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ALDH2 and CYP2E1 genotypes, urinary acetaldehyde excretion and the health consequences in moderate alcohol consumers

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Abstract Deficiencies in mitochondrial low-Km aldehyde dehydrogenase (ALDH2) activity, and consequently high blood acetaldehyde levels, have been suggested to relate to various diseases in Japanese, including esophageal cancer. In the present study, 200 men aged 35–59 years randomly selected from an occupational population were analyzed for the association of ALDH2 genotypes and cytochrome P450-2E1 (CYP2E1) genotypes with the urinary excretion of acetaldehyde (which is bound to some chemicals in the urine) and with common alcohol-related health consequences. Urinary acetaldehyde excretion was increased, reflecting increased alcohol consumption even in this moderate alcohol-consuming population. Neither the ALDH2 nor the CYP2E1 genotypes showed significant influence on the elevation of urinary acetaldehyde excretion. Neither these genotypes nor urinary acetaldehyde concentration significantly affected blood pressure, serum aspartate aminotransferase and gamma-glutamyl transferase activities, or serum HDL-cholesterol and lipid peroxide concentrations. It was concluded that acetaldehyde accumulates in moderate alcohol consumers irrespective of ALDH2 and CYP2E1 genotype, and that the implications of these genotypes and acetaldehyde accumulation in terms of common alcohol-related health consequences were obscure. The results also suggest that the carcinogenicity of acetaldehyde on esophageal mucosa depends greatly upon repeated exposure to high blood acetaldehyde, even through transient rather than chronic exposure.

Keywords Moderate alcohol consumption · Low-Km aldehyde-dehydrogenase (ALDH2) · Cytochrome P450-2E1 (CYP2E1) · Urinary acetaldehyde · Blood pressure · Fasting serum glucose · Serum lipid peroxides · Esophageal cancer

Introduction

Around half of all Orientals, including Japanese, have a hereditary deficiency in mitochondrial low-Km aldehyde dehydrogenase (ALDH2) activity, where acetaldehyde generated from ethanol is promptly converted into acetate. ALDH2 is normally composed of four subunit proteins encoded by ALDH2*1 allele, but it loses the activity if it includes just one false inactive subunit protein encoded by ALDH2*2 allele. Therefore, the enzyme activity in around 8% of Japanese who are homozygous for ALDH2*2 allele is nil, and in nearly 40% of those who are heterozygous for ALDH2*2 allele it is theoretically as low as 1/16 of those without this allele (Enomoto et al. 1991). A marked elevation of blood acetaldehyde is observed in these groups soon after alcohol ingestion, which causes the characteristic symptoms of facial flushing, palpitation, headache and nausea (Mizoi et al 1979; Harada et al 1981; Enomoto et al 1991; Takeshita 1999).

Acetaldehyde is a highly active and toxic substance that has been surmised to play an etiological role in the development of alcoholic liver damage (Lieber 1980). A pathogenic role of acetaldehyde has even been suggested in hypertension in alcohol consumers from animal studies (Vasdev et al. 1995, 1999) and human observations (Itoh et al. 1997). Possible contributions of ALDH2 genotypes have also been reported in myocardial infarction (Takagi et al. 2002), diabetes mellitus (Murata et al. 2000), esophageal cancer (Yokoyama et al. 1996) and Alzheimer's disease (Kamino et al. 2000) in Japanese, and high blood acetaldehyde is speculated to be involved in the pathogenesis of the diseases. However,

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acetaldehyde production depends greatly on the volume of alcohol consumed, and the high blood acetaldehyde in those showing ALDH2 deficiency is observed only temporarily after alcohol intake because of the work of other acetaldehyde metabolizing enzymes, such as a high-K_m ALDH. Therefore, the pathogenic roles of ALDH2 deficiency and consequent high blood acetaldehyde in such chronic and multifactorial illnesses remain uncertain, and further investigations are required.

Imai et al. (2002) reported a higher urinary excretion of acetaldehyde, which is bound to some chemicals in the urine, in heavy drinkers consuming nearly 90 g or more of alcohol daily, but a lower excretion in alcoholic patients after cessation of alcohol consumption for 12 months or longer, in comparison with nondrinkers. These findings suggest that the urinary chemical-bound acetaldehyde reflects accumulated acetaldehyde in the bodies of alcohol consumers, and can be regarded as a marker of chronic exposure to acetaldehyde. However, the number of subjects examined by Imai et al. (2002) was small, and the exact associations with the volume of alcohol consumed, ALDH2 genotypes and alcohol-related health consequences have not yet been fully evaluated. The aim of the present study was to clarify the implications of ALDH2 genotypes on the accumulation of acetaldehyde and health of alcohol consumers recruited from a general Japanese population, usually consuming a moderate volume of alcohol, by determining the effects of ALDH2 genotypes on urinary acetaldehyde excretions and common alcohol-related health consequences, such as elevation of blood pressure, serum hepatic enzyme activities, and serum lipid, uric acid (UA) and glucose concentrations. In this study, the effects of genotypes of cytochrome P450-2E1 (CYP2E1) on urinary acetaldehyde excretions and alcohol-related health effects were also analyzed, because CYP2E1 is another enzyme that produces acetaldehyde upon alcohol intake and it is known to display genetic polymorphism (Hayashi et al. 1991).

Subjects and methods

Two hundred subjects were randomly selected from 853 male workers aged between 35 and 59 years from a factory producing electronic components. Among the 200 subjects, whose mean age was 41.2 years, hypertension and impaired glucose tolerance (IGT) had been detected in 23 and 3 subjects, including 5 and 1 treated with medicines, respectively. The frequencies of hypertension and IGT were in accordance with those for all 853 workers of the age group in the factory. The subjects were requested to visit a health care station in the factory for health check-ups in the morning after overnight fasting, and those who had been treated with medicine were requested to refrain from taking it until the examinations had been completed. Data collections were undertaken after obtaining written informed consent from all the subjects.

Urine samples were kept frozen at -80°C until analysis for ethanol and acetaldehyde concentrations. Blood samples were collected to determine the ALDH2 and CYP2E1 genotypes of genomic DNA extracted from white blood cells, and for serum biochemical analyses. At the same time, blood pressure was measured in the sitting position using an automatic monometer (BP-103iII, Colin, Japan) after resting on a chair for 5 min or longer. Body mass index (BMI) was calculated from height and weight. Data on usual alcohol consumption over the past year (frequency, kinds of alcoholic beverage and volume consumed on each occasion), and consumption during the previous evening were collected in interviews by an experienced nurse, who also ascertained cigarette consumption and the frequency and intensity of physical activity at leisure time.

According to the alcohol consumption data, the subjects were divided into four groups of usual alcohol consumption and were scored with 1–4 following our previous studies (Tsuritani et al. 1995; Tanaka et al. 1997). Those who had consumed only a small volume of alcohol less than once a month during the past year were categorized as nondrinkers, in addition to those who declared not to have consumed alcohol at all in the period (scored 1). Those who consumed alcohol once a month or more were categorized into three groups: consuming ethanol <200 g per week (scored 2), 200–399 g (3), 400 g or more (4). Similarly, alcohol consumption in the previous evening was categorized into four groups and scored: none (1), <30 g of ethanol (2), 30–59 g (3) and 60 g or more (4). Cigarette consumption was categorized into nonsmoker, ex-smoker, current smoker consuming up to 20 cigarettes per day, and those consuming more, and these were scored 1–4, respectively. The subjects who didn't perform any kind of physical exercise lasting 30 min or longer more than once a month were categorized as physically inactive and scored 1, and the remaining subjects were categorized into three groups of weakly active (score 2), active (3) and strongly active (4), considering the frequency: up to two times a week or three times or more, and intensity: light (walking, cycling and slow dancing) or heavy (jogging, fast dancing and ball games). They were classified as "weakly active" when they performed light exercise up to two times a week and "strongly active" when they performed heavy exercise three times a week or more, otherwise they were "active".

In brief, the procedure for measuring ethanol and acetaldehyde concentrations in urine was as follows. Urine specimens (2 ml) were freeze-dried for 24 h, and hydrolyzed with 2 ml of 3.7 N sulfuric acid at 60°C for 1 h. One milliliter of the resulting substance was mixed with 1 ml of perchloric acid (PCA) reagent (1 ml of saline containing ice-cold 0.6 N PCA, 30 mM thiourea and 0.1 mM EDTA), and centrifuged at 3,000 rpm for 15 min for deproteinization. One milliliter of the supernatant was transferred into a vial containing 0.1 ml of 0.003% isobutanol as the internal standard, and

subjected to head-space gas-chromatography analysis. On the other hand, the creatinine concentration in the urine was measured with Jaffe's method, and the urinary ethanol and acetaldehyde concentrations were expressed as nmol per mg of creatinine. ALDH2 alleles (ALDH2*1 and ALDH2*2) and CYP2E1 alleles (c1 and c2) were determined by polymerase chain reaction-restricted fragment length polymorphism (PCR-RFLP) methods, as described in our previous report (Sun et al. 1999). Measurements of the hepatic enzyme activities of aspartate aminotransferase (AST) and gamma-glutamyl transferase (GGT), and serum concentrations of total cholesterol (t-Chol), HDL-cholesterol (HDLc), triglycerides (TG), UA and glucose were conducted using an automatic analyzer (Hitachi 7450, Hitachi, Japan). Serum concentration of lipid peroxides (LPO) was determined using a commercial kit (Lipoperoxide test, Wako, Japan). Data on urinary ethanol and acetaldehyde concentrations, serum AST and GGT activities, and serum TG concentrations were logarithmically transformed in the following statistical analyses.

Associations between the genotypes of ALDH2 and CYP2E1 and distributions of the subjects among the four categories of usual alcohol consumption were tested using a χ^2 -test. Differences in the means of urinary ethanol and acetaldehyde concentrations for the four alcohol consumption groups (usual and for the previous evening) were tested by a one-way analysis of variance (ANOVA), and differences in the four usual alcohol consumption groups in terms of the three ALDH2 genotypes and the three CYP2E1 genotypes were tested by a two-way ANOVA. A generalized linear model (GLM) analysis was then conducted to test the associations of urinary ethanol and acetaldehyde with independent and interactive effects of the ALDH2 and CYP2E1 genotypes, adjusting for the effects of alcohol consumption, age, BMI, cigarette consumption and physical activity. Differences in the means of blood pressure, serum hepatic enzyme activities, and serum lipid, UA and glucose concentrations among the four usual alcohol consumption groups were tested using a GLM analysis, adjusting for age, BMI, cigarette consumption and physical activity. These were also tested for using a GLM analysis where the genotypes of ALDH2 and CYP2E1 and urinary acetaldehyde

concentration were included as independent variables in the models. Finally, contributors to serum LPO concentrations in the subjects were determined by a step-wise multiple regression analysis. All of the statistical analyses were performed using the SPSS v.11 software package.

Results

For the usual alcohol consumption over the past year, 45 (23%) of the subjects were defined as nondrinkers, 123 (62%) consumed <200 g of ethanol per week, 25 (13%) consumed 200–399 g and 7 (4%) consumed 400 g or more. In the previous evening, 67 (34%) did not consume alcohol, 56 (28%) consumed <30 g of ethanol, 26 (13%) consumed 30–59 g and 12 (6%) consumed 60 g or more. The distribution of ALDH2 genotypes among the subjects were as follows: 109 (55%) of ALDH2*1/1, 76 (38%) of ALDH2*1/2 and 15 (8%) of ALDH2*2/2. For the CYP2E1 genotypes, 135 (68%) were c1/c1, 62 (31%) c1/c2 and 3 (2%) c2/c2. The associations of the ALDH2 and CYP2E1 genotypes with usual alcohol consumption are shown in Table 1. It is clear that much fewer of the subjects who usually consume 200 g or more of ethanol per week had the ALDH2*2 allele than didn't have it ($P < 0.001$). On the other hand, the subjects that had the c2 allele of CYP2E1—especially those with the c2/c2 genotype—seemed to consume a large volume of alcohol, although the difference for the CYP2E1 genotypes did not reach a significant level ($P = 0.11$).

Means and standard errors (SE) of urinary ethanol and acetaldehyde concentrations among the four groups of usual alcohol consumption and among those for the previous evening's consumption are summarized in Table 2. In comparison with nondrinkers, elevated urinary ethanol and acetaldehyde mean concentrations were observed in the subjects usually consuming 200 g or more of alcohol a week. However, the increases in urinary ethanol concentrations observed upon increased usual alcohol consumption were significant ($P = 0.002$) while the increases in acetaldehyde concentrations were not ($P = 0.06$). Similarly, although the mean ethanol and acetaldehyde concentrations in urine were higher in the

Table 1 Distributions of usual alcohol consumption according to the genotypes of aldehyde dehydrogenase (ALDH2) and cytochrome P450-2E1 (CYP2E1)

Genotype	Usual alcohol consumption (weight of ethanol consumed per week)				P^a
	None	1–199 g	200–399 g	400 g or more	
ALDH2*1/1	10 (9.1%)	76 (69.7)	16 (14.7)	7 (6.4)	< 0.001
ALDH2*1/2	22 (28.9)	46 (60.5)	8 (10.5)		
ALDH2*2/2	13 (86.7)	1 (6.7)	1 (6.7)		
CYP2E1 c1/c1	30 (22.2)	85 (63.0)	15 (11.1)	5 (3.7)	0.107
CYP2E1 c1/c2	15 (24.2)	36 (58.1)	10 (16.1)	1 (1.6)	
CYP2E1 c2/c2		2 (66.7)		1 (33.3)	

^aResults of χ^2 -test

Table 2 Means (M) and standard errors (SE) of ethanol and acetaldehyde concentrations in the urine of middle-aged men, divided up according to usual alcohol consumption and the previous evening's consumption

Usual alcohol consumption (weight of ethanol per week)	None (<i>n</i> = 45)		1–199 g (<i>n</i> = 123)		200–399 g (<i>n</i> = 25)		400 g or more (<i>n</i> = 7)		<i>P</i> ^b
	M	SE	M	SE	M	SE	M	SE	
Ethanol (nmol/mg.cr) ^a	37.5	1.2	38.7	1.1	90.8	1.3	96.8	1.8	0.002
Acetaldehyde (nmol/mg.cr) ^a	1.83	1.1	1.73	1.1	2.56	1.2	2.76	1.4	0.059
Alcohol consumption on the previous evening (weight of ethanol)	None (<i>n</i> = 97)		1–29 g (<i>n</i> = 70)		30–59 g (<i>n</i> = 20)		60 g or more (<i>n</i> = 13)		<i>P</i> ^b
	M	SE	M	SE	M	SE	M	SE	
Ethanol (nmol/mg.cr) ^a	33.0	1.1	48.6	1.1	86.0	1.3	80.7	1.5	0.001
Acetaldehyde (nmol/mg.cr) ^a	1.75	1.1	1.80	1.1	2.54	1.2	2.44	1.3	0.124

^aGeometric means and geometric standard errors^bResults of one-way analysis of variance

subjects who consumed 30 g or more of alcohol on the previous evening, the increases in urinary ethanol excretion were significant ($P=0.001$) but those for acetaldehyde ($P=0.12$) were not. Although not shown in the Table, urinary acetaldehyde concentration showed a strong correlation with ethanol concentration ($r=0.47$; $P<0.001$).

The means and standard deviations (SD) of the urinary ethanol and acetaldehyde concentrations among the four usual alcohol consumption groups and three ALDH2 genotypes are illustrated in Fig. 1. No significant effects of the three genotypes on the mean concentrations of urinary ethanol or acetaldehyde were detected in a two-way ANOVA ($P=0.89$ and 0.91 , respectively). Figure 2 shows urinary ethanol and acetaldehyde concentrations among the four alcohol consumption groups and three CYP2E1 genotypes. A tendency to higher urinary ethanol in those with a c2 allele was observed ($P=0.06$), but not for urinary acetaldehyde ($P=0.16$). The effects of the ALDH2 and CYP2E1 genotypes on urinary ethanol and acetaldehyde concentrations were further analyzed by a GLM method including independent and interactive effects of ALDH2 and CYP2E1 genotypes as variables, adjusting for the effects of alcohol consumption, age, BMI, cigarette consumption and physical activity. However, the results obtained by the GLM analyses were almost equal to those obtained in the one-way and two-way ANOVA.

Table 3 shows the means and SEs for blood pressure, serum activities of AST and GGT, and serum concentrations of t-Chol, HDLc, TG, LPO, UA and glucose in the four usual alcohol consumption groups, adjusting for age, BMI, cigarette consumption and physical activity. Diastolic blood pressure, serum AST and GGT activities, and serum LPO and UA concentrations were significantly elevated with increased alcohol consumption. A borderline significant elevation was observed in systolic blood pressure and serum HDLc concentrations. The effects of the genotypes of ALDH2 and CYP2E1 and urinary acetaldehyde concentrations on the associations

of alcohol consumption with blood pressure, serum AST and GGT, and serum HDLc, LPO and UA were further analyzed by a GLM method, but no significant effects of the genotypes and urinary acetaldehyde concentration were obtained, except for a marginally significant effect of urinary acetaldehyde concentration on the elevation of serum UA concentration ($P=0.03$).

The means and SDs of serum LPO concentrations among the four usual alcohol consumption groups and three ALDH2 genotypes are illustrated in Fig. 3. The means were not statistically different among the non-drinkers of the three ALDH2 genotypes, and the elevations with increased alcohol consumption also did not differ among the three genotypes. Table 4 shows the results of a step-wise multiple regression analysis to determine the variables contributing to the serum LPO level. Serum t-Chol and alcohol consumption were shown to be major contributors to serum LPO levels in the subjects.

Fig. 1 Means and standard deviations (SD) of urinary ethanol and acetaldehyde excretion in middle-aged men divided up by alcohol consumption and aldehyde dehydrogenase (ALDH2) genotypes. Open and closed symbols indicate ethanol and acetaldehyde, respectively. Circles, triangles and squares indicate ALDH2*1/1, ALDH2*1/2 and ALDH2*2/2, respectively

Fig. 2 Means and SD of urinary ethanol and acetaldehyde excretion in middle-aged men divided up by alcohol consumption and cytochrome P450-2E1 (CYP2E1) genotypes. *Open and closed symbols indicate ethanol and acetaldehyde, respectively. Circles, triangles and squares indicate c1/c1, c1/c2 and c2/c2, respectively*

Discussion

As shown in Table 2, the means of the urinary ethanol and acetaldehyde concentrations were elevated in both the subjects who usually consumed 200 g or more of alcohol a week and in those who consumed 30 g or more on the previous evening in comparison with nondrinkers. However, the relation of increased urinary acetaldehyde excretion to increased self-reported alcohol consumption was not significant (in contrast to that for in urinary ethanol excretion, which showed a significant association with the self-reported alcohol consumption), probably due to the small number of subjects who consumed a large volume of alcohol and the small difference between the mean urinary acetaldehyde concentrations of nondrinkers and drinkers; the mean

Fig. 3 Means and SDs of serum lipid peroxide concentration in middle-aged men divided up by alcohol consumption and ALDH2 genotype. *Circles, triangles and squares indicate ALDH2*1/1, ALDH2*1/2 and ALDH2*2/2, respectively*

urinary acetaldehyde concentration in nondrinkers was consistent with 66% of the mean concentration exhibited by drinkers consuming 400 g or more of alcohol a week. These results suggest that a large part of the chemical-bound acetaldehyde in the urine of the subjects is not derived from their current alcohol consumption. The major origin of chemical-bound acetaldehyde in the urine of moderate alcohol consumers, therefore, remains to be elucidated in further studies. At present, however, since urinary acetaldehyde concentration showed a strong correlation with urinary ethanol in the subjects, it can be said that urinary acetaldehyde excretion will increase, at least partly, upon increased current alcohol consumption, even in middle-aged Japanese men consuming only a moderate volume of alcohol.

On the other hand, no significant effects of ALDH2 genotypes were found upon urinary acetaldehyde concentration. A rapid and marked elevation of blood

Table 3 Means (M) and standard errors (SE) of blood pressure (BP), serum hepatic enzymes, lipids, uric acid and glucose in middle-aged men divided by usual alcohol consumption, adjusting for age, body mass index, cigarette consumption and physical activity

	Alcohol consumption (weight of ethanol consumed per week)								<i>P</i> ^b
	None (<i>n</i> = 45)		1–199 g (<i>n</i> = 123)		200–399 g (<i>n</i> = 25)		400 g or more (<i>n</i> = 7)		
	M	SE	M	SE	M	SE	M	SE	
Systolic BP (mmHg)	123	2.3	126	1.4	128	3.1	139	6.0	***
Diastolic BP (mmHg)	79	1.5	80	0.9	83	2.0	89	3.8	*
Serum									
AST (U/l) ^a	21.1	1.05	22.7	1.03	28.3	1.07	33.7	1.14	**
GGT (U/l) ^a	35.1	1.09	44.7	1.05	54.8	1.13	80.7	1.25	**
TG (mg/dl) ^a	113	1.08	117	1.05	130	1.11	139	1.23	NS
t-Chol (mg/dl)	198	4.6	201	2.7	207	6.2	181	11.7	NS
HDLc (mg/dl)	52.1	1.8	56.7	1.1	58.1	2.5	61.7	4.7	***
LPO (nmol/ml)	2.9	0.10	3.1	0.06	3.5	0.14	3.4	0.26	**
UA (mg/dl)	5.6	0.17	5.9	0.10	6.3	0.23	7.2	0.43	**
Glucose (mg/dl)	91	2.5	96	1.5	95	3.3	100	6.3	NS

^aGeometric means and geometric standard errors

^bResults from generalized linear model analyses

For the abbreviations, refer to text

NS Not significant

P* < 0.05, *P* < 0.01, ***0.10 < *P* ≤ 0.05

Table 4 Results from a step-wise multiple regression analysis of significant contributors to serum lipid peroxides concentration in 200 middle-aged men

	Standardized		<i>P</i> ^a
	β	<i>t</i> value	
Usual alcohol consumption (1–4)	0.228	3.557	<0.001
Serum t-Chol (mg/dl)	0.242	3.486	0.001
Serum TG (mg/dl) ^b	0.188	2.708	0.007
Age (years)	0.137	2.140	0.034

^aMultiple correlation coefficient $r^2=0.200$, $P<0.001$ ^bLogarithmically transformed

acetaldehyde after ingesting alcohol, although transient, is a characteristic finding in those with an ALDH2*2 allele (Mizoi et al. 1979; Harada et al. 1981; Enomoto et al. 1991; Takeshita 1999). However, urinary acetaldehyde excretion did not seem to reflect this transient high concentration in the blood, but instead reflected chronically accumulated acetaldehyde, which depended on the total volume of alcohol consumed. For CYP2E1, those having c2 allele showed somewhat higher urinary concentrations of ethanol, which may reflect increased alcohol consumption in those with the c2 allele, but the effects of the c2 allele on acetaldehyde were not clear. Furthermore, neither the ALDH2 and the CYP2E1 genotypes nor increased in acetaldehyde in the urine affected common alcohol-related health consequences in the moderate alcohol consumer, such as elevation of blood pressure, serum AST and GGT activities, or serum HDLc and LPO concentrations. Increased urinary acetaldehyde concentration showed a marginal promoting effect on serum UA elevation, but the implications of this association are not clear at present. From these results, it can be concluded that acetaldehyde accumulates even in moderate alcohol consumers irrespective of ALDH2 and CYP2E1 genotypes, and that neither those genotypes nor urinary acetaldehyde affects common health consequences related to moderate alcohol consumption.

A possible etiological role of ALDH2 deficiency and consequent high blood acetaldehyde in alcohol-related hypertension has been suggested by Itoh et al. (1997), but effects of ALDH2 genotypes on the alcohol–blood pressure relationship have been ruled out in many studies (Okayama et al. 1994; Tsuritani et al. 1995; Tanaka et al. 1997; Takagi et al. 2001; Amamoto et al. 2002; Yamada et al. 2002), similar to the present results. A high incidence of myocardial infarction in those having ALDH2 deficiency has been demonstrated to be a reflection of the low serum HDLc due to low alcohol consumption (Takagi et al. 2002). Therefore, direct effects of ALDH2 deficiency and consequent high blood acetaldehyde on the elevation of blood pressure and the occurrence of myocardial infarction are questionable.

The association of ALDH2 with diabetes mellitus seems to be more complicated. A higher HbA1c (Murata

et al. 2000) and more neuropathy (Suzuki et al. 2004) have been reported in diabetic patients showing ALDH2 deficiency. A more specific association is a high prevalence of the ALDH2*2 allele in diabetic patients who have a diabetic mother (Suzuki et al. 1996a) and in those who have a mitochondrial tRNA mutation at position 3243 (Suzuki et al. 1996b), suggesting that high acetaldehyde in mitochondria due to ALDH2 deficiency may cause mitochondrial DNA mutations, and those mutations may be transmitted together to the offspring. ALDH2 deficiency may have some links with mitochondrial abnormalities related to diabetes mellitus, but it is not necessarily prominent in all patients with type 2 diabetes mellitus (Murata et al. 2000) and in those having nephropathy (Kuriyama et al. 1998). In the middle-aged men studied here, no significant effects of ALDH2 and CYP2E1 genotypes or urinary acetaldehyde concentrations were observed on fasting serum glucose levels. However, the diabetogenic effects of those genotypes and acetaldehyde, even if present, may not be detectable in moderate alcohol consumers since elevations of fasting serum glucose concentration would only be observed in drinkers consuming 400 g or more of ethanol per week (Yamada et al. 2004).

A high incidence of Alzheimer's disease in those with the ALDH2*2 allele has been observed in a case-control study in Japanese (Kamino et al. 2000). However, it was not confirmed in a Korean population (Kim et al. 2004), and so further investigations are required. A possible mechanism underlying the association involves ALDH2 playing a protective role against oxidative stress; this was proposed on the basis of results from an experimental cell study (Ohsawa et al. 2003b). The same group of researchers observed increases in serum LPO in women with ALDH2 deficiencies (Ohsawa et al. 2003a), and speculated that it was evidence for the anti-oxidative role of ALDH2. However, it was not observed in the male subjects, whose serum LPO was mainly increased with increased alcohol consumption, which is consistent with the present and previous findings (Araki et al. 1994; Shimmura et al. 1995) in Japanese men. The factors contributing to serum LPO in women have not yet been fully evaluated. Although the present subjects were all middle-aged men, a multiple regression analysis in them showed that serum t-Chol is a major contributor to serum LPO level in addition to alcohol consumption. In women, serum t-Chol levels are markedly elevated and sometimes higher than those in men after the menopause (Bush 1990; Gorodeski 1994; Noborisaka et al. 1998). The association between ALDH2 genotypes and serum LPO in middle-aged women, therefore, should be investigated in studies that take the effects of menopause and serum t-Chol levels into consideration.

A high incidence of esophageal cancer in those with an ALDH2 deficiency has been observed in studies among Japanese that consume various volumes of alcohol (Yokoyama et al. 1996, 1998, 2001), and the odds ratio of esophageal cancer even in moderate drinkers with ALDH2*2 allele was reported to be nearly

six times higher than those without it (Yokoyama et al. 2002). Heavy alcohol consumption is a well-known risk factor for esophageal cancer, and very poor ALDH2 expression in esophageal mucosa (Yin et al. 1993) is supposed to cause the vulnerability to the carcinogenetic effects of acetaldehyde generated from ethanol. However, the specific vulnerability of those showing ALDH2 deficiency remains obscure. If we assume from the present results that acetaldehyde accumulates similarly depending on the total volume of alcohol consumed irrespective of ALDH2 genotype, it is speculated that the carcinogenesis of esophageal mucosa cells must critically depend on repeated (even if transient) exposure to a high concentration of acetaldehyde, rather than chronic exposure to a certain level of acetaldehyde. To prevent esophageal cancer in those showing ALDH2 deficiency, therefore, reducing the number of occasions of that alcohol is consumed as well as the volume consumed should be recommended.

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