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The Role of Alcohol Dehydrogenase 2 and Aldehyde Dehydrogenase 2 Genotypes in Alcohol-Induced Vasospastic Angina

TAKAFUMI SEKI, HIROSHI OKAYAMA, SHOGEN ISOYAMA, YUTAKA KAGAYA, KUNIO SHIRATO, KEI MUNAKATA,[1] MASAHARU KANAZAWA,[1] KENJI TAMAKI,[2] TETSUYA HIRAMOTO,[3] MICHIKO OKAYAMA[4] and SHINYA KASAHARA[4]

The First Department of Internal Medicine, Tohoku University School of Medicine, Sendai 980-8574, [1]Cardiology Division, Sendai City Medical Center, Sendai 983-0824, [2]Cardiology Division, Iwate Prefectural Central Hospital, Morioka 020-0066, [3]Cardiology Division, Tohoku Kosai Hospital, Sendai 980-8674, and [4]Sendai Cardiovascular Center, Sendai 981-3107

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—— Alcohol ingestion often provokes attacks in patients with vasospastic angina. Type 2 aldehyde dehydrogenase (ALDH2) deficiency, which is based on a single point mutation (Glu487Lys) of the ALDH2 gene, is common in the Japanese population, but rare among the Caucasian population. We investigated how the genotype of ALDH2 affects the characteristics of alcohol-induced vasospastic angina. Ninety-one patients with vasospastic angina who had ingested alcohol daily or occasionally were studied. Patients had been diagnosed as vasospastic angina by a provocation test with an intracoronary injection of ergonovine or acetylcholine during coronary angiography. The Glu487Lys mutation was detected by allele specific PCR. We interviewed the patients to obtain information concerning the relationship between alcohol ingestion and anginal attacks. Alcohol ingestion induced attacks in 16 of 66 patients without the Glu487Lys mutation, 8 of 22 in heterozygotes, and 1 of 3 in mutant homozygotes. The intervals between alcohol ingestion and the onset of anginal attacks were shorter in homozygotes (0.17 hours) and heterozygotes (1.5 ± 0.6 hours) for ALDH2*2 than in normal homozygotes for ALDH2*1 (5.4 ± 0.6 hours). The amount of ethanol which induced attacks was significantly greater in normal homozygotes than in homozygotes (11 ml) and heterozygotes (42.5 ± 7.1 ml) for ALDH2*2 (96.1 ± 13.4 ml in normal patients). The frequency of anginal attacks induced by alcohol

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Address for reprints: Shogen Isoyama, M.D., Ph.D., Faculty of Medical Science and Welfare, Tohoku Bunka Gakuen University, 6-45-16 Kunimi, Aoba-ku, Sendai 981-8550, Japan.

e-mail: shogen@rehab.tbgu.ac.jp

ingestion did not differ between ALDH deficient and normal homozygotes. In ALDH deficient patients, however, anginal attacks were induced by a smaller amount of alcohol immediately after its ingestion. Thus, the ALDH2 genotype modifies the characteristics of the anginal attacks as a co-factor for the induction of vasospastic angina after alcohol ingestion. ———— alcohol ingestion; vasospastic angina; aldehyde dehydrogenase genotype; alcohol dehydrogenase genotype

Coronary artery spasm plays an important role in the induction of ischemic attacks in some types of angina pectoris. Coronary artery spasm is precipitated by several factors including alcohol ingestion. In 1973 Fernandez et al. (1973) reported a case with angina who had experienced attacks only following alcohol ingestion. Thereafter, only Japanese cases with alcohol-induced vasospastic angina were reported (Sato et al. 1981; Kashima et al. 1982; Matsuguchi et al. 1984; Tanabe et al. 1992; Ando et al. 1993; Oda et al. 1994). Furthermore, Matsuguchi et al. (1984), Oda et al. (1994), and Takizawa et al. (1984) studied the effects of alcohol ingestion on vasospastic angina in patients with a history of chest pain occurring following alcohol ingestion, and reported that in 19 of 71 patients, 4 of 8 patients, and 5 of 6 patients, respectively, attacks were provoked by alcohol ingestion. Thus, the frequency of alcohol-induced vasospastic angina is high in the Japanese population. There seem to be racial differences in the frequency of alcohol-induced vasospastic angina.

The main pathway of ethanol metabolism in the human liver has alcohol dehydrogenase (ADH) oxidizing ethanol to acetaldehyde and aldehyde dehydrogenase (ALDH) converting acetaldehyde to acetate. ADH is an enzyme composed of subunits that can be divided into three different classes: I, II and III. The ADH molecular forms in class I comprise the homo- and heterodimeric isozymes with α, β and γ subunit chains derived from three separate genes, ADH1, ADH2, and ADH3 (Smith 1986; Tsukahara and Yoshida 1989; Yasunami et al. 1990). Genetic polymorphism is observed at the ADH2 locus due to three alleles, ADH2*1, ADH2*2 and ADH2*3. Most Caucasians and Orientals fall into three genotype groups designated ADH2*1/ADH2*1, ADH2*1/ ADH2*2 and ADH2*2/ADH2*2. The β1 subunit encoded by the ADH2*1 allele (normal type, lower enzymatic activity) is common among Caucasians, and the β2 subunit encoded by the ADH2*2 allele (mutant type, higher enzymatic activity) appears predominantly in Orientals (Goedde et al. 1979, 1982).

Ordinary human livers contain two major ALDH isozymes, cytosolic ALDH1 component and mitochondrial ALDH2 component. The ALDH2 gene is about 44 kbp in length and contains at least 13 exons which encode 517 amino acid residues. The ALDH2 gene has two types, normal ALDH2*1 and atypical ALDH2*2 which is based on a point mutation (Glu487Lys) (Yoshida et al. 1984; Hsu et al. 1985). Almost all Caucasians are normal homozygotes for ALDH2*1, and have normal ALDH activity (Goedde et al. 1979, 1982). On the other hand, 50% of Japanese

are heterozygotes or homozygotes for ALDH2*2 (Goedde et al. 1979, 1982; Crabb et al. 1989). The individuals who are homozygotes for ALDH2*2 have no enzymatic activity of ALDH. Even though they are heterozygotes for ALDH2*2, they have only a small amount of enzymatic activity of ALDH (Mizoi et al. 1979, 1983; Impraim et al. 1982).

The mechanisms responsible for the high frequency of alcohol-induced attacks in the Japanese with vasospastic angina are not clear: higher sensitivity of the coronary artery to alcohol ingestion, or different metabolism of ethanol derived from ADH2 and ALDH2 genotypes. In this study, we attempted to clarify the relationship between alcohol-induced attacks and the genotypes of ADH2 and ALDH2 in Japanese patients with vasospastic angina.

Subjects and Methods

Ninety-one patients with vasospastic angina were recruited from institutions participating in this study from December 1994 through December 1995. Coronary angiography was performed in all of them. Spasm of a large coronary artery was demonstrated after intracoronary infusion of acetylcholine or ergonovine. All patients complained of chest pain, and elevation or depression of the ST-segment appeared during attacks and disappeared with the subsidence of the attacks in all of them. Since we attempted to clarify the relationship between alcohol-induced anginal attacks and the genotypes, patients without a history of alcohol intake were not included. Patients with liver disease or neoplasm were also excluded.

We interviewed patients and asked them their history of alcohol intake, the kind and amount of alcohol beverage, and the relationship of the alcohol ingestion to anginal attacks. The amount of ethanol was calculated from the ethanol concentration and the amount of alcohol beverage.

According to the study by Takizawa et al. (1984), anginal attacks were considered to have been induced by alcohol ingestion when one of the following criteria was fulfilled:

1. attacks occurred only after alcohol ingestion,
2. attacks always occurred after alcohol ingestion,
3. attacks at a time and under conditions in which spontaneous attacks would not occur without alcohol ingestion,
4. attacks occurred at least two times more frequently after alcohol ingestion, and
5. attacks after alcohol ingestion and with symptomatic characteristics different from those in spontaneous attacks.

The information from the interview was obtained before the gene analysis in order to negate the bias by the result of the gene analysis. Informed consent was obtained from each patient.

Detection of ALDH2 genotype

Genomic DNA was prepared from monocytes of fresh peripheral blood by the method of Ciulla et al. (1988). We modified allele-specific polymerase chain reaction (PCR) to detect the ALDH2 genotype. Allele-specific PCR is a simple, rapid, nonradioactive method to facilitate the direct detection of point mutations (Okayama et al. 1989). In this allele-specific PCR false positive DNA fragments are sometimes produced by inadequately purified template DNA, an incorrect amount of template DNA, unsuitable temperature and time conditions for PCR, and so on. To decrease the false positive DNA amplification and to increase the efficiency of PCR, we designed new allele-specific primers which were shorter than usual allele-specific primers and to which four other base sequences not complemental to template DNA were added (Fig. 1). Usual allele-specific PCR was performed with allele-specific primers which were designed to correspond to 3′-end base with the mutant point on exon 12: 5′-CCACACTCACAGTTTTCACTTC-3′ as a normal gene specific primer and 5′-CCACACTCACAGTTTTCACTTT-3′ as a deficient gene specific primer. Furthermore, we designed other allele-specific primers for modified allele-specific PCR: 5′-GAGGCACTCACAGTTTTCACTTC-3′ as a normal gene-specific primer and 5′-GAGGCACTCACAGTTTTCACTTT-3′ as a deficient gene specific primer. The 5′-end sequence GAGG is non-specific and not complemental to template DNA. We performed PCR for the ALDH2 gene with each allele-specific primer and a common primer which was complemental to the template DNA sequence on exon12, 5′-CAAATTACAGGGTCAACTGCT-3′. The reagent concentration in the 10 μl PCR was 5 pmol each for sense and antisense primers, 200 μmol/L deoxynucleotide triphosphates, 1.5 mmol/L $MgCl_2$, 50 mmol/L KCl, 10 mmol/L Tris-HCl (pH 8.4), and 0.25 units of Taq DNA

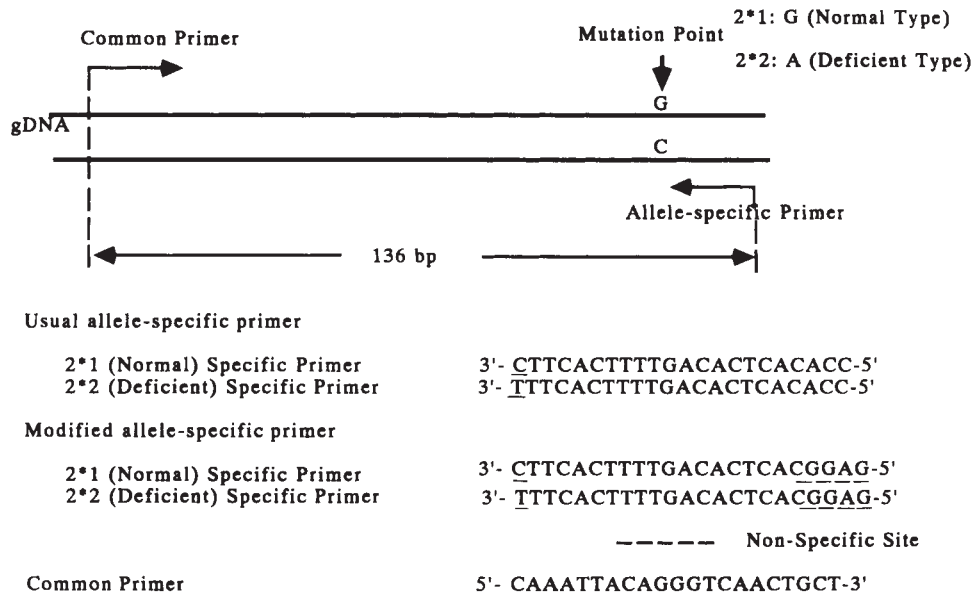

Fig. 1. ALDH2 gene and modified allele-specific PCR.

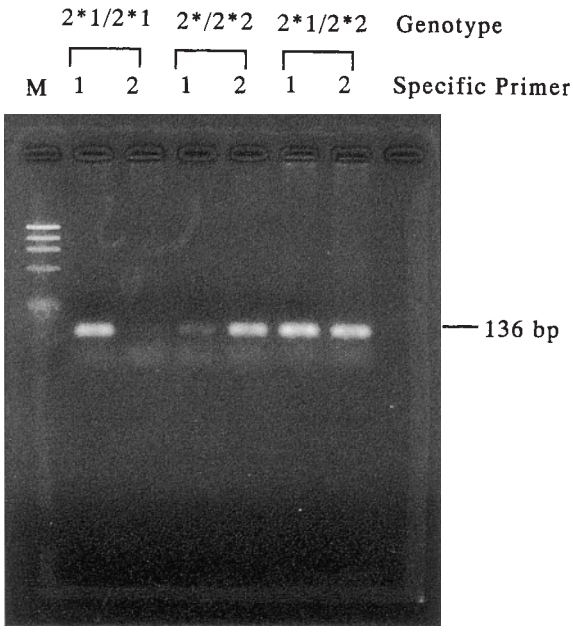

Fig. 2. Electrophoresis of amplified ALDH2 by usual allele-specific PCR. False positive DNA amplification was recognized on lane 2 of 2*1/2*1 and lane 1 of 2*2/2*2.
M, DNA marker (Φ X174/Hae III digest).
2*1/2*1, ALDH homozygotes; 2*2/2*2, ALDH2*2 homozygotes; 2*1/2*2, heterozygotes for ALDH2*1 and ALDH2*2.

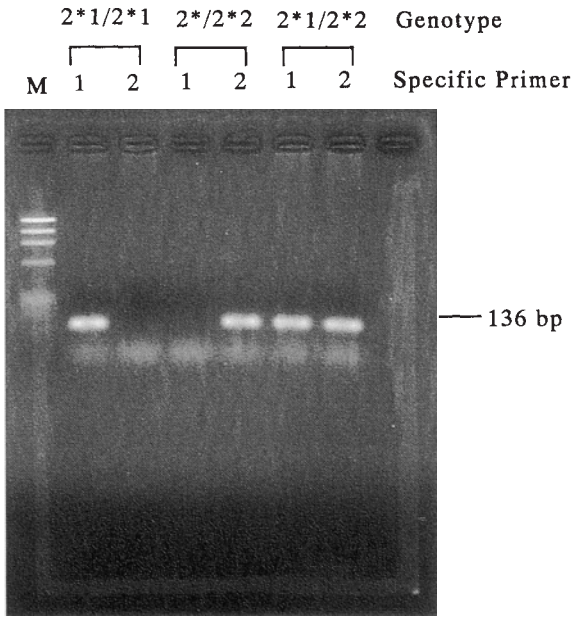

Fig. 3. Electrophoresis of amplified ALDH2 gene by modified allele-specific PCR. False positive DNA amplification was not recognized on lane 2 of 2*1/2*1 or lane 1 of 2*2/2*2.
2*1/2*1, ALDH homozygotes; 2*2/2*2, ALDH2*2 homozygotes; 2*1/2*2, heterozygotes for ALDH2*1 and ALDH2*2.

polymerase. Twenty ng of DNA were amplified for 30 cycles consisting of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 45 seconds, followed by a final extension step at 72°C for 4

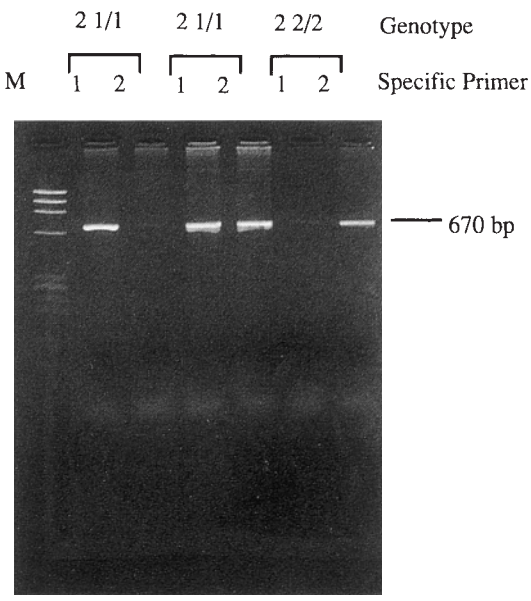

Fig. 4. Electrophoresis of amplified ADH2 gene by modified alle-specific PCR. False positive DNA amplification was not recognized on lane 2 of 2*1/2*1 or lane 1 of 2*2/2*2.
M, DNA marker (Φ X174/Hae III digest).
2*1/2*1, ADH homozygotes; 2*2/2*2, ADH2*2 homozygotes; 2*1/2*2, heterozygotes for ADH2*1 and ADH2*2.

minutes. The amplified PCR products were analyzed by 3% agarose gel electrophoresis in the presence of ethidium bromide. We recognized false positive DNA amplification in ALDH2*1 homozygotes and ALDH2*2 homozygotes by the usual allele-specific PCR (Fig. 2). On the other hand, false positive DNA amplification was not recognized and the efficiency of PCR was not decreased by the modified allele-specific PCR (Fig. 3).

Detection of ADH2 genotype

We also analyzed ADH2 genotypes by the modified allele-specific PCR. We designed two allele-specific primers: 5′-GTGCACGTGGTCATCTGTGC-3′ as an ADH2*1 specific primer and 5′-GTGCACGTGGTVATCTGTGT-3′ as an ADH2*2 specific primer. The GTG sequence of the 5′-end was the site that was not complemental to template DNA. The base of the 3′-end, C and T corresponded to the mutant point of exon3 on the ADH2 gene. We performed PCR with each allele-specific primer and common sense primer on exon 2. The reagent concentration in the 10 μl PCR was the same as described above for the ALDH2 genotype analysis. Twenty ng of DNA were amplified for 24 cycles consisting of denaturation at 94°C for 45 seconds, annealing at 60°C for 75 seconds, and extension at 72°C for 60 seconds, followed by a final step at 72°C for 4 minutes. We determined the ADH2 genotype by the pattern of electrophoresis on 2% agarose gel (Fig. 4).

Statistical analysis

The statistical differences in the amount of ethanol in each genotype of ALDH2, and the differences in the intervals between alcohol ingestion and the onset of anginal attack were analyzed by the Student's *t*-test with Bonferroni correction. Other statistical analysis was performed by χ^2 test. Values are expressed as mean±S.E.

Results

As summarized in Table 1, 77 of 91 patients were male, and 28% of patients were sensitive to alcohol. None of the female patients were alcohol-sensitive. Twelve patients fulfilled item 1, 2 patients item 2, 3 patients item 3, 3 patients item 4, one patient item 5, 2 patients items 1 and 2, and 2 patients items 4 and 5. In one patient who had experienced anginal attacks frequently after alcohol ingestion, myocardial infarction was induced when he did not take medicine and took alcohol. In all patients, spontaneous or alcohol-induced anginal attacks completely disappeared after administration of nitrates and/or calcium blockers.

ALDH2 genotype and alcohol-induced anginal attacks

As summarized in Table 2, 66 of 91 patients (73%) were homozygotes, 22 patients (24%) were heterozygotes for ALDH2*1, and only 3 patients (3%) were

Table 1. *Patient characteristics*

	Age (years)	Alcohol-sensitive	Non-sensitive	Total
Male	62±1	25 (33%)	52 (68%)	77
Female	61±2	0 (0%)	14 (100%)	14
Total		25 (28%)	66 (73%)	91

Values are Mean±S.E.

Table 2. *The relationship between ALDH2 genotypes and anginal attacks*

Genotype	Alcohol sensitivity		Usual amount of ethanol ingestion (ml/day)	Ethanol amount related to induction of angina (ml)	Interval between alcohol ingestion and attack (hour)
ALDH2*1/*1	Yes	16	31.3±7.2	96.1±13.4[a,b]	5.4±0.6[a,b]
	No	50	23.7±3.	—	—
ALDH2*1/*2	Yes	8	6.6±2.9	42.5±7.1[c]	1.5±0.6[c]
	No	14	8.7±3.9	—	—
ALDH2*2/*2	Yes	1	5	11	0.17
	No	2	0.5±0.4	—	—

Values are mean±S.E. a, $p<0.01$ vs. ALDH2*1/*2; b, $p<0.01$ vs. ALDH2*2/*2; c, $p<0.01$ vs. ALDH2*2/*2

homozygotes for ALDH2*2. The gene frequency of ALDH2*1 to ALDH2*2 allele was 0.85/0.15.

In 24% of ALDH2*1 homozygotes, 36% of ALDH2*1 heterozygotes and 33% of ALDH2*2 homozygotes, anginal attacks were induced after alcohol ingestion. There was no difference in the frequency of alcohol-induced anginal attacks among patients with the three different ALDH2 genotypes.

One alcohol-sensitive homozygote for ALDH2*2, and 8 alcohol-sensitive heterozygotes for ALDH2*2 were all facial flushers, but only 2 of 16 alcohol-sensitive homozygotes for ALDH2*1 were flushers. The usual amount of ethanol intake was smaller in heterozygotes for ALDH2*1, and the smallest in homozygotes for ALDH2*2, compared with that in homozygotes for ALDH2*1. The ethanol amount which related to the occurrence of anginal attacks was much greater than the usual one; the amount was greatest in ALDH2*1 homozygotes, and the smallest in ALDH2*2 homozygotes.

The interval between alcohol ingestion and the onset of anginal attacks was longer than 1 hour in all of alcohol-sensitive patients of ALDH2*1 homozygotes, and the mean value was 5.4 ± 0.6 hours. In 7 of 8 alcohol-sensitive heterozygotes for ALDH2*1 the interval was shorter than 1 hour, and the mean value was 1.5 ± 0.6 hours. In the homozygotes for ALDH2*2, the interval was shortest.

ADH2 genotype and alcohol-induced anginal attacks

As summarized in Table 3, there was no correlation between ADH2 genotypes and the frequency of alcohol-induced anginal attacks. Also, there was no difference in the usual amount of ethanol intake, the amount which related to the occurence of attacks, or the intervals between alcohol ingestion and the onset of

TABLE 3. *The relationship between ADH2 genotypes and alcohol ingesiton*

ADH2 genotypes	Alcohol-sensitive	non-sensitive	Total
ADH2 1/1	1 (12.5%)	7 (87.5%)	8
ADH2 1/2	7 (25.9%)	20 (74.1%)	27
AIH2 2/2	17 (30.4%)	39 (69.6%)	56
Total	25 (27.5%)	66 (72.5%)	91

TABLE 4. *The incidence of alcohol-induced anginal attacks in groups of different genotypes of ADH2 and ALDH2 genotypes*

	ADH2*1/*1	ADH2*1/*2	ADH2*2/*2	Total
ALDH2*1/*1	0/6 (0 %)	4/17 (23.5%)	12/43 (27.9%)	16/66 (24.2%)
ALDH2*1/*2	1/2 (50.0%)	3/9 (33.3%)	4/11 (36.4%)	8/22 (36.4%)
ALDH2*2/*2	0/0 (0 %)	0/1 (0 %)	1/2 (50.0%)	1/3 (33.3%)
Total	1/8 (12.5%)	7/27 (25.9%)	17/56 (30.4%)	25/91 (27.5%)

attacks among the three ADH2 genotypes. There was no correlation between the frequency of anginal attacks and the groups of different genotypes of ADH2 and ALDH2.

Discussion

Using new, shorter primers and adding several non-specific DNA sequences, we successfully developed a modified allele-specific PCR to improve the specificity for amplification without inefficiency. With this modified method, we examined the relationship between alcohol-induced attacks and the genotypes of ALDH2 and ADH2 in Japanese patients with vasospastic angina. Our important findings are as follows: (i) There was no correlation between the frequency of alcohol-induced attacks and the genotype of ALDH2 or ADH2. (ii) In patients with ALDH deficiency, the amount of ethanol which related to the occurence of anginal attacks was smaller, and the interval between alcohol ingestion and the onset of attacks was shorter. The amount or the interval was not affected by the genotype of ADH2. Thus, the genotype of ALDH2 but not ADH2 affects the characteristics of alcohol-induced attacks in patients with vasospastic angina.

In this study, the frequency of the ALDH2*2 allele in 91 vasospastic angina (15%) was lower than that in the healthy Japanese population (24 %) (Goedde et al. 1979, 1982), probably because only patients with vasospastic angina and a history of alcohol intake were included. The low frequency of the ALDH2*2 allele might simply indicate that the homozygotes or heterozygotes for ALDH2* 2 rarely take alcohol. Before this study we supposed that the frequency of the alcohol-induced attacks was higher in ALDH deficient patients with vasospastic angina compared with that in ALDH normal patients, because almost one half of the Japanese population are ALDH deficient and because almost all cases with alcohol-induced vasospastic angina had been Japanese, not Caucasian. Contrary to our expectations, anginal attacks were induced by alcohol ingestion even in 32% of ALDH normal patients, and there was no significant difference in alcohol sensitivity between ALDH deficient and non-deficient patient groups.

In this study, alcohol ingestion induced anginal attacks in 28% of patients with vasospastic angina, but did not induce attacks in any of 14 female patients. Furthermore, no female patient with alcohol-induced anginal attacks has been reported. There seems to be a gender-related difference in the frequency of alcohol-induced anginal attacks. It is not clear whether the gender-related difference is due to the lower sensitivity to alcohol or the fewer occasions of alcohol intake.

The mechanism(s) responsible for the occurrence of anginal attacks after alcohol ingestion has not been clarified. Several possibilities are raised: The direct action of ethanol or acetaldehyde metabolized from ethanol in the liver (Fernandez et al. 1973; Ando et al. 1993), and the indirect action by vasoactive substances such as catecholamine, bradykinin, serotonin, histamine, thromboxane

B2, 6-keto prostaglandin F1α, and cyclin nucleotides which may be increased or decreased after alcohol ingestion (Mizoi et al. 1979; Matsuguchi et al. 1984; Hatake et al 1990; Ando et al. 1993; Oda et al. 1994). Previous studies revealed that the intervals between alcohol ingestion and the onset of attacks varied from 1.5 to 17.5 hours. The direct action of ethanol or acetaldehyde would not always be responsible for the induction of these anginal attacks. In the reports of Sato et al. (1981), Matsuguchi et al. (1984) and Oda et al. (1994), anginal attacks occurred after the plasma level of ethanol had already decreased to zero. Furthermore, the blood level of acetaldehyde peaked 30–90 minutes after alcohol ingestion and declined rapidly thereafter (Ando et al. 1993). Therefore, the direct action of ethanol or acetaldehyde may play an important role in the induction of attacks relatively immediately after alcohol ingestion, but would not be directly related to those that occur much later.

In the present study, the intervals between alcohol ingestion and the onset of anginal attacks and the amount of ethanol which related to the induction of anginal attacks depended on the genotype of ALDH2. The interval was shortest in homozygotes for ALDH2*2, and longest in homozygotes for ALDH2*1; the amount was lowest in homozygotes for ALDH2*2 and highest in homozygotes for ALDH2*1. The wide variety of the intervals in the previous and present studies may be explained by the genotypes of ALDH2. The attacks immediately after the ingestion of smaller amounts of alcohol in homozygotes or heterozygotes for ALDH2*2 might be induced by the direct action of ethanol or acetaldehyde, because the maximum level of plasma acetaldehyde after alcohol ingestion has been reported to be 6-15 times higher in the heterozygotes for ALDH2*2 than that in normal homozygotes (Yamamoto et al. 1993). Matsuguchi et al. (1984) reported that the plasma levels of norepinephrine and serotonin were still maintained higher late after the ethanol levels were decreased to zero. The high plasma levels of these substances may be associated with the attacks that occur after alcohol ingestion in homozygotes for ALDH2*1.

We also analyzed the ADH2 genotypes in patients with vasospastic angina, because ADH2 affects the plasma level of acetaldehyde through the oxydation process of ethanol (Yoshida et al. 1981). The gene frequency of ADH2*1 to ADH2*2 was 0.236/0.746 in the present study, which is comparable to that in the study of Goedde et al. (1979). There was no significant correlation between the frequency of attacks induced by alcohol ingestion and the ADH2 genotype. In addition, the ADH2 genotype did not alter the characteristics of the anginal attacks, i. e., the ethanol amount which related the anginal attacks or the interval between alcohol ingestion and the onset of anginal attacks.

In summary, there was no difference in the frequency of anginal attacks between ALDH2 deficient and normal patients. In the ALDH2 deficient patients, smaller amounts of alcohol induced angina attacks more immediately after its ingestion. The ALDH2 genotype does not affect the frequency of anginal

attacks, but does alter the characteristics of anginal attacks as a co-factor for the induction of vasospastic angina. The ADH2 genotype did not modify the characteristics of anginal attacks.

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