

Screening for acetaldehyde dehydrogenase 2 genotype in alcohol-induced asthma by using the ethanol patch test

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Background: We have previously reported that alcohol-induced asthma in Japanese patients is caused by increased blood acetaldehyde concentration resulting from abnormalities of acetaldehyde dehydrogenase 2 (ALDH2) enzyme activity on the basis of *ALDH2* genotype differences.

Objectives: The purpose of the present study was to determine whether the ethanol patch test could predict the *ALDH2* genotype in Japanese asthmatic subjects.

Methods: An ethanol patch test on the upper arm and a questionnaire survey addressing the past history of alcohol-induced asthma were administered to 148 adult Japanese asthmatic subjects. The *ALDH2* genotypes in these 148 subjects were also determined by means of PCR.

Results: The genotype distribution of *ALDH2* determined by PCR in 68 subjects with positive ethanol patch test results was 4 (5.9%), 56 (82.4%), and 8 (11.8%) for genotypes NN (normal homozygote), NM (mutant heterozygote), and MM (mutant homozygote). The *ALDH2* genotype in 80 subjects with a negative test result was only NN. The distribution of *ALDH2* genotype in 78 (52.7%) subjects who had experienced alcohol-induced asthma symptoms on the basis of the questionnaire was 27 (34.6%), 44 (56.4%), and 7 (9.0%) for genotypes NN, NM, and MM, respectively. On the other hand, 70 subjects had never experienced alcohol-induced asthma symptoms. In these subjects the *ALDH2* genotype was NN in 51 (72.9%), NM in 18 (25.7%), and MM in 1 (1.4%).

Conclusions: Our results indicate that the results of ethanol patch testing correlate well with *ALDH2* genotype, as determined by means of PCR, suggesting that the ethanol patch test is useful for the screening of alcohol-induced asthma. (*J Allergy Clin Immunol* 2001;108:715-9.)

Key words: Alcohol-induced asthma, acetaldehyde dehydrogenase 2, ethanol patch test, questionnaire, polymerase chain reaction

Abbreviation used

ALDH: Aldehyde dehydrogenase

A small group of European white subjects and half of the Japanese population are estimated to experience exacerbation of asthma after consumption of alcoholic drinks^{1,2}; however, the underlying mechanisms differ in these 2 groups. Certain antigens, preservatives, or both that are present in alcoholic beverages cause asthma exacerbation in the former group.³⁻⁸ In contrast, we have previously reported that acetaldehyde, a metabolite of alcohol, causes bronchoconstriction in the Japanese population.² The metabolism of acetaldehyde differs among races. The enzymatic activity of acetaldehyde dehydrogenase 2 (ALDH2), a primary catabolic enzyme of acetaldehyde, is normal in European white subjects, whereas its activity is genetically reduced in approximately half of the Japanese subjects.⁹ Thus the significant increase in serum acetaldehyde triggered by alcohol drinking induces histamine release from mast cells and basophils and results in bronchoconstriction.

Although the *ALDH2* genotype can be determined by means of PCR with deoxyribonucleic acid isolated from peripheral blood, this method requires specific equipment and is time consuming. Because alcohol dehydrogenase and ALDH2 exist systemically, including the skin,¹⁰ the skin-patched ethanol is converted into acetaldehyde. Acetaldehyde is not catabolized in individuals with low ALDH2 activity and causes larger skin erythema caused by vasodilatation compared with individuals with normal ALDH2 activity. On the basis of this phenomenon, the ALDH2 activity can be determined by using the ethanol patch test.^{11,12} We hypothesized that the ethanol patch test might be a useful screening tool for the diagnosis of alcohol-induced asthma. To test this hypothesis, ethanol patch tests, questionnaire surveys, and PCRs for *ALDH2* genotypes were performed in Japanese asthmatic subjects. Our results indicated that the ethanol patch test correlates well with the results of PCR and shows a higher sensitivity and specificity compared with the questionnaire.

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METHODS

Patients

Subjects in the present study included 148 adult Japanese asthmatic subjects who were managed at the Second Department of Internal Medicine, Nagasaki University School of Medicine (male/female ratio, 72:76; mean $\pm$ SD age, 40.9 $\pm$ 4.2 years). All subjects had consumed alcoholic drinks previously. The severity and diagnosis of bronchial asthma was based on the updated National Heart, Lung, and Blood Institute Expert Panel II on Asthma.¹³ The severity of asthma was mild and persistent in 24, moderate and persistent in 96, and severe and persistent in 8 patients. Atopy, defined by a presence of positive skin prick test responses to one or more common aeroallergens, was found in 82 patients. The present study was approved by the ethics committee of our institution, and written informed consent was obtained from all patients.

PCR for the human *ALDH2* gene

Gene analysis of *ALDH2* was performed as described previously.¹⁴ Briefly, DNA samples were isolated from the peripheral blood by means of standard procedures. The amplification refractory mutation system analysis was designed so that only the terminal 3'-nucleotide of the PCR primer was allele specific. The forward primer was CAA ATT ACA GGG TCA ACT GCT ATG, the reverse normal primer was CCA CAC TCA CAG TTT TCA CTT C, and the reverse mutant primer was CCA CAC TCA CAG TTT TCA CTT T 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, and 65°C for 2 minutes and a final single cycle at 65°C for 8 minutes. The PCR products were separated on a 3% agarose gel and stained with ethidium bromide. The 135 bp of PCR product is amplified with only reverse normal primer in the normal homozygote (NN), with both normal and mutant reverse primers in the mutant heterozygote (NM) and with only reverse mutant primer in the mutant homozygote (MM, Fig 1).

Ethanol patch test

In all subjects antiasthmatic medications were withheld during the 48-hour period before the patch test. The ethanol patch test was performed as described previously.^{11,12} We used a patch plaster (Torii, Tokyo, Japan) of 2 round lint pads 15 mm in diameter and 1 mm thick fixed on an adhesive tape. Just before application of the plaster, 100 μ L of 70% ethanol was placed on one of the lint pads, and as a control, the same volume of distilled water was put on the second pad. The patches were taped on the inner surface of the upper arm for a 7-minute period and then removed. A patch area that showed erythema of 15 mm after removal represented a positive test result for reduced ALDH2 activity.

Questionnaire survey

Subjects were asked as to whether they had ever experienced asthma symptoms associated with alcohol drinking. To further clarify the clinical features of alcohol-induced asthma, individuals with a positive alcohol patch test result were requested to provide further details on the time of onset, amount of alcohol, baseline condition before drinking, and type of drink that elicited worsening of asthma (Table I). In the section addressing baseline condition before drinking, "poorly controlled asthma" and "bad physical condition" were defined and shown on the questionnaire as follows: Asthma is poorly controlled when asthma-related symptoms are present before drinking, such as cough, stridor, chest tightness, dyspnea, and nocturnal awakening, and bad physical condition is defined as the absence of asthma-related symptoms with such physical conditions as sleeplessness and overworking before drinking. In the section addressing type of drink, the respondents were allowed to indicate any specific alco-

holic drinks (eg, beer, wine, or sake [a Japanese alcoholic drink made from rice]) that had previously induced asthma symptoms.

Data analysis

The distribution of the *ALDH2* genotype determined by PCR in asthmatic subjects with a positive or negative ethanol patch test result and questionnaire result were compared by using the χ^2 test. The false-positive ratio (ie, the proportion of subjects with a positive patch test or questionnaire result but a normal *ALDH2* genotype determined by means of PCR) and the false-negative ratio (ie, the proportion of subjects with a negative patch test or questionnaire result but a mutant *ALDH2* genotype determined by means of PCR) were also determined. Differences between groups were considered significant at a *P* value of less than .05.

RESULTS

ALDH2 genotype determined by PCR

The distribution of *ALDH2* genotype in the present study, as determined with PCR, was 74 (50.0%) with type NN, 65 (43.8%) with type NM, and 9 (6.2%) with type MM, respectively. Thus the distribution of the *ALDH2* genotype in the present study was similar to that of our previous report,¹⁴ which showed that the results of the oral ethanol provocation test could be predicted by PCR.

Correlation between results of ethanol patch test and *ALDH2* genotype

Sixty-eight (45.9%) of 148 subjects had a positive ethanol patch test result. The distribution of the *ALDH2* genotype in subjects with a positive test result was 4 (5.9%) with type NN, 56 (82.4%) with type NM, and 8 (11.8%) with type MM (Fig 2). Thus the mutant *ALDH2* genotype (NM and MM) was found in 94.1% of subjects with a positive ethanol patch test result. In contrast, the *ALDH2* genotype in those with a negative ethanol patch test result was only type NN. The proportion of patients with a mutant *ALDH2* genotype (NM and MM) in the positive test result group was significantly higher than that in the negative test result group (*P* < .01).

Correlation between questionnaire and *ALDH2* genotype

The questionnaire identified 78 (52.7%) patients who had experienced at least one episode of worsening of asthma symptoms after consumption of alcohol, and the distribution of *ALDH2* genotype in these patients was 27 (34.6%) with type NN, 44 (56.4%) with type NM, and 7 (9.0%) with type MM (Fig 3). The distribution of the *ALDH2* genotype in 70 patients who had never experienced alcohol-triggered asthma symptoms was 51 (72.9%) with type NN, 18 (25.7%) with type NM, and 1 (1.4%) with type MM. Thus the proportion of subjects with the mutant *ALDH2* genotype was significantly higher in those with a positive questionnaire response compared with those with a negative response (*P* < .05).

False-positive and false-negative ratios

We also determined the false-positive and false-negative ratios for ethanol patch test and questionnaire

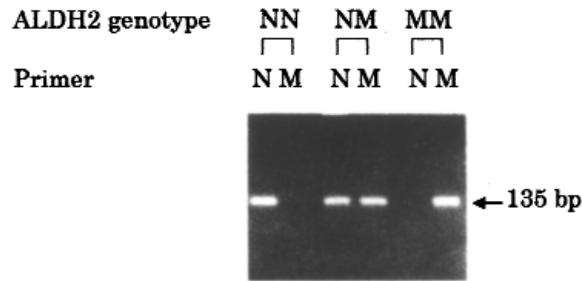


FIG 1. Representative PCR gel for *ALDH2* genotypes. The *ALDH2* genotype was determined by means of PCR with DNA isolated from peripheral blood (see "Methods" section). The 135 bp of PCR product is amplified with only normal primer (N) in type NN (normal homozygote), with both normal and mutant (M) primers in type NM (mutant heterozygote), and with only mutant primer in type MM (mutant homozygote).

responses. The false-positive ratio for the ethanol patch test was 5.9%, and the false-negative ratio was 0%. In comparison, the false-positive and false-negative ratios for the questionnaire were 46.6% and 27.1%, respectively. Thus both the sensitivity and specificity of the ethanol patch test were significantly higher than those of the questionnaire for the screening of *ALDH2* genotype in adult Japanese asthmatic subjects ($P < .05$).

Questionnaire-based clinical features of alcohol-induced asthma

Seventy-eight asthmatic subjects who had experienced at least one episode of worsening of asthma symptoms after alcohol ingestion provided details of clinical features of alcohol-induced asthma (Table I). Symptoms occurred within 30 minutes of alcohol ingestion in 51 (65.4%) patients. These results were consistent with our previous findings of a significant decline in FEV_{1.0} 15 minutes after oral ethanol provocation.² Interestingly, 51 (65.4%) patients reported experiencing asthma symptoms, even after ingestion of a small amount of alcohol. Asthma always worsened in 23 (29.5%) patients, irrespective of the baseline condition, whereas it worsened in 34 (43.6%) patients only when their asthma was poorly controlled. Furthermore, 9 (11.5%) patients reported worsening of their asthma after alcohol ingestion only when in bad physical condition. These results suggest that the baseline state of asthma before drinking alcohol influences the development of alcohol-induced asthma. The present study failed to find any specific alcoholic beverage that precipitated alcohol-induced asthma. Nine (10%) patients reported exacerbation of asthma only when they drank beer or sake, but none reported exacerbation after drinking wine. Future studies are necessary to determine whether these findings are due to the reduced likelihood of asthma after drinking of wine in Japanese subjects or whether they reflect the Japanese drinking custom, in which wine is less commonly consumed than beer and sake.

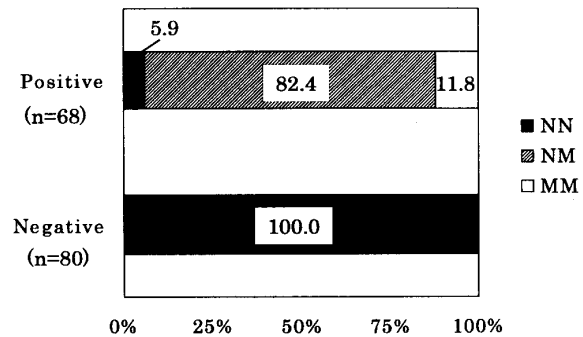


FIG 2. Relationship between ethanol patch test and *ALDH2* genotype. The distribution of *ALDH2* genotype in asthmatic subjects with a positive or negative ethanol patch test result was determined by PCR. Results are shown as proportions (%) of types NN, NM, and MM.

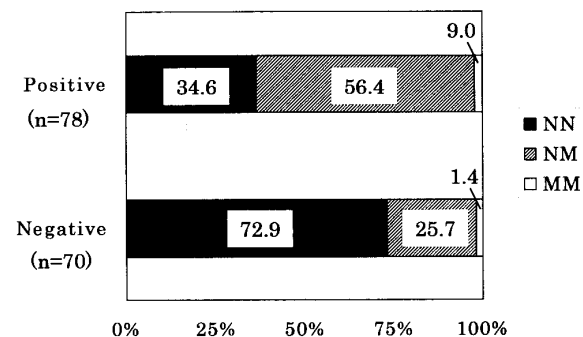


FIG 3. Relationship between questionnaire and *ALDH2* genotype. The distribution of *ALDH2* genotype in asthmatic subjects with a positive or negative response to the part of the questionnaire addressing the past history of alcohol-induced asthma was determined by means of PCR. Results are shown as proportions (%) of types NN, NM, and MM.

TABLE I. Characteristics of alcohol-induced asthma in 78 subjects who had ever experienced asthma symptoms triggered by alcohol

Characteristics	n (%)
Time of onset	
<30 min	51 (65.4)
30 min-1 h	12 (15.4)
1-2 h	2 (2.6)
>2 h	9 (11.5)
Unknown	4 (5.1)
Amount of alcohol	
Small	51 (65.4)
Large	9 (11.5)
Irrespective of amount	7 (9.0)
Unknown	11 (14.1)
Baseline condition before drinking	
Always	23 (29.5)
Asthma is poorly controlled	34 (43.6)
Bad physical condition	9 (11.5)
Unknown	12 (15.4)
Type of drink	
Any	53 (67.9)
Specific	9 (11.5)
Beer	6 (7.7)
Sake	3 (3.8)
Unknown	16 (20.5)

DISCUSSION

The major findings of the present study were the following. First, the results of the ethanol patch test correlate well with those of the *ALDH2* genotype, as determined by using PCR. Second, the specificity and sensitivity of the ethanol patch test to determine the *ALDH2* genotype are significantly higher than those of the questionnaire.

Alcohol-induced asthma is diagnosed by means of the oral ethanol provocation test.² We have previously reported that it is possible to predict the results of the oral ethanol provocation test by means of the *ALDH2* genotype, as determined by PCR.¹⁴ Thus in the present study patients were tested with the ethanol patch test, questionnaire, and PCR but not with the oral ethanol provocation test.

The metabolism of acetaldehyde differs among races, and symptoms associated with alcohol ingestion differ. Mongoloid populations often show face flushing, palpitation, and nausea after drinking a small amount of alcohol. Approximately half of the Japanese population experience facial flushing after consumption of alcohol.¹⁵⁻¹⁷ Japanese persons can rarely drink large amounts of alcohol because of these alcohol-related symptoms, resulting in a low prevalence of chronic alcoholism in Japan.¹⁸ These differences in acetaldehyde metabolism are based on differences in ALDH2 enzymatic activity among various races. The gene encoding ALDH2 is located in the long arm of chromosome 12. ALDH2 enzyme becomes inactivated when the amino residue 487 (glutamic acid) is replaced with lysine as a result of point mutation of the 12th exon (GAA→AAA). ALDH2 is a tetramer, and all 4 subunits must be normal for the enzyme to retain its activity. In the type MM *ALDH2* gene, all 4 tetramers are absent, and thus no ALDH2 activity is present. In the type NM *ALDH2* gene, there are only a few normal tetramers, resulting in a low ALDH2 activity.¹⁹ Types NN, NM, and MM make up 56.4%, 39.4%, and 4.2% of the Japanese population, respectively. In contrast, type NN is present in all European white subjects.⁹

The prevalence of alcohol-induced asthma also differs among races.²⁰ Although only a few studies examined the prevalence of alcohol-induced asthma, a small group of European white subjects and half of Japanese asthmatic subjects are estimated to show exacerbation of asthma triggered by alcohol drinking.^{1,2} However, the mechanism of alcohol-induced asthma is different in the 2 races; bronchoconstriction develops in European white subjects in response to an allergic reaction by IgE induced by a variety of allergens, such as fungi, yeast, and hops, contained in alcoholic beverages.⁴ Aspirin, dyes, and preservatives can also trigger asthma.⁵ Furthermore, consumption of relatively large quantities of alcohol within a short period of time or inhalation of concentrated alcoholic beverages is known to induce asthma attacks caused by direct stimulation of the cough receptor in the airway.⁶⁻⁷ Sulfur dioxide in red wine may also cause asthmatic attacks.⁸ On the other hand, we previously reported a specific mechanism of alcohol-induced asthma in Japanese individuals.² In that study the oral

ethanol provocation test resulted in comparable serum ethanol concentrations among patients with a positive test result who showed a significant fall in FEV_{1,0} after ethanol challenge and those with a negative test result who did not show a significant decline in FEV_{1,0}. In contrast, the serum concentrations of acetaldehyde and histamine after ethanol challenge were significantly higher in subjects with a positive test result than in those with a negative test result. Oral administration of azelastine, a histamine H₁ receptor antagonist, almost completely inhibited the reduction of FEV_{1,0} in those with positive test results.²¹ In addition, stimulation by acetaldehyde, but not by ethanol, induces histamine release in a dose-dependent manner from peripheral blood white blood cells obtained from those with a positive ethanol challenge test result. The determination of the *ALDH2* genotype by means of PCR demonstrated a higher distribution of mutant *ALDH2* in those with a positive ethanol provocation test result.¹⁴ Considered together, it seems that alcohol-induced asthma in Japanese individuals is caused by increased blood acetaldehyde concentration as a result of abnormalities of ALDH2 enzyme activity.

A previous study of 193 Japanese adults and children showed that the ethanol patch test is sensitive and specific for determination of the *ALDH2* genotype.¹¹ The present study also demonstrated that results of the ethanol patch test correlated well with those of PCR and that the test was more sensitive and more specific than the questionnaire. It is possible that in a subset of subjects with normal *ALDH2* genotype who had alcohol-induced asthma symptoms that certain substances in alcoholic beverages caused bronchoconstriction in a manner similar to that seen in European white subjects. Other factors associated with drinking habit, such as smoking, insomnia, and hyperventilation, may also cause bronchoconstriction in some asthmatic subjects. Furthermore, acetaldehyde-induced histamine release may not be of a sufficient degree to cause bronchoconstriction in subjects with mild or well-controlled asthma with the mutant *ALDH2* genotype. Thus the sensitivity and specificity of the questionnaire are lower than those of the ethanol patch test.

In the present study all subjects with a negative patch test result were of *ALDH2* genotype NN. In contrast, 4% of subjects with a positive test result had normal *ALDH2*. These false-positive cases may be due to direct, nonspecific stimulation of ethanol or patch tape. The specificity of the ethanol patch test may be increased by changes in ethanol concentration, patch tape, and timing of evaluation.

The prevalence of bronchial asthma is increasing worldwide. In addition to various therapeutic strategies, such as early intervention with anti-inflammatory drugs, avoidance of a trigger of exacerbation is also important. In this context we have previously reported fatal alcohol-induced asthma.²² The ethanol patch test is a simple, quick, and safe method that can be applied to children. Screening for the *ALDH2* genotype by using the ethanol patch test may be useful to prevent asthma exacerbation in adult patients and to educate young asthmatic subjects about the possible dangers of alcoholic drinks.

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