Rapid Detection of ALDH2 Genotypes by Constant Denaturant Gel Electrophoresis, *Andrew N. Zybenko, Brian N. Nightingale, and John B. Whitfield*[1] (Dept. of Clin. Biochem., Royal Prince Alfred Hosp., Missenden Rd., Camperdown, 2050, Australia; [1] author for correspondence: fax Int + 61-2-515-8427, E-mail molgen03@angis.su.az.au)

During ethanol metabolism, mitochondrial aldehyde dehydrogenase (ALDH2; EC1.2.1.3) catalyzes the oxidation of acetaldehyde to acetate. The enzyme, a homotetramer encoded by a single gene locus on chromosome 12 (*1*), has two allelic forms, ALDH2-1 and ALDH2-2. The 2-2 allele is due to a G→A nucleotide transition in exon 12 (*2*), producing a Glu→Lys exchange in the enzyme. Individuals homozygous for the 2-2 allele lose all catalytic activity in the ALDH2 enzyme, and heterozygotes have ~6% of the activity of 2-1 homozygotes (*3*). Alcohol ingestion by individuals with this diminished or absent ALDH2 activity results in accumulation of acetaldehyde, which produces dysphoric symptoms known as the alcohol flush reaction (*4, 5*). The flush reaction is a strong inhibitor of alcohol consumption (*6, 7*).

The relationship between ALDH2 genotype and sensitivity to alcohol, alcohol abuse, and alcohol-related morbidities is still emerging. Compared with 2-1 homozygotes, 2-2 homozygotes rarely abuse alcohol or develop alcoholic liver disease (*8*). Heterozygotes also have a lower incidence of alcohol abuse than 2-1 homozygotes, but the rate of alcohol abuse by heterozygotes appears to be increasing (*9*). Moreover, compared with 2-1 homozygotes who consume excessive amounts of alcohol, heterozygotes who abuse alcohol may be more susceptible to alcoholic liver disease (*10*). Further elucidation of these relationships requires efficient methods of genotyping the ALDH2 locus.

We consider Southern transfer (*11*), allele-specific oligonucleotide probing (*12*), single-stranded conformation polymorphism analysis (*13*), and restriction fragment length polymorphisms (*14, 15*) impractical for large-scale ALDH2 genotyping because of time, cost, or safety considerations. Here we report the use of constant-denaturant gel electrophoresis (CDGE) (*16*) as a suitable alternative method.

We prepared genomic DNA from whole blood by the method of Higuchi (*17*). We studied 4 Chinese and 30 Caucasian humans previously ALDH2-genotyped by the method of Goedde et al. (*12*) in accordance with the standards of the ethics committee of Royal Prince Alfred Hospital. A blood sample was also collected from a baboon because baboon DNA will potentially amplify with the same PCR primers as human DNA but also display some sequence variation from the human DNA, variation that makes it a suitable control for CDGE analysis.

Exon 12 of the ALDH2 gene was amplified by PCR in 20-μL capillary tubes containing 0.5 μg of genomic DNA, 0.5 μg of forward and reverse primers (from DNA Xpress, Fort Collins, CO), 200 μmol/L of each dNTP, 2 mmol/L $MgCl_2$, 1 U of Taq DNA polymerase (Perkin-Elmer, Branchburg, NJ) in a buffer of 15 mmol/L $(NH_4)_2SO_4$ and 60 mmol/L Tris-HCl, pH 9. PCR was performed in a Corbett Research (Sydney, Australia) FTS-1 thermal sequencer. Cycling conditions were: 1 cycle of 95 °C for 4 min, 52 °C for 1 min, and 70 °C for 1 min; then 35 cycles of 95 °C for 20 s, 52 °C for 20 s, and 70 °C for 20 s. The sequences of the forward and reverse primers are as follows (capital letters indicate the sequence homologous to the human ALDH2 gene):

forward 5′-CAGGGTCAACTGCTATGAT-3′

reverse 5′-cgcccgccgcgcccccgcccgtcccgcccccgcccccgCCACA-CTCACAGTTTTCA-3′

To determine the denaturant concentration that would differentiate the ALDH2 alleles, we electrophoresed ~2 μg of known 2-1/2-2 heterozygote PCR product on a 75 g/L acrylamide gel (37.5:1 acrylamide:bisacrylamide) that contained a linear gradient of denaturants perpendicular to the direction of electrophoresis. The concentration gradient of denaturants was 350–650 mL/L of 7 mol/L urea solution and 400 mL/L deionized formamide. Electrophoresis was at 80 V for 90 min on Bio-Rad (Richmond, CA) Mini-Protean II gels modified to run submerged in an 8-L buffer tank and maintained at 60 °C. Electrophoresis buffer was 1× TAE (Tris-acetate 40 mmol/L and $Na_2EDTA \cdot 2H_2O$ 2 mmol/L). DNA bands were visualized by ethidium bromide staining and ultraviolet transillumination.

The constant-denaturant gels used to determine ALDH2 alleles were made up of 60 g/L acrylamide (37.5:1 acrylamide:bisacrylamide) and 480 mL/L denaturant in 1× TAE buffer. After the gel solution was completely degassed, *N, N, N′, N′*-tetramethylethylenediamine, 1 mL/L, and ammonium persulfate, 0.5 g/L, were added (final concentrations). The gel was poured into the mold and overlayed with 1 g/L sodium dodecyl sulfate, and the well-forming comb was inserted. Polymerization time was 2 h. The gels were preelectrophoresed at 80 V and 60 °C for 15 min, the gel wells were rinsed with 1× TAE buffer, and the samples [3.5 μL of PCR product mixed with 1.5 μL of loading buffer (750 mL/L glycerol, 3.3× TAE, 60 g/L bromphenol blue)] were loaded onto the gel. Electrophoresis was performed at 80 V and 60 °C for 105 min in 1× TAE. The DNA bands were visualized as above.

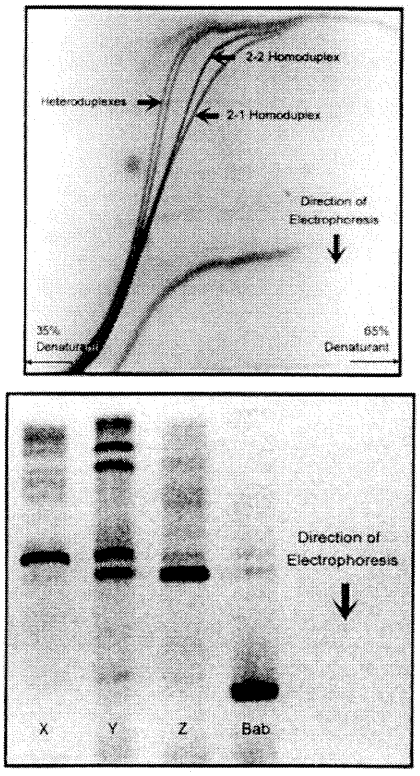

Fig. 1. (*Top*) ALDH2 exon 12 PCR product from a 2-1/2-2 heterozygote electrophoresed on a perpendicular denaturant gradient gel; (*bottom*) ALDH2 exon 12 PCR products electrophoresed on a 48% (480 mL/L) constant-denaturant gel: *X*, 2-2 human homozygote; *Y*, human heterozygote; *Z*, 2-1 human homozygote; *Bab*, homoduplex from baboon.

Figure 1 (top) shows the perpendicular denaturant gradient gel on which the known ALDH2-1/2 heterozygote was electrophoresed. Between the denaturant concentrations of 350 and 439 mL/L, the DNA fragment travels as a single band because the migration rate depends only on molecular mass. As the denaturant concentration increases above 439 mL/L, the DNA migration becomes dependent on the reversible melting behavior of the helix. The heteroduplexes were separated between 439 and 493 mL/L denaturant, the homoduplexes between 469 and 560 mL/L denaturant. The heteroduplex bands melt at a lower denaturant concentration than the homoduplexes because non-Watson–Crick base-pairing occurs in each heteroduplex (*18*). From analysis of the perpendicular gel we concluded that both heteroduplexes and both homoduplexes would be resolved at a denaturant concentration of 480 mL/L. Greater separation of the homoduplexes would be achieved at 510 mL/L denaturant, but the two heteroduplexes would then comigrate.

Figure 1 (bottom) shows a 480 mL/L constant-denaturant gel on which exon 12 PCR amplification products were electrophoresed. Lane Bab contains a single, fast-migrating homoduplex amplified from the baboon ALDH2 gene. The fast migration of the baboon homoduplex indicates that the baboon exon differs from the human exon by one or more nucleotides, and that these nucleotide alterations make the baboon exon more thermally stable than the

human exon. Lanes X, Y, and Z contain DNA amplified from the ALDH2 gene of different humans. The homoduplex in lane Z migrates behind the baboon homoduplex but ahead of homoduplex in lane X. This indicates that lane Z is a ALDH2-1 homozygote and lane X is a 2-2 homozygote. The 2-1 homoduplex is electrophoretically faster than the 2-2 homoduplex because of the higher thermal stability of the 2-1 component. The specimen in lane Y contains both the fast- and slow-migrating DNA homoduplexes, indicating ALDH2-1/2 heterozygosity; the additional two slower bands in lane Y are the heteroduplexes. For the 34 human samples examined, the results obtained for ALDH2 genotype by the CDGE method were identical to those previously obtained by allele-specific oligonucleotide probing.

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