 (42)	82 (22)	81 (22)

Mean level of alcohol consumption was increased from c1/1 to c1/2 in the subjects having the NN genotype while it was the opposite in the subjects having the M gene. This interactive effect of ALDH2 and P450 2E1 genes on alcohol consumption was shown to be significant ($P < 0.05$) by generalized linear model analysis.

tion in the subjects having the c2 gene than in those having the c1 gene was observed when they were homozygous for the N gene of ALDH2, while an inverse relationship was observed in the subjects with the M gene. This interactive effect of ALDH2 and P450 2E1 genes on alcohol consumption levels was significant ($p < 0.05$), together with the main effect of ALDH2 genotypes ($p < 0.001$), by GLM analysis scoring the four levels of alcohol consumption from 0 to 3.

The mean values, standard deviations and ranges of BP and

Table 3 Means (M), standard deviations (SD) and ranges of blood pressure and blood biochemical parameters in 367 middle-aged male workers.

Parameter	M	SD	Range
SBP (mmHg)	122	15	84 - 180
DBP (mmHg)	83	10	50 - 124
AST (U/l) ^a	25	1.4	11 - 125
ALT (U/l) ^a	28	1.7	9 - 212
GGT (U/l) ^a	31	2.1	7 - 232
ALP (U/l)	166	44	14 - 319
ChE (U/l)	1.89	0.35	0.97 - 3.47
Amyl (U/l)	87	27	24 - 258
Tch (mg/dl)	213	35	122 - 327
TG (mg/dl) ^a	132	1.6	42 - 507
HDLc (mg/dl)	47	11	24 - 90
UA (mg/dl)	6.1	1.3	0.7 - 10.5
Glc (mg/dl)	105	15	78 - 186
HbA1c (%)	5.7	0.6	4.1 - 8.6

a: Geometric mean and geometric standard deviation.

b: Abbreviations; SBP and DBP: systolic and diastolic blood pressure, AST: serum aspartate aminotransferase, ALT:alanine aminotransferase, GGT: gamma-glutamyl transferase, ALP: alkaline phosphatase, ChE: choline esterase, Amyl: amylase, Tch: total cholesterol, TG: triglyceride, HDLc: HDL-cholesterol, UA: uric acid, Glc: glucose, HbA1c: hemoglobin A1c.

blood biochemical parameters in the 367 subjects are shown in Table 3. These values were basically consistent with those in the general population. Though 10 to 15% of the subjects showed values higher than the normal range in each laboratory test, the subjects were considered to be generally healthy and not to show specific pathological abnormalities.

GLM analyses were performed to test the contributions of alcohol consumption, the three genotypes of ALDH2, those of P450 2E1 and the interaction between alcohol consumption and the genotypes to the levels of BP and blood biochemical parameters, adjusting for the effects of age and BMI. The model equation was as follows; Parameter = $\beta_0 + \beta_1 \times \text{age} + \beta_2 \times \text{BMI} + \beta_3 \times \text{alc-s} + \beta_4 \times \text{ALDH2 gtp} + \beta_5 \times \text{P450 2E1 gtp} + \beta_6 \times (\text{alc-s} \times \text{ALDH2 gtp}) + \beta_7 \times (\text{alc-s} \times \text{P450 2E1 gtp})$, where alc-s is the score of the alcohol consumption level, 0 to 3 are continuous variables, and gtp represents the three genotypes of ALDH2 and P450 2E1 as nominal variables.

The results showed that all the parameters except serum Alp and HbA1c were closely related to BMI, and SBP, DBP, serum ALT, ChE, Amyl, Glc and HbA1c were also significantly related to age. The levels of alcohol consumption were positively related to SBP, DBP, serum AST, GGT and HDLc levels. DBP, serum ALT and GGT levels showed a significant relation to the genotypes of ALDH2, and SBP, DBP and serum UA showed a significant relation to the genotypes of P450 2E1. The association between serum HDLc and the genotypes of P450 2E1 was of borderline significance. No significant association was found between these parameters and the interactions of alcohol consumption and the genotypes of ALDH2 and P450 2E1.

Least square means (LSMs) of DBP, serum AST and GGT in the subjects with the three ALDH2 genotypes, and those of SBP and serum UA and HDLc in the three genotypes of P450 2E1 according to the four levels of alcohol consumption were obtained by GLM analyses, adjusting for the effects of age and BMI. As illustrated in Fig. 2, none of the MM genotype subjects drank 200g or more of alcohol per week. The LSMs of DBP and

Fig. 2 Least square means and standard errors of diastolic blood pressure and serum alanine aminotransferase and gamma-glutamyl transferase levels divided by alcohol consumption levels and genotypes of ALDH2, adjusted for the effects of age and body mass index.

serum GGT were highest in NN genotypes and lowest in MM genotypes among the non-drinkers, and this was also found for serum GGT in the minimal drinkers who consumed <200g of alcohol per week. DBP and serum ALT were somewhat higher in NN genotypes than in NM genotypes among the subjects consuming 200 g or more of alcohol per week.

The LSMs of SBP and serum UA and HDLc in the subjects categorized by P450 2E1 genotypes and alcohol consumption levels are illustrated in Fig. 3. SBP and serum UA were higher in persons with c2/2 genotypes than in the other two genotypes among alcohol consumers, especially in drinkers who consumed 200g or more per week. On the other hand, serum HDLc levels were lower for those with c2/2 genotypes than with the other genotypes among the non-drinkers and drinkers who consumed <400g per week, but not in the drinkers who consumed more alcohol.

The LSMs of DBP and serum ALT and GGT, analyzed by the two ALDH2 genotypes, NN and NM, in the subjects who consumed 200 g or more of alcohol per week obtained by GLM

Fig. 3 Least square means and standard errors of systolic blood pressure and serum uric acid and HDL-cholesterol levels divided by alcohol consumption levels and genotypes of P450 2E1, adjusted for the effects of age and body mass index.

Table 4 Least square means (LSM) and standard errors (SE) of DBP and serum ALT and GGT levels, adjusted for age, body mass index and alcohol consumption, in the subjects who consumed 200g or more of alcohol per week by genotypes of ALDH2.

Genotype of ALDH2	N	Parameters		
		DBP	ALT ^a	GGT ^a
		LSM (SE)	LSM (SE)	LSM (SE)
NN	129	85.2 (0.8)	29.5 (1.04)	40.1 (1.06)
NM	34	83.0 (1.5)	23.2 (1.09)	37.8 (1.13)
MM	0	-	-	-

a: Geometric mean and geometric standard error

b: Significant difference in the means; *: $P < 0.05$.

c: The MM genotype was not found in the subjects who consumed 200g or more of alcohol per week.

d: Abbreviations; N: number of subjects, DBP: diastolic blood pressure, ALT: serum alanine aminotransferase, GGT: gamma-glutamyl transferase.

Table 5 Least square means (LSM) and standard errors (SE) of SBP and serum UA and HDLc levels, adjusted for age, body mass index and alcohol consumption, in the subjects who consumed 200g or more of alcohol per week by genotypes of P450 2E1.

Genotype of P450 2E1	N	Parameters		
		SBP	UA	HDLc
		LSM (SE)	LSM (SE)	LSM (SE)
c1/1	90	126 (1.5)	6.06 (0.13)	50.0 (1.06)
c1/2	66	122 (1.8)	5.86 (0.15)	49.9 (1.24)
c2/2	7	135 (5.5)	6.98 (0.46)	48.1 (3.80)

a: Significant difference in the means; *: $P < 0.05$, †: $P < 0.10$.

b: Abbreviations; N: number of subjects, SBP: systolic blood pressure, UA: serum uric acid, HDLc: HDL-cholesterol.

Table 6 Prevalence (%) of hypertension (BP $\geq 140/90$ mmHg) in the 367 middle-aged men by alcohol consumption levels and genotypes of P450 2E1.

Genotype of P450 2E1	Alcohol consumption (g/week)				Total
	None	<200	200-399	400-	
c1/1	28.0%	25.8	32.1	48.6	27.0
c1/2	8.7	22.8	38.5	17.5	21.9
c2/2	0.0	42.9	66.7	50.0	41.2
Total	17.6	25.5	35.4	33.3	28.3

a: Number of subjects in each category is shown in Table 2.

b: Increases in the prevalence of hypertension with increasing alcohol consumption levels were significant ($P < 0.05$), but the difference in the three P450 2E1 genotypes was not significant by multiple logistic regression analysis.

Table 7 Prevalence (%) of hyperuricemia (≥ 8.0 mg/dl) in the 367 middle-aged men by alcohol consumption levels and genotypes of P450 2E1.

Genotype of P450 2E1	Alcohol consumption (g/week)				Total
	None	<200	200-399	400-	
c1/1	12.0%	9.0	11.3	2.7	8.8
c1/2	4.3	3.5	3.8	2.5	3.4
c2/2	0.0	14.3	33.3	25.0	17.6
Total	7.8	7.2	9.8	3.7	7.1

a: Number of subjects in each category is shown in Table 2.

b: No statistically significant difference in the prevalence of hyperuricemia was detected among the different alcohol consumption levels and the different P450 2E1 genotypes by multiple logistic regression analysis.

analyses are shown in Table 4. Only serum ALT showed a significantly higher value in NN genotypes than in NM genotypes. No significant differences were found in DBP and serum GGT between the two genotypes. Table 5 shows LSMs of SBP and serum UA and HDLc analyzed by the three P450 2E1 genotypes in the drinkers consuming 200g or more per week. SBP and serum UA were both highest for the c2/2 genotypes and the differences from those in c1/2 genotypes were significant ($p < 0.05$) and differences from those in c1/1 genotypes were of borderline significance ($p < 0.10$). No significant difference was, however, found in serum HDLc levels.

The associations of alcohol consumption and the genotypes of P450 2E1 with the prevalence of hypertension (BP $\geq 140/90$ mmHg) and that of hyperuricemia (serum UA ≥ 8 mg/dl) are shown in Tables 6 and 7, respectively. Multiple logistic regression analyses adjusting for the effects of age and BMI showed that the prevalence of hypertension was increased significantly with increasing BMI and the levels of alcohol consumption. The prevalence of hyperuricemia was related to BMI, but not to the four levels of alcohol consumption. Both hypertension and hyperuricemia were somewhat more prevalent in alcohol consumers having the c2/2 genotype of P450 2E1 than in those with the other genotypes, but the differences were not statistically significant.

Discussion

We analyzed for polymorphism in ALDH2 and P450 2E1 genotypes as related to sensitivity to mild-to-moderate alcohol consumption in middle-aged Japanese men recruited from an occupational population. A marked difference in alcohol consumption was observed in the subjects having different genotypes of ALDH2. Those with NN genotype drank most followed by the NM genotype. In the 24 MM genotypes, 15 (63%) were essentially non-drinkers, and the remaining 9 did not drink more than 200g/week. Their alcohol consumption was thus not large enough to show adverse alcohol-induced health effects. These data are consistent with previous findings that polymorphism in the ALDH2 gene is closely related to drinking behavior^{25,26} and that homozygotes for mutant ALDH2 genes are generally free of adverse alcohol-induced health effects owing to their limited ability to consume alcohol¹⁴⁻¹⁶.

On the other hand, mean alcohol consumption was not different among the subjects with three different genotypes of P450 2E1. However, alcohol consumption was larger in the subjects having the c2/2 genotype in the case of normal homozygotes for the ALDH2 gene, while it was smaller in those having the c2 gene and mutant ALDH2 gene. This interactive effect of ALDH2 and P450 2E1 genes on alcohol consumption was statistically significant when the four levels of alcohol consumption in the subjects were scored from 0 to 3. Though this interactive effect has not been reported by other researchers, it may not be unreasonable. If people having the c2 gene catabolize alcohol more rapidly and produce more acetaldehyde as suggested by some researchers^{4,11,12}, blood acetaldehyde may soon reach an intolerable level after alcohol consumption when ALDH2 is inactive and cannot catabolize acetaldehyde in people having a mutant ALDH2 gene, while people homozygous for the normal ALDH2 gene may catabolize it effectively and be able to drink more.

Drinking habits in people are strongly affected by various socio-cultural factors other than genetic background. Though all the present subjects were recruited from the same workplace, and their socioeconomical and cultural status might not have been so different, the influence of sociocultural factors on alcohol consumption was not analyzed for the present subjects. This should be taken into account in interpreting the study results. Further, possible artificial effects due to the poor reliability of self-reported alcohol consumption data should also be considered.

GLM analyses showed that ALDH2 genotypes were significantly related to higher DBP and serum ALT and GGT levels

even after adjusting for the effects of age and BMI, and that P450 2E1 genotypes were related to higher SBP and serum UA levels and lower serum HDLc level. As shown in Fig. 2, higher DBP and serum ALT and GGT levels were most marked in non-drinkers having the NN genotype of ALDH2. The differences in DBP and serum GGT between the NN and NM genotypes were not significant in the subjects consuming 200g or more of alcohol per week, though there was still a significant difference in serum ALT levels (Table 4). Thus, the higher DBP and serum GGT levels in the NN genotypes were not related to alcohol consumption. Even in these elevated BP and serum hepatic enzyme are assumed to reflect manifestations induced by alcohol consumption, these results contradict the assumption that elevated blood acetaldehyde due to the mutant ALDH2 gene exerts toxic effects on liver cells⁸⁻¹⁰. Although the exact reasons for these contradictory findings are unclear, one possible explanation is underreporting of alcohol consumption in the NN subjects, who could drink much more than the other genotypes of ALDH2.

On the other hand, as shown in Fig. 3, alcohol consumers, especially mild to moderate alcohol consumers having the c2/2 genotype of P450 2E1 showed higher SBP and serum UA levels than those having the other genotypes. The differences in SBP and serum UA between c2/2 and c1/2 genotypes of P450 2E1 were statistically significant in the subjects who consumed 200g or more of alcohol per week. Those differences between c2/2 and c1/1 genotypes were both of borderline significance (Table 5). Prevalences of hypertension and hyperuricemia were also somewhat higher in the alcohol consumers with the c2/2 genotype than in the other P450 2E1 genotypes, but the differences were not significant (Tables 6 and 7).

In the present subjects, elevation of SBP and the prevalence of hypertension were significantly related to increases in alcohol consumption while those of serum UA and hyperuricemia were not related to it. Serum UA levels and hyperuricemia in the present subjects were mainly related to increased obesity. Elevation of serum UA is, however, one of the typical early health effects caused by excessive alcohol consumption^{27,28} as well as BP elevation^{29,30}, and elevated SBP and serum UA in the subjects having the c2/2 genotype of P450 2E1 were found in alcohol consumers but not in non-drinkers, suggesting the relation of alcohol to the higher values. To confirm that P450 2E1 genotype of c2/2 is related to higher sensitivity to elevations of BP and serum UA, in other words, to prove different dose-response relationships among the different P450 2E1 genotypes, the interaction between alcohol consumption and the genotypes as related to these parameters should be significant in statistical analyses. However, the interactions were not significant as mentioned above.

On the other hand, serum HDLc is also well known to increase with alcohol consumption^{31,32}. Indeed, serum HDLc levels were positively related to increases in alcohol consumption in the present subjects. However, in contrast to the findings on SBP and serum UA, the subjects having the c2/2 gene of P450 2E1 showed rather lower serum HDLc levels in comparison with the other genotypes. A lower level of serum HDLc was, however, observed in non-drinkers and minimal to mild drinkers, but not in moderate drinkers. The differences in HDLc among the three P450 2E1 genotypes in the subjects who consumed 200g or more of alcohol per week were not statistically significant (Table 5). Thus the relation of lower serum HDLc in the c2/2 subjects to

their alcohol consumption remains unclear.

Some results of the present study suggested the possibility that the c2/2 genotype of P450 2E1 is related to a higher sensitivity to elevation of BP and serum UA after alcohol consumption in middle-aged Japanese men, but the biological links are obscure. The exact biological mechanisms underlying the elevations of BP and serum UA due to alcohol consumption also remain unclear, but our previous studies suggested the possible association of progression of ALD, mainly hepatic steatosis, and related insulin resistance and hyperinsulinemia with BP elevations³³⁻³⁵. Elevation of serum UA has also been suggested to be a sign of hyperinsulinemia³⁶. If the c2 gene produces more acetaldehyde after alcohol consumption because of higher alcohol oxidation and results in higher sensitivity to ALD as suggested by some researchers^{4, 11, 12}, the mechanism may also be involved in BP and serum UA elevations in alcohol consumers having the c2/2 genotype. However, serum hepatic enzymes levels were not statistically different in the present drinkers having different genotypes of P450 2E1, and the link between higher sensitivity to ALD and elevations of BP and serum UA remains unclear.

The present study results can be summarized as follows: polymorphism in the ALDH2 gene strongly effects drinking

behavior of middle-aged Japanese men, and different P450 2E1 genotypes may also affect it interactively with the ALDH2 gene. Polymorphism in the ALDH2 gene does not affect sensitivity to alcohol-induced health effects, but people having the c2/2 type of P450 2E1 gene may be more sensitive to BP and serum UA elevations after alcohol consumption. These results are, however, not conclusive at present since the c2/2 genotype was only found in 5% of the subjects and the effects of the genotype on alcohol consumption and sensitivity to alcohol-induced health effects were statistically very small. Larger-scale studies are thus required to confirm these findings.

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