Short Communication

Reliability of a Flushing Questionnaire and the Ethanol Patch Test in Screening for Inactive Aldehyde Dehydrogenase-2 and Alcohol-related Cancer Risk

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Abstract

Molecular epidemiology of esophageal and upper aerodigestive tract cancers revealed that alcohol is more carcinogenic in persons with inactive aldehyde dehydrogenase-2 (ALDH2) than in those with active ALDH2. A simple questionnaire has been developed to screen for the facial flushing that occurs in persons with inactive ALDH2 when they drink even a single glass of beer. In this study, 266 of 284 consecutive male Japanese clinic patients (age, ≥50 years) completed the flushing questionnaire, and 239 underwent the ethanol patch test (a cutaneous model for the flushing response). Blinded genotyping showed inactive ALDH2 for 94.4% (102 of 108) of subjects who reported always flushing (early in their drinking history or currently) and for 47.7% (21 of 44) of those who reported sometimes flushing, whereas 95.6% (109 of 114) of subjects reporting that they never exhibited facial flushing had active ALDH2. When all three categories of flushing (current always, former always, and sometimes) were collapsed into one, the questionnaire's sensitivity and specificity for identifying inactive ALDH2 were 96.1 and 79.0%, respectively, compared with 72.4 and 71.4% for the ethanol patch test. The results suggest the utility of this simple flushing questionnaire in daily practice, as well as large-scale studies to assess cancer risks associated with drinking and ALDH2 and for activities aimed at preventing alcohol-related cancer.

Introduction

There is much evidence of the carcinogenicity of alcoholic beverages in the oral cavity, pharynx, larynx, and esophagus in humans (1). Recent studies have revealed that alcohol is more carcinogenic in persons who have the inactive form of ALDH2[2] (2–5). This enzyme is crucial in the elimination of acetaldehyde, a recognized animal carcinogen (6) that is generated during the metabolism of p.o.-ingested ethanol (7). In an earlier study, we found that the odds ratios for esophageal cancer in persons with inactive ALDH2 were 7.4 among alcoholics and 12.1 among everyday drinkers, provided their ethanol consumption were similar to that in persons with active ALDH2 (2). The significance of the increased risk underscores the potential benefit of identifying persons who have the inactive enzyme, in terms of both intervention and cost-effective cancer screening, particularly in Asian countries. For example, in Japan and China, approximately 50% of the population have the inactive phenotype (8–10).

The gene for ALDH2 is located on chromosome 12, where a single point mutation results in the inactive isozyme (11, 12). Because the mutant allele, *ALDH2*2*, is dominant over the normal *ALDH2*1* allele, persons who are either homozygous or heterozygous for the *ALDH2*2* allele cannot metabolize acetaldehyde effectively (13). In *ALDH2*2/2*2* homozygotes and *ALDH2*1/2*2* heterozygotes, blood acetaldehyde concentrations are approximately 19 and 6 times that in *ALDH2*1/2*1* homozygotes, respectively (14). After drinking alcohol, such persons exhibit the so-called flushing response, which includes facial flushing, tachycardia, headache, and other unpleasant symptoms (15).

Despite research evidence of the relationship between inactive ALDH2 and the flushing response, practitioners do not always associate the two, and no reliable indicator has been available for general use in screening for ALDH2 genotype, particularly in persons who have a long history of drinking. This study was designed to evaluate the reliability of a simple questionnaire about facial flushing and of the ethanol patch test (a cutaneous model of the flushing response; Ref. 15) to identify for individuals at high risk of developing alcohol-related cancers.

Materials and Methods

The study sample consisted of 284 consecutive male Japanese patients (age, ≥50 years; mean, 60 years) who visited a Tokyo clinic for annual health check-ups in 1996. The study was reviewed and approved by the Ethics Committee of the National Institute on Alcoholism, Kurihama National Hospital. After giving informed consent, each subject was asked to fill out a simple questionnaire concerning drinking habits and the flushing response. The flushing questions were as follows: (*a*) Do you flush in the face immediately after drinking a glass of beer: always, sometimes, or never? (b) Did you flush in the face

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[2] The abbreviation used is: ALDH, aldehyde dehydrogenase.

Table 1 Drinking habits and *ALDH2* genotypes[a] in 284 Japanese men, age ≥50 years

Drinking habit	*ALDH2*2/2*2* (*n* = 19)	*ALDH2*1/2*2* (*n* = 122)	*ALDH2*1/2*1* (*n* = 143)
Nondrinker	19 (100%)	24 (19.7%)	4 (2.8%)[b]
Exdrinker	0 (0%)	5 (4.1%)	9 (6.3%)
Drinker	0 (0%)	93 (76.2%)	130 (90.9%)

[a] *ALDH2*2*, mutant allele; *ALDH2*1*, normal allele.
[b] $P < 0.001$ *versus* exdrinker; $P < 0.001$ *versus* drinker, by the Mantel-extension test.

Table 2 Facial flushing and *ALDH2* genotypes[a] in 266 Japanese men, age ≥50 years

Flushing questionnaire	*ALDH2*2/2*2* (*n* = 15)	*ALDH2*1/2*2* (*n* = 113)	*ALDH2*1/2*1* (*n* = 138)
Current always flushing	15 (100%)	73 (64.6%)	3 (2.2%)
Former always flushing	0 (0%)	14 (12.4%)	3 (2.2%)
Sometimes flushing	0 (0%)	21 (18.6%)	23 (16.7%)
Never flushing	0 (0%)	5 (4.4%)	109 (79.0%)

[a] *ALDH2*2*, mutant allele; *ALDH2*1*, normal allele.

immediately after drinking a glass of beer during the first to second year after you started drinking: always, sometimes, or never? "Current always flushing" was applied to individuals who answered "always" to question *a*; "former always flushing" to those who answered "always" to question *b* but not to question *a*; and "never flushing" to those who answered "never" to both questions. The remaining subjects were classified as "sometimes flushing." The flushing questionnaire was completed by 266 subjects.

Ethanol patch testing was perfomed in 239 of these subjects by medical staff in the clinic, according to the method described previously (15). Each patch consists of two 15 × 15 × 1 mm lint pads fixed side by side to adhesive tape. Just before application, 100 μl each of an aqueous solution of ethanol (70% v/v) and distilled water (as a control) were pipetted onto the first and second lint pad. The patches were attached to the inner surface of the lower arm for 5 min and then removed. One of the authors (Y. K.) served as sole judge of the results. Areas showing erythema 5 min after removal of the patches were judged to be positive; those without erythema, negative.

ALDH2 genotyping was performed on lymphocyte DNA samples from all 284 subjects by PCR-RFLP method (16) without knowledge of the information obtained from the questionnaire and the ethanol patch test.

Results

Table 1 depicts the relationship between drinking habits and *ALDH2* genotypes and shows the inhibitory effect of *ALDH2*2* allele. The genotype distributions were in Hardy-Weinberg equilibrium. There were significant differences between *ALDH2* genotype frequencies in nondrinkers and drinkers ($P < 0.001$) and in nondrinkers and exdrinkers ($P < 0.001$).

Table 2 shows the relationship between *ALDH2* genotypes and the results of the flushing questionnaire for all 266 subjects who completed the questionnaire. Inactive *ALDH2* was found in 94.4% (102 of 108) of current or former always flushing individuals and active *ALDH2* in 95.6% (109 of 114) of those who had never exhibited facial flushing. "Sometimes flushing" was reported by 16.5% (44 of 266) of the subjects, of whom 47.7% had inactive *ALDH2*. When all three categories of flushing individuals (current always, former always, and sometimes) were considered to have inactive *ALDH2*, the questionnaire's sensitivity (a ratio of true inactive to all inactive *ALDH2*) and specificity (a ratio of true active to all active *ALDH2*) were 96.1% (123 of 128) and 79.0% (109 of 138), respectively.

Table 3 Ethanol patch test and *ALDH2*[a] genotypes in 239 Japanese men, age ≥50 years

Ethanol patch test	*ALDH2*2/2*2* (*n* = 16)	*ALDH2*1/2*2* (*n* = 108)	*ALDH2*1/2*1* (*n* = 115)
Positive	15 (93.8%)	77 (71.3%)	35 (30.4%)
Negative	1 (6.3%)	31 (28.7%)	80 (69.6%)

[a] *ALDH2*2*, mutant allele; *ALDH2*1*, normal allele.

As shown in Table 3, the sensitivity (a ratio of true inactive *ALDH2* to all positive results) and specificity (a ratio of true active *ALDH2* to all negative results) of the ethanol patch test in identifing inactive *ALDH2* were 72.4% (92 of 127) and 71.4% (80 of 112), respectively.

There were no differences in the distributions of *ALDH2* genotype and the results of the flushing questionnaire or the ethanol patch test among the 50- to 70-year age brackets.

Discussion

The number of genes identified as responsible for susceptibility to certain cancers has been rapidly increasing. Although the identification of these genes is a major achievement, offering the possibility of genetic screening, challenges lie ahead, both in cost-effective testing procedures and in the development of preventive measures before genetic testing becomes widespread. Laborious methods of laboratory gene analysis are clearly not practical for mass screening. Extensive testing for high-risk individuals might be the strategy of choice, but identification of those subjects is often the first and most difficult work. Such problems have complicated efforts aimed at widespread predictive testing for genetic susceptibility to specific cancers.

In theory, the way to prevent alcohol-related cancers appears simple: do not drink or drink less. However, prevention is anything but straightforward because of all of the complicating individual and environmental factors. Accordingly, the identification of individuals genetically at high risk for these cancers should lead to the immediate design and implementation of effective intervention strategies. Recently, inactive ALDH2 has been demonstrated to be a strong risk factor for the development of certain alcohol-related cancers, especially esophageal cancers (2–5). Because almost one-half of the Asians have the inactive ALDH2 (8–10), the method of screening for this genotype could serve as a powerful tool in prevention strategies.

Previously established as a reliable indicator for the phenotype of ALDH2 (15), the ethanol patch test is especially useful in health education for youth because it can reveal the phenotype even in persons who have never consumed alcohol. However, some studies have shown that this test is less reliable for heavy drinkers, probably because of some acquired tolerance to acetaldehyde after long-term exposure to ethanol.[3] In fact, in the present study, the reliability of the ethanol patch test

[3] Unpublished observation.

was unsatisfactory in men over age 50, many of who had a long history of drinking.

In contrast, the simple questionnaire tested in this study proved useful in identifying the ALDH2 phenotype in persons over age 50. The results revealed the 94.4% of those who reported current or former always flushing had inactive ALDH2, in direct contrast to the 95.6% of those who reported never flushing had active ALDH2. Nevertheless, the questionnaire's ALDH2 predictive power among subjects who reported sometimes flushing was poor; of 44 such persons (16.5% of all subjects), nearly one-half had the mutant allele. However, high sensitivity for inactive ALDH2 for the purpose of identifying susceptible individuals is crucial for this method. When all three categories of flushing (current always, former always, and sometimes) were collapsed into one, the sensitivity and specificity were 96.1% and 79.0%, respectively. The reliability of the flushing questionnaire is clearly satisfactory for identification of high-risk individuals for extensive intervention.

This simple questionnaire with high sensitivity for detecting inactive ALDH2 can be used in a number of interventions against alcohol-related cancer. The first application of this knowledge will likely involve education of normal drinkers concerning susceptibility. A more direct and cost-effective application of the knowledge derived from the questionnaire should be endoscopic surveillance (3, 17), concentrated on persons with double risk factors, *i.e.*, a history of heavy drinking plus inactive ALDH2. Furthermore, the questionnaire's demonstrated reliability makes it possible to begin a large-scale prospective study based on risk assessment of alcohol-related cancers.

The mutant *ALDH2* is an exceptional allele in that knowledge concerning its phenotypic impact is considerable and its association with certain cancers has already been elucidated (2–5). Moreover, owing to the mutation's prevalence in Asian populations (8–10), new knowledge about it has public health implications. Scientific progress based on the genotypic variation of *ALDH2* has provided unprecedented opportunities for the quick application of knowledge in practical interventions. The results obtained in ALDH2 cancer studies may, in fact, serve as a model for demonstration of the potential public benefit of continuing investigation of cancer genetics.

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