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Research Article

Many Orientals lack the mitochondrial aldehyde dehydrogenase (ALDH2) activity responsible for the oxidation of acetaldehyde produced during ethanol metabolism. These individuals suffer the alcohol-flush reaction when they drink alcoholic beverages. The alcohol-flush reaction is the result of excessive acetaldehyde accumulation, and the unpleasant symptoms tend to reduce alcohol consumption. The subunit of this homotetrameric enzyme was sequenced and the abnormality in the inactive enzyme shown to be a substitution of lysine for glutamate at position 487. We have used the polymerase chain reaction to determine the genotypes of 24 livers from Japanese individuals. Correlating genotype with phenotype leads to the conclusion that the allele (ALDH2(2)) encoding the abnormal subunit is dominant.

Genotypes for Aldehyde Dehydrogenase Deficiency and Alcohol Sensitivity

The Inactive *ALDH2*^{2} Allele Is Dominant

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Abstract

Many Orientals lack the mitochondrial aldehyde dehydrogenase (ALDH2) activity responsible for the oxidation of acetaldehyde produced during ethanol metabolism. These individuals suffer the alcohol-flush reaction when they drink alcoholic beverages. The alcohol-flush reaction is the result of excessive acetaldehyde accumulation, and the unpleasant symptoms tend to reduce alcohol consumption. The subunit of this homotetrameric enzyme was sequenced and the abnormality in the inactive enzyme shown to be a substitution of lysine for glutamate at position 487. We have used the polymerase chain reaction to determine the genotypes of 24 livers from Japanese individuals. Correlating genotype with phenotype leads to the conclusion that the allele (*ALDH2*^{2}) encoding the abnormal subunit is dominant.

Introduction

Mitochondrial aldehyde dehydrogenase (EC 1.2.1.3; hereafter referred to as ALDH2)[1] is a tetrameric protein that catalyzes the NAD^+-dependent oxidation of acetaldehyde and other aliphatic aldehydes (1). Although there are multiple forms of ALDH in liver, the mitochondrial enzyme, encoded by the *ALDH2* locus on chromosome 12 (2, 3), has a very low K_m for acetaldehyde ($\sim$ 1 μM) and is believed to be responsible for the oxidation of most of the acetaldehyde generated during alcohol metabolism. About half of Orientals lack ALDH2 activity, as assayed in hair root or liver specimens (4–6), and experience facial flushing, dysphoria, tachycardia, nausea, and hypotension owing to acetaldehyde accumulation when they drink (7, 8). The ALDH2-deficient phenotype appears to be very uncommon in Caucasian and Black Americans, Europeans, and most North American Indians, but it has been detected in $\sim$ 40% of some South American Indians (9–12).

ALDH2 has been purified from Caucasian livers (13) and from a Japanese liver with the ALDH2-deficient phenotype (14). The Japanese liver contained an immunologically cross-reacting protein with subunit molecular weight and amino acid composition similar to that of enzymatically active ALDH2 purified from a Caucasian liver, suggesting that the deficient phenotype results from the production of a catalytically inactive enzyme (14). The sequences of the two proteins differ only in a glutamate to lysine substitution at residue 487 (15). The *ALDH2* alleles encoding the active and inactive subunits have been termed *ALDH2*^{1} and *ALDH2*^{2}, respectively. It had been proposed that the two alleles are expressed codominantly, and that only individuals homozygous for *ALDH2*^{2} are ALDH2-deficient (16, 17). However, studies of the inheritance of alcohol-induced flushing in families suggest that this trait is dominant (18). To establish the genetic basis for ALDH2 deficiency, we developed a genotyping method based upon enzymatic amplification of genomic DNA (19). We found that both heterozygotes and homozygotes for *ALDH2*^{2} are deficient in ALDH2 activity; that is, the *ALDH2*^{2} allele is dominant.

Methods

ALDH2 phenotyping. Frozen liver samples were homogenized in an equal volume of 50 mM Hepes buffer, pH 7.6, containing 0.1 mM DTT, and the samples were centrifuged for 45 min at 100,000 *g*. ALDH isoenzymes were identified by electrophoresis of the supernatant in a 13% starch gel containing Tris-maleate, pH 7.6. The enzyme activity was visualized by staining with 100 mM propionaldehyde, 1 mM NAD^+, 0.14 mM phenazine methosulfate, 0.3 mM thiazolyl blue, and 0.01 mM 3-bromopyrazole (20, 21).

DNA extraction and amplification. DNA extractions and amplifications were performed essentially as described for genotyping of the alcohol dehydrogenase loci (22). Amplification primers were designed based on the sequence of the *ALDH2* gene (23), and allele-specific oligonucleotides were those originally used by Hsu et al. (24). The primers (Fig. 1) were annealed for 2 min at 48°C, and the extension step was carried out using *Taq* polymerase (Perkin-Elmer/Cetus, Norwalk, CT) for 3 min at 56°C, followed by a 1-min denaturation step at 93°C. The DNA was amplified for 34 cycles. 6 μl of the reaction mixture was subjected to electrophoresis in a 2% agarose gel. The DNA was then transferred to nitrocellulose filters (25).

Determination of genotype. The filters were baked and prehybridized at 50°C for 3 h in 5× SSPE (1× SSPE is 180 mM NaCl, 10 mM sodium phosphate, and 1 mM EDTA, pH 7.4) with 0.1% SDS. The labeled probes (2×10^6 cpm/ml) were then added and the filters were hybridized overnight at 50°C. All washes were in 6× standard saline citrate (1× standard saline citrate is 150 mM NaCl and 15 mM sodium citrate, pH 7). The filters were first washed three times at room temperature. Filters hybridized with the *ALDH2*^{2} probe were washed at 54°C for 10 min; those hybridized with the *ALDH2*^{1} probe were washed at 56°C for 3 min. The filters were then exposed to x-ray film for several hours to overnight, typically with one or two intensifying screens.

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1. *Abbreviations used in this paper:* ALDH2, mitochondrial aldehyde dehydrogenase.

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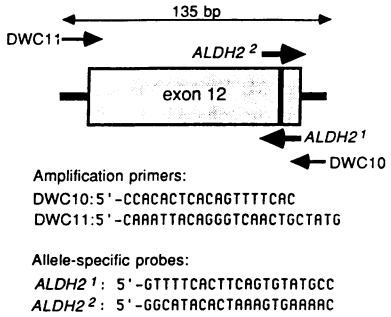

Figure 1. Amplification strategy for genotyping the *ALDH2* locus. The amplification primers were based on the sequence of the *ALDH2* gene (23) and the allele-specific oligonucleotide probes were those used by Hsu et al. (24).

Results and Discussion

We determined the ALDH phenotype of 24 Japanese livers by starch gel electrophoresis and also determined their genotypes. For the latter, we first amplified a 135-bp segment containing exon 12 of the *ALDH2* gene by the polymerase chain reaction with *Taq* DNA polymerase, and then used allele-specific oligonucleotide probes to determine the genotype by hybridization (Figs. 1 and 2). This method of genotyping can be performed faster and with smaller amounts of genomic DNA (2 μg or less) than methods that use probing of Southern blots of restriction endonuclease-digested genomic DNA. 10 of the specimens had the active ALDH phenotype, and all 10 were homozygous for $ALDH2^1$. The remaining 14 specimens had the ALDH2-deficient phenotype. 13 of these ALDH2-deficient specimens were heterozygous for the $ALDH2^2$ allele, while only one was homozygous for $ALDH2^2$.

We also prepared DNA from blood samples from two Caucasians. As expected from previous population studies, they were homozygous for $ALDH2^1$. Two Chinese individuals who almost invariably experience alcohol-flush reactions when they drink were heterozygous for $ALDH2^2$ (Fig. 3).

A previously proposed genetic model of ALDH2 deficiency suggested that the deficient phenotype and alcohol-flushing occurred only in individuals homozygous for $ALDH2^2$ (14, 16, 17). This was based on the presence of protein that cross-reacted with antibody against ALDH2 in ALDH2-deficient liver samples (presumed to be homozygous $ALDH2^2$), and the presence of both ALDH2 enzyme activity and cross-reacting protein (detected by rocket immunoelectrophoresis) in the livers of some Japanese individuals (presumed to represent heterozygotes) (17). However, the interpretation of these studies was complicated by the cross-reaction of the antiserum with cytosolic ALDH (17). Our results indicate that the presence of a single $ALDH2^2$ allele results in the ALDH2-deficient phenotype; that is, this phenotype is inherited as an autosomal dominant trait. This would agree with the dominant inheritance of the flushing trait revealed by family studies (18). The mechanism by which the presence of the $ALDH2^2$ gene product renders heterozygous individuals deficient in ALDH2 activity is not clear. However, ALDH2 is a homotetrameric enzyme. Random association of active and inactive subunits, equally expressed, should generate ~ 6% normal tetramers; the remainder would contain at least one mutant subunit. If all tetramers containing at least one mutant subunit were inactive, there would only be 6% activity in individuals who are heterozygous. This low amount of activity is likely to be below the detection limit of activity staining of the gels.

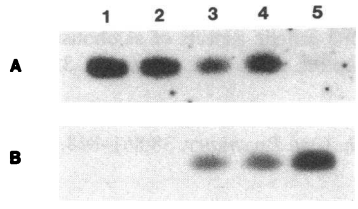

Figure 2. Determination of *ALDH2* genotype in livers of various ALDH2 phenotypes. Genomic DNA was amplified, fractionated on agarose gels, Southern blotted, and hybridized with $ALDH2^1$- (*A*) or $ALDH2^2$- (*B*) specific oligonucleotides. Liver samples *1* and *2* were from a Caucasian and a Japanese, respectively, with the active ALDH2 phenotype. Livers *3–5* were from Japanese with the ALDH2-deficient phenotype.

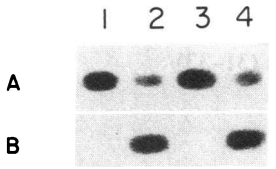

Figure 3. Determination of *ALDH2* genotypes in Caucasian and Chinese individuals. Leukocyte DNA was used to genotype two Caucasians (*1* and *3*) and two Chinese individuals (*2* and *4*). The amplified DNA was hybridized with the probe for $ALDH2^1$ (*A*) or $ALDH2^2$ (*B*).

There have been conflicting reports about the enzymatic activity of ALDH2 cross-reacting material isolated from ALDH2-deficient livers: one reported that the protein from a Japanese liver was completely inactive (14), whereas another reported that this protein exhibited ~ 15% of the specific activity of the enzyme from Caucasian livers (26). It is not known whether the individuals from whom these proteins were purified were homo- or heterozygous for $ALDH2^2$. The possibility that the two types of ALDH2 subunit exhibit an allosteric interaction that renders the heterotetramers inactive is interesting and awaits studies with purified and reconstituted enzymes. It is also possible that the mutant subunit destabilizes the holoenzyme, as suggested by the low recovery of ALDH2 protein from a deficient Oriental liver (26).

Population studies that used electrophoresis of hair root or liver extracts revealed a prevalence of ~ 0.41 for the ALDH2-deficient phenotype in the Japanese (27). Using the older recessive genetic model, gene frequencies of ~ 0.36 for $ALDH2^1$ and 0.64 for $ALDH2^2$ were calculated. In light of our findings, the actual gene frequencies would be 0.77 for $ALDH2^1$ and 0.23 for $ALDH2^2$ in the Japanese. These gene frequencies need to be directly determined, as it has not been possible to differentiate the homozygous and heterozygous ALDH2-deficient phenotypes by the starch or isoelectric focusing gel methods currently used.

This genetic variant in ALDH2 appears to play a major role in determining the prevalence of alcoholism in the Japanese, and presumably in other Oriental populations. It has been shown that the ALDH2-deficient phenotype is quite uncommon in Japanese alcoholics (27–29) and in Japanese individuals with alcoholic liver disease (30). Only 2.3% of alcoholics and 2.8% of alcoholics with alcoholic liver disease were ALDH2-deficient when phenotyped by hair-root analysis, compared with 41% of the general population in Japan (27, 28). It is likely that the alcohol-flush reaction, similar to the alcohol-disulfiram reaction, discourages alcohol ingestion and thereby markedly reduces the risk of alcoholism. This is thus far the best characterized genetic trait that influences alcohol-drinking behavior; in fact, the $ALDH2^1$ allele may be considered a well-defined positive risk factor and the $ALDH2^2$ allele a strong negative risk factor for alcoholism. It would be ex-

tremely interesting to study the interaction between this trait and other genetic factors that appear to increase the risk of alcoholism, such as those for the type II form of alcoholism described by Cloninger and Bohman (31–33).

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