

Clinical aspects of allergic disease

Correlation between alcohol-induced asthma and acetaldehyde dehydrogenase-2 genotype

Atsuko Takao, MD,^a Terufumi Shimoda, MD,^a Shigeru Kohno, MD,^a
Sadahiro Asai, MD,^b and Syoji Harda, MD^c *Nagasaki, Sasebo, and Ibaragi, Japan*

Background: Alcohol-induced asthma, a phenomenon characteristic of Asians, is due to differences in alcohol metabolism, particularly acetaldehyde metabolism. We investigated the effect of polymorphism in acetaldehyde dehydrogenase 2 (ALDH2) gene on the response to alcohol challenge testing in a group of Japanese asthmatic subjects and normal subjects.

Methods: Subjects were 32 asthmatic subjects and 30 healthy individuals. We measured the change in FEV₁ after ingestion of 30 gm of ethanol. Blood ethanol, acetaldehyde, and histamine concentrations were determined. ALDH2 gene type was established by polymerase chain reaction.

Results: Ethanol provocation test results were positive in 15 (47% responders) asthmatic subjects. The blood ethanol concentration was similar in responders and nonresponders.

The fall in FEV₁ was associated with a rise in blood acetaldehyde and histamine concentrations. The response to oral ethanol challenge was positive in three (19%) of 16 patients with normal homozygote ALDH2 genotype, 10 (71%) of 14 patients with type mutant heterozygote, and two (100%) of two patients with type mutant homozygote ALDH2 genotype.

Conclusions: Alcohol-induced asthma is probably caused by increased blood acetaldehyde concentration resulting from abnormalities of ALDH2 enzyme activity based on ALDH2 genotype differences. (*J Allergy Clin Immunol* 1998;101:576-80.)

Key words: *Bronchial asthma, alcohol, Asians, histamine release, ALDH2 genotype*

Approximately half of Japanese patients have exacerbation of asthma after drinking alcohol. Asthmatic attacks may be induced by oral intake of pure ethanol in Asians^{1,2} but not in Caucasians. Worsening of asthma in the latter group is rather due to hypersensitivity to additives in alcoholic beverages.³ We have recently identified the pathogenic mechanism of alcohol-induced asthma.⁴ Mast cells, basophils, or both are degranulated as the blood concentration of acetaldehyde increases after alcohol ingestion to abnormally high levels, resulting in the release of histamine, which in turn causes contraction of bronchial smooth muscles. Responses to ethanol show racial differences,⁵⁻¹⁴ possibly caused by

Abbreviations used

ALDH:	Aldehyde dehydrogenase
MM:	Mutant homozygote
NM:	Mutant heterozygote
NN:	Normal homozygote
PCR:	Polymerase chain reaction

differences in acetaldehyde metabolism. Genetic polymorphism of acetaldehyde dehydrogenase-2 (ALDH2), which is the most important enzyme in acetaldehyde metabolism, has been well documented, and the frequency of inactive genotypes (type NM, normal homozygote and type MM, mutant homozygote) is 43.6% in the Japanese but almost zero in the white European population.¹⁵

In this study we evaluated the relation between the response to oral ethanol provocation test and genetic analysis of ALDH2 to clarify the pathogenic mechanisms of alcohol-induced asthma.

METHODS

Subjects

We examined 32 Japanese adults with bronchial asthma (16 men and 16 women) 20 to 72 years old (mean, 40.9 years). The diagnosis of bronchial asthma was established on the basis of criteria of the American Thoracic Society.¹⁶ The severity of asthma graded according to the Internal Consensus Report (National Institutes of Health)¹⁷ was mild in six, moderate in 24, and severe in two patients. The disease type was atopic in 19 and nonatopic in the other 13 patients. The oral ethanol loading test was performed in stable symptom-free asthmatic subjects with FEV₁ greater than 75%. Bronchodilators were suspended 24 hours before and steroids or antiallergic drugs were suspended 48 hours before the ethanol loading test. The tests were also conducted in a group of 30 healthy volunteers with no airway hypersensitivity (15 men and 15 women; mean age, 39.3 years) (See Table I).

Oral ethanol challenge test

The oral ethanol provocation test was performed according to the method of Shimoda et al.⁴ Briefly, pure ethanol free of possible antigens was diluted with 5% glucose solution, and a 10% ethanol solution was prepared. While seated, the subject ingested 300 ml (containing 30 gm of ethanol) of ethanol solution over 5 to 10 minutes. The study was conducted after providing a detailed explanation of the contents and significance of the test and expected side effects, and a signed consent form was obtained.

From ^athe Second Department of Internal Medicine, Nagasaki University School of Medicine; ^bSasebo Municipal Central Hospital; and ^cthe Department of Legal Medicine, Tsubaki University, Ibaragi.

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Reprint requests: Atsuko Takao, MD, The Second Department of Internal Medicine, Nagasaki University School of Medicine, 1-7-1 Sakamoto-machi, Nagasaki 852, Japan.

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TABLE I. Clinical characteristics of asthmatic patients

Case No.	Age/sex	Severity of asthma	Type of asthma	History of ethanol-induced asthma
1	37/F	Mild	A	(+)
2	28/M	Mild	A	(+)
3	20/F	Mild	A	(+)
4	56/F	Moderate	A	(+)
5	25/F	Moderate	A	(+)
6	22/M	Moderate	A	(+)
7	63/M	Moderate	N	(+)
8	46/M	Moderate	A	(-)
9	60/M	Moderate	N	(+)
10	38/F	Moderate	A	(+)
11	39/M	Moderate	N	(+)
12	39/M	Moderate	N	(+)
13	55/F	Moderate	N	(-)
14	24/F	Moderate	A	(+)
15	46/F	Severe	A	(+)
16	38/M	Mild	A	(-)
17	26/F	Mild	A	(-)
18	22/F	Mild	A	(-)
19	22/M	Moderate	A	(-)
20	69/M	Moderate	N	(-)
21	29/M	Moderate	A	(-)
22	42/M	Moderate	A	(+)
23	37/F	Moderate	N	(-)
24	33/M	Moderate	A	(-)
25	52/F	Moderate	N	(-)
26	59/M	Moderate	N	(-)
27	22/M	Moderate	A	(-)
28	52/F	Moderate	N	(+)
29	72/F	Moderate	N	(-)
30	48/F	Moderate	N	(-)
31	42/F	Moderate	N	(-)
32	46/M	Severe	A	(-)

A, Asthmatic type; N, nonasthmatic.

Spirometry

FEV₁ was measured before and 15, 30, 60, and 120 minutes after ethanol ingestion with an autspirometer (Spiro Analyzer ST200, Fukuda Electronics).

The percentage changes in FEV₁ after alcohol ingestion were calculated in each individual by using the following equation:

Change in FEV₁ (%) =

$$\frac{\text{Baseline FEV}_1 - \text{Postethanol FEV}_1}{\text{Baseline FEV}_1} \times 100$$

The response to the oral dose of ethanol was considered positive when the percentage change in FEV₁ was greater than 20%.

Blood sampling and measurements

Blood samples were withdrawn from the antecubital vein before and 15, 30, 60, and 120 minutes after ethanol ingestion to measure the concentration of ethanol, acetaldehyde, and histamine. Blood ethanol and acetaldehyde concentrations were measured by gas chromatography with 5 ml of frozen heparinized whole blood. The plasma histamine concentration was measured by radioimmunoassay after isolating serum from

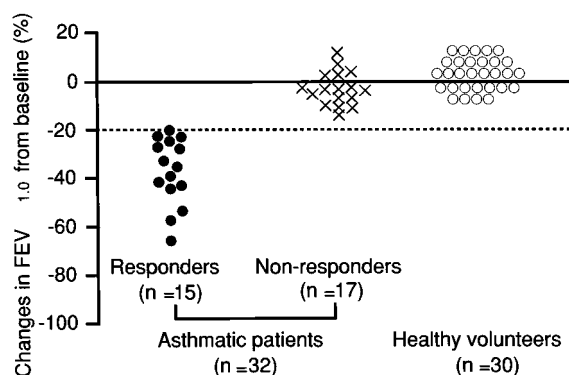


FIG. 1. Percentage change in FEV₁ after ethanol challenge.

5 ml of whole blood supplemented with ethylene diaminetetra acetic acid-2 sodium.

Gene analysis of ALDH2

DNA samples were isolated from the blood by standard procedures.¹⁸ The amplification refractory mutation system analysis¹⁹ was designed so that only terminal 3'-nucleotide of the polymerase chain reaction (PCR) primer was allele specific. The forward primer was CAAATTACAGGGTCAACTGC-TATG, the reverse normal primer was CCACACTCA-CAGTTTTCACCTTC, and the reverse mutant primer was CCA-CACTCACAGTTTTTCACCTTT to amplify 135 bp. PCR conditions were set as follows: one cycle of 94° C for 6 minutes, followed by 25 cycles each at 94° C for 1 minute, 60° C for 1 minute, 65° C for 2 minutes, and finally one cycle of 65° C for 8 minutes. The PCR products were separated on a 3% agarose gel and stained with ethidium bromide.

Simultaneous reproducibility and interday reproducibility were evaluated four times in two cases each for the normal homozygote (NN), NM, and MM types, and the same type was detected in the same samples. In addition, both this method (PCR-ARMS) and the oligonucleotide hybridization method (PCR-ASO) were performed for the NN type (six cases), NM type (seven cases), and the MM type (two cases), and the same results were obtained. These results suggest good reproducibility of this method.

Statistical analysis

Data were expressed as mean ± SD. Differences between matched groups were examined by the paired Student's *t* test and those between unmatched groups by the unpaired Student's *t* test. The percentage values were examined by the Fisher exact probability test. Correlations were considered significant at *p* values less than 0.05.

RESULTS

Changes in spirometry

Fifteen (47%) of the 32 asthmatic subjects had a positive response (responders); the remaining 17 patients were nonresponders. The percentage change in FEV₁ was 5% or less in healthy subjects (Fig. 1). Fig. 2 shows the actual changes in FEV₁ before, during, and after ethanol challenge test. At baseline, FEV₁ was similar in responders and nonresponders. Examination of the serial changes in FEV₁ showed that in responders,

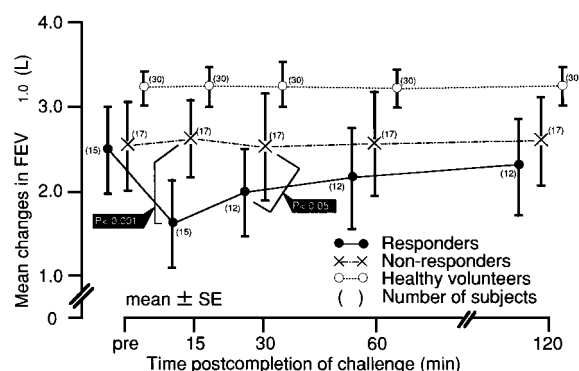


FIG. 2. Serial changes in FEV₁ after ingestion of ethanol.

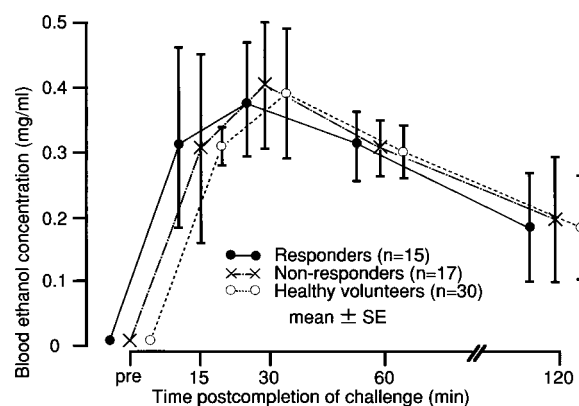


FIG. 3. Mean change in blood ethanol concentration after ingestion of ethanol.

FEV₁ decreased markedly 15 to 30 minutes after ingestion (-0.43 L to approximately -1.11 L) but recovered spontaneously in 12 of the 15 responders and returned to the baseline level after 120 minutes. However, in the remaining three responders, FEV₁ remained low (-1.02 to approximately -2.07 L) 15 minutes after the challenge, necessitating treatment with inhaled salbutamol for marked dyspnea. Accordingly, spirometric data from these patients recorded after 30 minutes were not included in the analysis (Fig. 2).

Blood ethanol concentration

There were no significant differences in blood ethanol concentrations among the three groups throughout the entire test (Fig. 3). Blood alcohol concentration reached a peak 15 to 30 minutes after ethanol ingestion in all groups and decreased thereafter.

Blood acetaldehyde concentration

The concentration of blood acetaldehyde reached a peak 15 to 30 minutes after alcohol ingestion and decreased thereafter (Fig. 4). It was significantly higher in responders than in nonresponders at 15 to

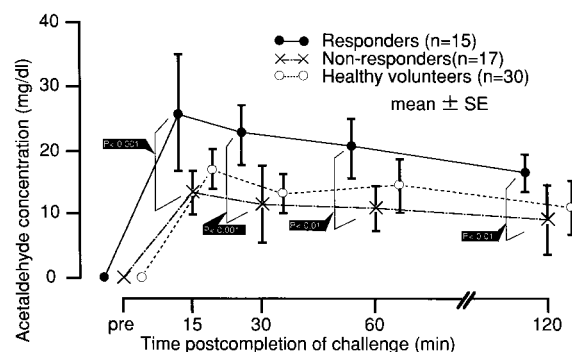


FIG. 4. Mean change in blood acetaldehyde concentration after ingestion of ethanol.

120 minutes after ingestion. The mean peak concentration in responders (25.50 ± 11.96 mg/dl) was significantly different from that in nonresponders (13.27 ± 6.54 mg/dl, $p < 0.001$). The peak concentration in the control subjects (17.40 ± 6.58 mg/dl) was not statistically different from that in responders or nonresponders.

Plasma histamine concentration

The histamine concentration was significantly higher in responders than in nonresponders 15 to 60 minutes after the alcohol challenge test (Fig. 5). The peak concentration in responders (26.00 ± 7.81 ng/ml) was significantly different from that of nonresponders (4.25 ± 3.28 ng/ml, $p < 0.001$). The peak concentration in the control group (3.74 ± 0.47 ng/ml) was significantly lower than that of responders ($p < 0.001$).

ALDH2 genotype

The ALDH2 genotype was NN in 16 (50%), NM in 14 (44%), and MM in two (6%) of the 32 asthmatic subjects (Fig. 6). In normal subjects, the ALDH2 genotype was NN in 17 (57%), NM in 12 (40%), and MM in one (3%). The frequency of ALDH2 genotypes in the two groups was not different.

Response to ethanol challenge test and ALDH2 genotype

The response to oral ethanol loading was positive in only three (19%) of the 16 patients with type NN ALDH2 compared with 10 (71%) of the 14 type NM patients and in two of two (100%) patients with genotype MM (Fig. 6). Thus the percentage of patients with positive test results was significantly higher among those with inactive ALDH2 types (NM and MM) than those with the normal active type (NN) ($p < 0.01$).

DISCUSSION

Asthma is an inflammatory airway disease. Bronchoconstriction develops in response to an allergic reaction by IgE induced by a variety of allergens such as fungi, yeast, and hops contained in alcoholic beverages.^{20, 21}

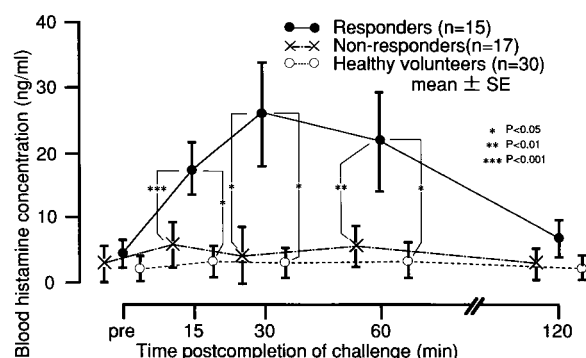


FIG. 5. Mean change in blood histamine concentration after ingestion of ethanol.

Other substances can also trigger asthma such as aspirin, dyes, and preservatives in alcoholic beverages.^{4,17} In addition, sudden drinking or inhalation of concentrated alcoholic beverages is known to induce asthma attacks of a short duration caused by direct stimulation of the airway.^{2,3} Sulfur dioxide in red wine may also cause asthmatic attacks.²² We have also examined the effect of pure alcohol free of common additives present in alcoholic beverages and found that ethanol intake induced a rapid rise in blood acetaldehyde concentration associated with increased histamine concentrations and development of bronchoconstriction.⁴

In this study we analyzed the response to oral ethanol provocation testing by using the method described by Shimoda et al.,⁴ according to ALDH2 genotype, to clarify the pathogenic mechanisms of alcohol-induced asthma. The response to oral administration of ethanol was positive in 15 (47%) asthmatic subjects. Although blood ethanol concentrations after ethanol ingestion were similar in responders and nonresponders, the mean concentration of blood acetaldehyde was significantly higher in responders than in nonresponders 15 to 120 minutes after ethanol administration. The concentration of plasma histamine was also significantly higher in responders than in nonresponders 15 to 60 minutes after ethanol administration, and acetaldehyde and histamine concentrations increased in parallel with each other. According to Shimoda et al.,⁴ acetaldehyde induces a dose-dependent release of histamine by peripheral white blood cells at concentrations of 2, 10, 20, and 100 $\mu\text{mol/L}$, although ethanol has no direct histamine-release effect. In addition, no difference was observed in the response to acetaldehyde in asthma patients between responders and nonresponders, but the response was enhanced in asthmatic subjects as a whole group compared with healthy control subjects.⁴ These early findings, together with those of this study, suggest that the concentration of blood acetaldehyde increases more after drinking in responders than in nonresponders and that histamine is released in larger amounts in the first group to induce airway narrowing.

We also examined the genetic mechanisms causing

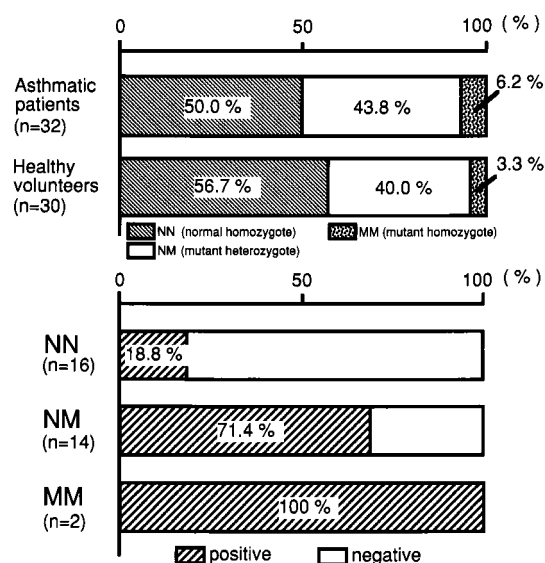


FIG. 6. Comparison of ALDH2 genotype between asthmatic subjects and healthy subjects.

increased levels of acetaldehyde. Acetaldehyde is catabolized mainly by ALDH2. The gene for ALDH2 is located in the long arm of chromosome 12. ALDH2 enzyme is inactivated when the amino residue 487 (glutamic acid) is replaced with lysine due to point mutation of the 12th exon (GAA Δ AAA). ALDH2 is a tetramer, and all four subunits must be normal for the enzyme to retain its activity. In type MM ALDH2 gene all four tetramers are absent, hence no ALDH2 activity is present. In type NM ALDH2 gene there are only few normal tetramers resulting in a low ALDH2 activity.²³ According to Harada,²⁴ the distribution of ALDH2 polymorphism varies among different races and ethnic groups in various regions of the world. Thus types NN, NM, and MM make up 56.4%, 39.4%, and 4.2% of the Japanese population, respectively. In contrast, type NN was present in all European whites. In this study the genotype distribution of ALDH2 in our population sample was 50%, 44%, and 6% in asthmatic subjects for genotypes NN, NM, and MM, respectively. The frequency of each genotype was not different from that of the control sample. Thus in essence, the frequency of different ALDH2 genotypes is similar to that of Harada in the Japanese population at large.²⁴ We also extended these results by examining the relation between the genotype and response to ethanol in asthma patients. Our results showed that 19% of patients with type NN, 71% of those with type NM, and 100% of those with type MM ALDH2 gene showed a positive response to ethanol challenge. Furthermore, the percentage of responders was higher in ALDH2 types associated with delayed acetaldehyde metabolism and increased blood acetaldehyde concentrations. Therefore in asthma patients with inactive ALDH2 genotypes (types NM and MM), the lack of enzyme activity is associated with increased blood

acetaldehyde concentrations. This leads to mast cell (or basophil) degranulation and the release of histamine, which in turn causes contraction of bronchial smooth muscles. In European whites, alcohol is less likely to exacerbate asthma through this mechanism because all have the type NN ALDH2 gene.²⁴

It should be noted that the response to ethanol challenge was also positive in a few patients with type NN ALDH2 gene in this study. It is possible that these positive responses are due to nonspecific airway stimulation by ethanol consumption. Alternatively, if enzyme activity is normal, individual differences in the total amount of the enzyme (i.e., enzyme production) may differ from one person to another. Because alcohol-induced asthma cannot be suppressed completely by antihistamines,⁴ it is also possible that chemical mediators other than histamine may be involved, and further studies are necessary to identify such mediators.

In conclusion, abnormalities of the ALDH2 activity based on gene polymorphism are likely to play an important role in the pathogenesis of alcohol-induced asthma.

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