Original Article

Common Single Nucleotide Polymorphisms in Japanese Patients with Essential Hypertension: Aldehyde Dehydrogenase 2 Gene as a Risk Factor Independent of Alcohol Consumption

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Essential hypertension (EH) is a multifactorial disorder determined by the interaction of environmental and genetic factors. EH patients' responses to these factors may vary, depending on differences in their genes that determine the physiological systems that mediate the response. The purpose of this investigation was to clarify the contributions of genetic background and lifestyle to EH through an association study using some common single nucleotide polymorphisms (SNPs) that should have functional effects on EH phenotypes. We studied the associations between common SNPs of some causal genes related to EH and lifestyle in a Japanese population. The variants of the causal genes were selected based on their functions, including: obesity (adrenergic, β-3-, receptor: ADRB3), alcohol consumption (aldehyde dehydrogenase 2: ALDH2), water-electrolyte metabolism (guanine nucleotide binding protein [G protein], β polypeptide 3: GNB3), glycometabolism (peroxisome proliferator–activated receptor γ: PPARG), lipometabolism (cholesteryl ester transfer protein, plasma: CETP), atherosclerosis (5,10-methylenetetrahydrofolate reductase [NADPH]: MTHFR), and cellular behavior (gap junction protein, α 4, 37 kD: GJA4). Case-control association analysis showed a significant association between EH and both the ALDH2 (Lys487Glu) and GNB3 (C825T) variants. Logistic regression analysis indicated that body mass index (BMI) is an important risk factor for EH, and that the GG (Glu/Glu) genotype of ALDH2 was an independent risk factor for EH overall and especially for EH in males. There was no interaction between the ALDH2 genotype and alcohol consumption overall or in male subjects. Our results suggest that the ALDH2 genotype is associated with EH independently of alcohol consumption. (*Hypertens Res* 2007; 30: 585–592)

***Key Words*: single nucleotide polymorphisms, essential hypertension, gene-environment interaction, common variants**

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Introduction

Hypertension affects 1 billion people worldwide and is implicated in 7.1 million deaths each year due to ischemic heart disease and stroke (www.who.int/en/index.html). Essential hypertension (EH) is a multifactorial disorder caused by the interaction of environmental and genetic factors. It is most likely that there are several causal genes, which together account for 30% to 50% of the blood pressure variation found among individuals (*1*). It is clear from familial and epidemiological studies that hypertension occurs as a result of a complex interplay between genetic and environmental lifestyle exposures (*2*). EH subjects happen to have inherited an aggregate of genes related to hypertension and/or have been exposed to exogenous factors that predispose them to hypertension.

It is quite remarkable that obesity and salt intake have consistently been shown to be risk factors for hypertension worldwide. Some of the other well-recognized risk factors are alcohol intake, inactivity, and psychosocial stress (*3*). Lifestyle factors have long been recognized as playing an important role in the pathogenesis of EH. Individuals may vary in their responses to these factors depending on differences in their genes that determine the way their physiological systems mediate responses.

During the last several years, many genetic susceptibility variants have been reported to be associated with multifactorial diseases. A few causal variants have been proven to have a functional effect on gene expression or protein structure, resulting in phenotypic differences. Furthermore, among the variants mentioned above, very few are considered common variants. To the best of our knowledge, the human aldehyde dehydrogenase 2 (ALDH2) gene (*4*) is the most famous variant proven to have a relationship between a genetic variant and alcohol consumption as a phenotype. No reports have examined the relationship between multiple common variants and EH.

The purpose of this investigation was to clarify the contributions of genetic background and lifestyle to EH through an association study using common variants that are known to have functional effects in the phenotypes of multifactorial disorders. In particular, we studied the associations between common variants of some causal genes related to lifestyle and EH in a Japanese population. The candidate genes were selected based on their functions, and included: alcohol consumption (aldehyde dehydrogenase 2: ALDH2) (*4*), obesity (adrenergic, β-3-, receptor: ADRB3) (*5*), atherosclerosis (5,10-methylenetetrahydrofolate reductase [NADPH]: MTHFR) (*6*), glycometabolism (peroxisome proliferator–activated receptor γ: PPARG) (*7*), water-electrolyte metabolism (guanine nucleotide binding protein [G protein], β polypeptide 3: GNB3) (*8*), lipometabolism (cholesteryl ester transfer protein, plasma: CETP) (*9*), and cellular behavior (gap junction protein, α 4, 37 kD: GJA4) (*10*). Based on their effects on gene expression or protein structure (Table 1), we chose seven common variants of these causal genes.

Methods

Subjects

The EH group consisted of 261 EH patients diagnosed according to the following criteria: sitting systolic blood pressure (SBP) >160 mmHg and/or diastolic blood pressure (DBP) >100 mmHg on three occasions within 2 months after the first blood pressure reading. None of the subjects were using antihypertensive medications. Subjects diagnosed as having secondary hypertension were excluded. We also studied 271 normotensive (NT) healthy subjects as controls. None of the NT subjects had a family history of hypertension, and they all had an SBP <130 mmHg and a DBP <85 mmHg. A family history of hypertension was defined as a prior diagnosis of hypertension in grandparents, uncles, aunts, parents, or siblings. Daily alcohol intake was 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" (180 mL), a traditional Japanese drinking unit; a "gou" of Japanese sake contains 20 g of ethanol, while a similar amount (180 mL) of Japanese "shochu" contains 50 g of ethanol, a medium-sized bottle of beer (550 mL) contains 22 g of ethanol, two single shots of whiskey (60 mL) contain 20 g of ethanol, and a glass (120 mL) of wine contains 12 g of ethanol. Both the EH patients and the NT control subjects were recruited from the northern part of Tokyo, and informed consent was obtained from each individual according to a protocol approved by the Human Studies Committee of Nihon University.

Biochemical Analysis

Plasma total cholesterol and high-density lipoprotein (HDL) cholesterol concentrations and serum creatinine and uric acid concentrations were measured at the Clinical Laboratory Department of Nihon University Hospital using previously described methods (*11*).

Genotyping of Single Nucleotide Polymorphisms

After consulting public databases, including PubMed and Online Mendelian Inheritance in Men (OMIM), we selected 7 causal genes that have been characterized and whose association with alcoholism, obesity, diabetes, lipid levels, salt intake, and other metabolic factors has been suggested. We further selected 7 common variants of these genes located in the exons or splice donors that might be expected to affect the function or expression of the encoded protein (Table 1). We

Table 1. Gene Polymorphisms Examined for Association

Gene	Symbol	mRNA position	Polymorphism	Amino acid change	Popular name	Region	dbSNP ID	Assay ID	Locus	Reference
Aldehyde dehydrogenase 2 family (mitochondrial)	ALDH2	1951	G→A	Lys504Glu	Lys504Glu	Exon 12	rs671	C_11703892_10	12q24.2	(*4*)
Adrenergic, β-3-, receptor	ADRB3	387	T→C	Trp64Arg	Trp64Arg	Exon 1	rs4994	C_2215549_20	8p12-p11.2	(*5*)
5,10-Methylenetetrahydrofolate reductase (NADPH)	MTHFR	716	C→T	Ala222Val	C677T	Exon 5	rs1801133	C_1202883_20	1p36.3	(*6*)
Peroxisome proliferator–activated receptor γ	PPARG	132	C→G	Pro12Ala	Pro12Ala	Exon 2	rs1805192	C_1129864_10	3p25	(*7*)
Guanine nucleotide binding protein (G protein), β polypeptide 3	GNB3	1230	C→T	41amino acids deletion	C825T	Exon 10	rs5443	C_2184734_10	12p13	(*8*)
Cholesteryl ester transfer protein, plasma	CETP	1506	A→G	Asp459Gly	D442G	Exon 15	rs2303790	C_790072_1_	16q21	(*9*)
Gap junction protein, α 4, 37 kD (connexin 37)	GJA4	1043	C→T	Ser 319 Pro	C1019T	Exon 2	rs1764391		1p35.1	(*10*)

Table 2. Characteristics of Study Participants

	Total			Men			Women		
	NT	EH	*p* value	NT	EH	*p* value	NT	EH	*p* value
Number of subjects	271	261		182	170		89	91	
Age (years)	51.5±8.6	51.1±5.6	0.525	52.0±6.7	51.0±5.8	0.145	50.4±11.5	51.1±5.3	0.563
BMI (kg/m^2)	22.7±3.6	24.4±4.4	<0.001*	22.8±3.6	24.4±4.6	<0.001*	22.5±3.5	24.4±4.1	0.002*
SBP (mmHg)	112.8±10.8	173.6±20.0	<0.001*	113.2±10.4	171.7±19.0	<0.001*	112.1±11.5	177.1±21.4	<0.001*
DBP (mmHg)	69.7±8.5	105.4±13.4	<0.001*	70.5±8.0	105.4±13.4	<0.001*	68.2±9.2	70.5±8.0	<0.001*
Pulse (beats/min)	73.9±14.3	77.6±15.4	0.011*	73.2±15.6	77.4±16.1	0.032	75.2±11.1	78.2±13.9	0.181
Creatinine (mg/dL)	0.8±0.2	0.9±0.3	0.434	0.9±0.2	0.9±0.2	0.114	0.7±0.2	0.7±0.2	0.569
Total cholesterol (mg/dL)	201.4±42.1	210.6±35.8	0.011*	196.8±39.0	204.9±32.9	0.057	210.8±46.6	220.5±38.5	0.133
HDL cholesterol (mg/dL)	56.2±17.4	57.4±17.8	0.459	53.9±15.7	55.8±17.0	0.973	61.0±19.6	63.6±17.6	0.372
Uric acid (mg/dL)	5.4±1.5	5.6±1.6	0.157	5.8±1.4	6.1±1.5	0.057	4.6±1.3	4.7±1.6	0.676
Hyperlipidmia (%)	19.2	25.3	0.096	15.4	22.4	0.102	27.0	30.8	0.624
Diabetes mellitus (%)	3.3	9.2	0.006*	3.9	10.6	0.021*	2.3	6.6	0.278
Alcohol frequency (%)	58.9	67.8	0.061	71.1	83.3	0.015*	33.9	33.9	0.570
Alcohol consumption (g/day)	21.7±37.3	32.7±45.5	0.010*	29.0±43.2	43.6±50.8	0.013*	7.2±11.5	12.5±22.3	0.101
Smoking (%)	41.7	53.8	0.013*	52.5	63.8	0.056	20.0	34.5	0.057

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high density lipoprotein; NT, normotension; EH, essential hypertension. *Indicates significant difference.

examined the relationship between the genotypes of these genes and hypertension in the study's 532 participants.

Blood samples were collected from all participants, and genomic DNA was extracted from the peripheral blood mononuclear cells by standard procedures. Genotyping was performed using an Assays-on-Demand® and Assays-on-Design kit (Applied Biosystems, Branchburg, USA) (*12*). Both kits included TaqMan PCR. In the 5′ nuclease assay, discrimination occurs during the polymerase chain reaction (PCR) because allele-specific fluorogenic probes, when hybridized to the template, are cleaved by the 5′ nuclease activity of Taq polymerase. The cleavage leads to increased emission of a reporter dye that otherwise is quenched by the dye TAMRA. Each 5′ nuclease assay requires two unlabeled PCR primers and two allele-specific probes. Each probe is labeled with a reporter dye at the 5′ end and TAMRA at the 3′ end. Both VIC and FAM were used as reporter dyes. The PCR method was done using the TaqMan Universal Master Mix (Applied Biosystems) in a 25 μL final reaction volume containing (final concentrations) 50 ng DNA, 700 nmol/L primer,

Table 3. Genotype Distribution in Normotensives (NT) and Patients with Essential Hypertension (EH)

	Total			Men			Women		
	NT	EH	*p* value	NT	EH	*p* value	NT	EH	*p* value
Number of participants	271	261		182	170		89	91	
Variants									
ALDH2									
Genotype									
AA	21 (0.077)	14 (0.054)	0.008*	14 (0.077)	7 (0.041)	0.001*	7 (0.079)	7 (0.077)	0.233
AG	114 (0.421)	81 (0.310)		78 (0.429)	45 (0.265)		36 (0.404)	48 (0.527)	
GG	136 (0.502)	166 (0.636)		90 (0.494)	118 (0.694)		46 (0.517)	36 (0.396)	
Allele									
A	156 (0.288)	109 (0.209)	0.003*	106 (0.291)	59 (0.174)	<0.001*	50 (0.281)	62 (0.341)	0.221
G	386 (0.712)	413 (0.791)		258 (0.709)	281 (0.826)		128 (0.719)	120 (0.659)	
ADRB3									
Genotype									
TT	198 (0.731)	170 (0.651)	0.11	136 (0.747)	110 (0.647)	0.093	62 (0.697)	60 (0.659)	0.853
TA	66 (0.243)	85 (0.326)		42 (0.231)	57 (0.335)		24 (0.270)	28 (0.308)	
AA	7 (0.026)	6 (0.023)		4 (0.022)	3 (0.018)		3 (0.033)	3 (0.033)	
Allele									
T	462 (0.852)	425 (0.814)	0.094	314 (0.863)	277 (0.815)	0.083	148 (0.831)	148 (0.813)	0.65
A	80 (0.148)	97 (0.186)		50 (0.137)	63 (0.185)		30 (0.169)	34 (0.187)	
MTHFR									
Genotype									
CC	104 (0.384)	83 (0.318)	0.275	70 (0.385)	61 (0.359)	0.875	34 (0.382)	22 (0.242)	0.121
CT	123 (0.454)	129 (0.494)		81 (0.445)	78 (0.459)		42 (0.472)	51 (0.560)	
TT	44 (0.162)	49 (0.188)		31 (0.170)	31 (0.182)		13 (0.146)	18 (0.198)	
Allele									
C	331 (0.611)	295 (0.565)	0.131	221 (0.607)	200 (0.588)	0.609	110 (0.618)	95 (0.522)	0.512
T	211 (0.389)	227 (0.435)		143 (0.393)	140 (0.412)		68 (0.382)	87 (0.478)	
PPARG									
Genotype									
CC	261 (0.963)	245 (0.939)	0.192	177 (0.973)	158 (0.929)	0.059	84 (0.944)	87 (0.956)	0.707
CG	10 (0.037)	16 (0.061)		5 (0.027)	12 (0.071)		5 (0.056)	4 (0.044)	
GG	0	0		0	0		0	0	
Allele									
C	532 (0.982)	506 (0.969)	0.198	359 (0.986)	328 (0.965)	0.063	173 (0.972)	178 (0.978)	0.71
G	10 (0.018)	16 (0.031)		5 (0.014)	12 (0.035)		5 (0.028)	4 (0.022)	
GNB3									
Genotype									
CC	72 (0.266)	78 (0.299)	0.036*	50 (0.275)	57 (0.335)	0.022*	22 (0.247)	21 (0.231)	0.732
CT	148 (0.546)	115 (0.441)		100 (0.549)	69 (0.406)		48 (0.539)	46 (0.505)	
TT	51 (0.188)	68 (0.260)		32 (0.176)	44 (0.259)		19 (0.214)	24 (0.264)	
Allele									
C	292 (0.539)	271 (0.519)	0.522	200 (0.549)	183 (0.538)	0.765	92 (0.571)	88 (0.484)	0.527
T	250 (0.461)	251 (0.481)		164 (0.451)	157 (0.462)		86 (0.483)	94 (0.516)	
CETP									
Genotype									
AA	256 (0.945)	240 (0.920)	0.247	170 (0.934)	153 (0.900)	0.321	86 (0.966)	87 (0.956)	0.612
AG	15 (0.055)	19 (0.073)		12 (0.066)	16 (0.094)		3 (0.034)	3 (0.033)	
GG	0	2 (0.007)		0	1 (0.006)		0	1 (0.011)	
Allele									
A	527 (0.972)	499 (0.956)	0.454	352 (0.967)	322 (0.947)	0.02*	175 (0.983)	177 (0.973)	0.494
G	15 (0.055)	23 (0.044)		12 (0.033)	18 (0.053)		3 (0.017)	5 (0.027)	

Table 3. Continued

	Total			Men			Women		
	NT	EH	*p* value	NT	EH	*p* value	NT	EH	*p* value
GJA4									
Genotype									
CC	164 (0.605)	170 (0.651)	0.271	110 (0.604)	112 (0.659)	0.382	54 (0.607)	58 (0.637)	0.672
CT	106 (0.391)	91 (0.349)		71 (0.039)	58 (0.341)		35 (0.393)	33 (0.363)	
TT	1 (0.004)	0		1 (0.006)	0		0	0	
Allele									
C	434 (0.801)	431 (0.826)	0.297	291 (0.799)	282 (0.829)	0.307	143 (0.803)	149 (0.819)	0.711
T	108 (0.199)	91 (0.174)		73 (0.201)	58 (0.171)		35 (0.197)	33 (0.181)	

*Indicates significant difference.

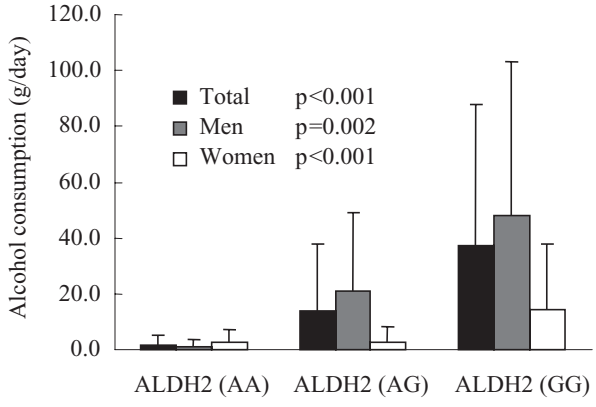

Fig. 1. *Comparison of alcohol consumption between the different ALDH2 genotype groups.*

and 100 nmol/L probe. The thermal cycling conditions were: 95°C for 10 min, then 50 cycles of 92°C for 15 s, and finally 60°C for 1 min. Thermal cycling was performed using the GeneAmp 9700 system.

Each 96-well plate contained 80 samples of unknown genotype and four reactions with reagents but no DNA. The homozygote and no-DNA control samples were necessary for the SDS 7700 signal processing, as outlined in the TaqMan Allelic Discrimination Guide (Applied Biosystems). Direct sequencing or single-stand conformation polymorphism (SSCP) was used to confirm control sample genotypes. The PCR plates were read on the ABI 7700 instrument using the end-point analysis mode of the SDS version v16.3 software package (Applied Biosystems). Genotypes were determined visually based on the dye-component fluorescent emission data depicted in SDS's *X*-*Y* scatter-plot. Genotypes were also determined automatically by the software's signal processing algorithms. The results of each scoring method were saved in two separate output files and compared later.

Statistical Analysis

The data are presented as means±SD. The Hardy-Weinberg equilibrium was assessed using χ^2 analysis. The overall distribution of alleles was analyzed using 2×2 contingency tables, and the distributions of the genotypes between EH patients and NT subjects were tested using a two-sided Fisher's exact test. Statistical significance was established at $p<0.05$. Differences in clinical data between the EH and NT groups were assessed by analysis of variance (ANOVA) followed by Fisher's protected least significant difference (PLSD) test.

To assess the contributions of the confounding factors, we performed logistic regression analysis with hypertension as a dependent variable and the following independent variables: body mass index (BMI), alcohol consumption status (0=nondrinker, 1=drinker), metabolic variables (0=no history of either diabetes mellitus or hyperlipidemia; 1=positive history of either), and genotype of each single nucleotide polymorphism (SNP; no susceptibility homozygote + heterozygote=0, susceptibility homozygote=1). The *p* value, odds ratios, and 95% confidence intervals (CIs) were calculated. Differences in alcohol consumption as continuous variables between genotypes were analyzed by one-way ANOVA. A *p* value of less than 0.05 was considered statistically significant. Statistical analyses were done using SPSS software for Windows, version 12 (SPSS Inc., Chicago, USA).

Results

The clinical characteristics of the EH patients and the NT subjects are shown in Table 2. The SBP, DBP, BMI, plasma total cholesterol concentrations, and pulse rate were significantly higher in the EH group than in the NT group. No significant differences in age, serum creatinine concentration, or serum uric acid concentration were observed between the two groups. Male subjects with EH had a higher prevalence of diabetes mellitus and were more likely to drink alcohol, but the prevalence of hyperlipidemia was not significantly different between EH and NT in men.

The distributions of the genotypes and alleles of each SNP

Table 4. Odds Ratios (OR) and 95% Confidence Intervals (CI) for Each Risk Factor and SNP Genotype Associated with Essential Hypertension

Risk factor	Total			Men			Women		
	OR	95% CI	*p* value	OR	95% CI	*p* value	OR	95% CI	*p* value
BMI	1.12	1.06–1.19	<0.001*	1.11	1.04–1.19	0.004*	1.14	1.04–1.25	0.004*
Total cholesterol	1.00	1.00–1.01	0.285		n.u.			n.u.	
Diabetes mellitus	1.81	0.73–4.50	0.204	1.83	0.67–5.04	0.241		n.u.	
Alcohol consumption	1.00	1.00–1.01	0.323	1.01	1.00–1.01	0.146		n.u.	
Smoking	1.60	1.02–2.51	0.041*		n.u.			n.u.	
ALDH2	1.60	1.02–2.51	0.039*	1.93	1.12–3.31	0.018*	0.98	0.53–1.79	0.934
GNB3	1.53	0.91–2.57	0.106	1.38	0.74–2.56	0.310	1.10	0.53–2.26	0.797
ALDH2 and alcohol consumption[#]	1.01	0.99–1.02	0.257	1.00	0.98–1.02	0.999	0.98	0.93–1.04	0.182

*Indicates significant difference. SNP, single nucleotide polymorphism; BMI, body mass index; n.u., not used. [#]The interaction between the ALDH2 genotype and alcohol consumption were analyzed.

in the 261 EH patients and 271 NT control subjects are displayed in Table 3. The overall genotype distributions of the ALDH2 (Lys504Glu) and GNB3 (C825T) variants were significantly different between the groups. The overall genotype distributions of other SNPs did not differ significantly. Among men, the allelic distributions of ALDH2 and CETP genes were significantly different between the groups. The distribution of the ALDH2 genotype was also significantly different between drinkers and those who drank rarely or never ($p<0.001$). The ALDH2 genotype was significantly associated with alcohol consumption overall among both males and females (Fig. 1).

A logistic regression analysis was done using variables that showed significant differences in the association studies: BMI, a history of diabetes mellitus, level of total cholesterol, smoking, ALDH2 and GNB3 genotypes, *etc*. The odds ratios, 95% CIs, and *p* values are shown in Table 4. Overall, BMI, smoking, and the GG (Glu/Glu) genotype of ALDH2 were independent risk factors for EH. There was no interaction between the ALDH2 genotype and alcohol consumption overall among either males or females. In men, the odds ratio for the presence of hypertension for the GG (Glu/Glu) genotype of ALDH2 compared with the other genotype was 1.93 (95% CI=1.12–3.31). In women, only BMI was significantly associated with EH.

Discussion

Hypertension is a common phenotype that is considered a multifactorial trait. In concert with environmental or biological factors, genetic factors are thought to raise or lower blood pressure (*3*).

Approximately 50% of hypertensive patients are salt-sensitive; their blood pressure increases in response to sodium intake or volume expansion. The mechanisms that underlie salt sensitivity have not been completely elucidated, although there is evidence that they may be genetically determined. The C825T genetic variant of the GNB3—which is the C-to-T base substitution in exon 9 of the gene that results in the protein lacking 41 amino acids—is considered a genetic risk for developing salt-sensitive hypertension (*13*). The frequency of GNB3/825T has been found to be significantly higher in the Japanese population than in the Caucasian population (*8*). Our results showed that the C825T genotype of GNB3 was significantly different between the EH and control groups ($p<0.05$). This suggests that the GNB3 gene variant is associated with EH in the Japanese population.

The relationship between alcohol consumption and blood pressure elevation is well documented (*14*). Although the mechanism is not clear, it may be mediated partly by the speed of alcohol metabolism, the types of alcoholic beverages consumed, the regularity of drinking, and nutritional status. In the present study, the prevalence of a drinking habit was significantly higher in the EH group in men than in the NT group in men.

ALDH2, the second enzyme of the ethanol metabolic pathway, converts acetaldehyde to acetic acid and plays a major role in acetaldehyde detoxification. A deficiency of ALDH2 activity results from a single nucleotide (G-to-A) substitution at codon 504, which produces a Glu→Lys change at position 504 on the β-subunit and causes the isozyme to be inactive (*15*). ALDH2 enzyme inactivation plays a major role in producing unpleasant symptoms after drinking, such as facial flushing, palpitations, headache, vomiting, and sweating. A study of racial differences in alcohol sensitivity demonstrated that about 50% of Japanese and Chinese populations had a defect in ALDH2 enzyme activity (*16*).

Those with the AA genotype of ALDH2 have a high intolerance to alcohol and do not generally drink alcoholic beverages. In contrast, ALDH2 heterozygotes have an intermediate tolerance and drink about half as much as GG (Glu/Glu) homozygotes overall. However, ALDH2 heterozygotes attain substantially higher blood concentrations of acetaldehyde if they drink alcohol (*17*). Thus, the ALDH2 variant can affect drinking behavior by affecting alcohol metabolism. In our experiment, the ALDH2 genotype actually affected the

amount of alcohol consumed (Fig. 1). Few drinkers had the AA genotype of ALDH2. Although our data showed that subjects with the GG (Glu/Glu) genotype were more likely to have a drinking habit and a higher prevalence of hypertension, the logistic regression analysis revealed that the GG (Glu/Glu) genotype was an independent risk factor for EH overall and especially for EH in males. There was no interaction between the ALDH2 genotype and alcohol consumption overall or in male subjects. Finally, our results suggest that the ALDH2 genotype is associated with EH independently of alcohol consumption.

Several studies in Japan have examined alcohol drinking in relation to hypertension (*18–20*). Two recent reports examined the relationship between ALDH2 genotypes and hypertension in the general population. Amamoto *et al*. found no causal relationship between hypertension and the ALDH2 genotypes *per se* after excluding some confounding factors, particularly alcohol drinking, in the general population (*21*). Takagi *et al*. determined the influence of the ALDH2 genotypes on blood pressure in a large cohort in a population-based study (the Suita study) (*22*). The results of that study also revealed that the GG (Glu/Glu) genotype was a potent risk factor for high blood pressure among men, and that the ALDH2 genotype does not affect sensitivity to alcohol's effect on blood pressure. Both investigations were performed in a general population in Japan, while our study design was a case-control association analysis using EH cases. Although many case-control studies have used logistic regression analysis (*23*), such analysis in case-control studies using population stratification can sometimes yield highly significant results (*24*). Therefore, in our study, it would be unwise to hastily conclude that the GG (Glu/Glu) genotype is a powerful factor independent of alcohol consumption for EH. Unexpectedly, the effect of the ALDH2 genotype on blood pressure or hypertension was almost the same in Takagi's results (*22*) as in our study. Thus, this result is very interesting for identifying EH susceptibility genes beyond lifestyle factors such as drinking.

Recently, the enzyme activity of ALDH2 has been reported to prevent acetaldehyde-induced cell injury *via* extracellular signal–regurated kinase (ERK)1/2 and p38 mitogen-activated protein (MAP) kinase in human vein endothelial cells (*25*). Moreover, it was reported that ALDH2 catalyzes mitochondrial bioactivation of nitroglycerin by the formation of a reactive nitric oxide–related intermediate that activates soluble guanylate cyclase (*26*). This may explain why ALDH2's effect on blood pressure or vessel dilation is independent of alcohol consumption.

Genetic association studies have identified genes associated with gender-specific susceptibility to EH (*27*). The underlying reason for the present study's finding of a positive association between EH and the ALDH2 genotypes in men is unclear. Female hormones may act to protect women from developing high blood pressure (*28*).

Further research involving studies with more detailed data may help clarify the unresolved interaction between alcohol consumption levels and patterns, the relevant ALDH2 genotypes, and hypertension.

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