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Authors
Duester, G
Hatfield, GW
Smith, M

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Molecular Genetic Analysis of Human Alcohol Dehydrogenase

GREGG DUESTER,* G. WESLEY HATFIELD* AND MOYRA SMITH*†

*Department of Microbiology and Molecular Genetics and †Department of Pediatrics
College of Medicine, University of California, Irvine, CA 92717

DUESTER, G., G. W. HATFIELD AND M. SMITH. Molecular genetic analysis of human alcohol dehydrogenase. ALCOHOL 2(1) 53-56, 1985.—Human alcohol dehydrogenase (ADH) consists of a complex group of isozymes encoded by at least five non-identical genes, two of which have previously been shown through enzymatic analysis to possess polymorphic variants. Using a cDNA probe the ADH2 gene encoding the β subunit of human ADH was mapped to human chromosome 4. The cDNA probe for ADH2 was also used to detect a restriction fragment length polymorphism present in human populations. This polymorphism may help establish whether certain ADH allele variants are linked with certain types of altered alcohol tolerance observed in various individuals. The restriction fragment length polymorphism may also be of use in genetic linkage studies of other genes located near ADH on human chromosome 4.

### Chromosome mapping
- Restriction fragment length polymorphism
- Gene family
- cDNA

IN humans three different classes of ADH isozymes have been described. Isozymes of the three classes differ from each other in terms of electrophoretic mobility, substrate specificities, inhibition characteristics and stability [6, 8, 10]. Peptide analyses have revealed that the peptide profiles of Class I ADH (homo- or heterodimers of α, β, and γ subunits), Class II ADH (dimers of π subunits), and Class III ADH (dimers of Χ subunits) differ significantly from each other while α, β, and γ subunits within Class I have very similar profiles [14]. Also, subunits from one class do not form heterodimers with subunits from the other two classes [6, 8, 10]. Class I ADH isozymes are the products of three gene loci, ADH1, ADH2, and ADH3, which encode the three different polypeptide chains designated α, β, and γ, respectively [10]. Amino acid sequence analysis of human β and γ ADH (H. Jornvall, personal communication), and hybridization analysis of human ADH genomic clones (G. Duester, unpublished data) indicate that the Class I ADH genes (ADH1, ADH2, and ADH3) are similar enough to be considered a gene family. Class II ADH π subunits are encoded by ADH4 and Class III ADH Χ subunits by ADH5 [1]. The relationship of ADH4 and ADH5 to the class I ADH genes is unknown.

Studies on the gene products of human Class I ADH genes have revealed genetic polymorphisms. Data on the gene frequencies of polymorphic variants have been derived from studies on post-mortem tissues [11]. Both the ADH2 and ADH3 gene loci have been shown to be polymorphic. About 10% of Caucasians express a variant form of ADH2 which differs in its electrophoretic mobility, pH optimum, stability and kinetic properties from the usual ADH2 isozyme. This variant form was called atypical ADH [13]. Studies on Oriental populations have revealed that the atypical ADH2 phenotype occurs in 85% of Japanese [5] and 89% of Chinese individuals [15]. The atypical phenotype is, however, infrequent in the Asian Indian population [15]. It has been suggested that the incidence of this atypical variant at the ADH2 locus in Oriental populations parallels the incidence of alcohol intolerance in these populations [16]. In the English population two common ADH3 alleles have been described. The ADH31 allele occurs with a frequency of 0.60 while the ADH32 allele occurs with a frequency of 0.40 [9]. In the Chinese and Japanese populations the frequency of the ADH32 allele is 0.91 [15].

Genetic studies on Class I ADH and studies aimed at determining the physiological consequences of the different Class I ADH genotypes have been hampered by the fact that these genotypes are not expressed in accessible tissues such as blood cells or cultured fibroblasts. In order to facilitate genetic studies in individuals with altered alcohol tolerance and to define the genomic organization and regulation of the human Class I ADH genes, we have isolated a cDNA probe for the ADH2 gene [4]. Nucleotide sequencing revealed that this cDNA (pADH12) contained a 593 base pair (bp) 3'-untranslated region in addition to a stretch of 273 nucleotides encoding 91 amino-acids at the carboxy terminal region of β ADH [4]. Here, we have used the β ADH cDNA to analyze the chromosomal location and polymorphism of the β ADH gene.

### Method

**Isolation of Plasmid DNA**

Plasmid DNA was isolated by using an alkaline lysis procedure [2] and further purified by CsCl/ethidium bromide density gradient centrifugation. The 1.0 kilobase pair (kb) cDNA insert of pADH12 (Fig. 1) was excised with PsiI and isolated by polyacrylamide gel electrophoresis. This cDNA insert was uniformly labeled by incorporation of [α-32P]dGTP in a nick-translation reaction [7] to a specific activity of approximately 10⁶ cpm per μg.
Hybridization Analysis of Human Genomic DNA

Genomic DNA was isolated from fresh human leukocytes, cultured Chinese hamster ovary (CHO) cells, and CHO-human hybrid cell lines by methods described elsewhere [7]. DNA was cleaved with either EcoRI or MspI, subjected to agarose gel electrophoresis, and transferred to nitrocellulose by the method of Southern [12]. A HindIII digest of λ bacteriophage DNA provided molecular size markers. Southern blots were hybridized to the β ADH cDNA probe, washed and subjected to autoradiography as described previously [7]. A high stringency wash was performed in 0.1 x SSC (1 x SSC is 0.15 M sodium chloride/0.015 M sodium citrate, pH 7.0) at 66°C for 1.0 to 2.5 hours.

RESULTS

Since knowledge of the chromosomal location of the Class I ADH genes will aid human genetic studies on ADH we undertook the mapping of ADH2 to a specific chromosome. Definitive information on the assignment was obtained using the cDNA probe pADH12 (Fig. 1). Genomic DNA was isolated from fresh human leukocytes, cultured Chinese hamster ovary (CHO) cells, and 2 different CHO-human hybrid cell lines. DNA was restricted with EcoRI and then subjected to agarose gel electrophoresis and Southern transfer [12]. Following hybridization to pADH12, nitrocellulose filters were washed under high stringency conditions and then autoradiographed. Using EcoRI six fragments were detected in human leucocyte DNA with molecular sizes of 9.6, 7.4, 4.2, 3.2, 2.9, and 2.2 kb. (Fig. 2). The multiple bands observed in DNA cleaved with EcoRI suggest that the β ADH cDNA probe cross-hybridizes with the genes encoding α and γ ADH. Analysis of genomic clones containing human ADH genes has revealed that the six EcoRI fragments observed in the human genomic blots are derived from the α, β, and γ ADH genes, each possessing two of the EcoRI fragments (G. Duester, unpublished results). Since all six EcoRI fragments are accounted for by the three Class I ADH genes, there is no evidence as yet for cross-hybridization of the β ADH cDNA to the Class II or Class III ADH genes, and no evidence for the existence of ADH pseudogenes.

Assignment of the ADH2 locus to chromosome 4 was obtained from Southern blot analysis of two CHO-human hybrids which contain human chromosome 4. The CHO-human hybrid HHW366 contains only human chromosomes 4 and 5. This hybrid cell line has locked in human chromo-
The sizes of the fragments are indicated in kilobase pairs (kb). Genomic DNA from three unrelated individuals (lanes 1 and 2, Caucasian, lane 3 Oriental) was restricted with MspI, and subjected to Southern blot analysis using the β ADH cDNA probe (pADH12). The sizes of the fragments are indicated in kilobase pairs (kb).

FIG. 3. Southern blot to detect polymorphism. Ten μg of leucocyte genomic DNA from three unrelated individuals (lanes 1 and 2, Caucasian, lane 3 Oriental) was restricted with MspI, and subjected to Southern blot analysis using the β ADH cDNA probe (pADH12). The sizes of the fragments are indicated in kilobase pairs (kb).

Some 5 since this chromosome is required to complement a leucyl-tRNA synthetase deficiency in the CHO mutant line thus allowing the hybrid cells to grow under selective conditions (i.e., in leucine deficient medium at 36°C) [3]. By growing the HHW 366 cell line under non-selective conditions and then recloning this cell line, it was possible to derive a CHO-human hybrid HHW 416, which contained only human chromosome 4. It is of interest to note that with a high stringency wash all of the EcoRI fragments detected by pADH12 in human leucocyte genomic DNA could be demonstrated in EcoRI digests of genomic DNA from the HHW 366 and HHW 416 cell lines (Fig. 2). These findings allow us to assign the ADH2 gene to human chromosome 4.

A more detailed description of the mapping of ADH2 to chromosome 4 using a panel of 20 human-rodent cell lines will be reported elsewhere (M. Smith, manuscript in preparation).

Since genetic linkage studies require the availability of polymorphic chromosomally assigned gene markers, we have undertaken studies to determine whether the pADH12 cDNA probe can be used to detect restriction fragment length polymorphisms in human genomic DNA. Using leucocyte DNA from 3 unrelated individuals we have been able to demonstrate a restriction fragment length polymorphism with the enzyme MspI (Fig. 3). The 8.5 kb MspI fragment is common to all three individuals (lanes 1-3, Fig. 3).

On the other hand the 9.6 kb MspI fragment is present only in the two Caucasian DNA samples (lanes 1 and 2, Fig. 3). Since the pADH12 probe hybridizes to all three Class I ADH genes, the particular ADH gene involved with the polymorphism is as yet unknown. This issue can be investigated using DNA probes specific for α, β, or γ ADH.

**DISCUSSION**

The results presented here indicate that the ADH2 gene is located on chromosome 4. In addition, based upon genomic cloning studies (G. Duester, unpublished results), we conclude that the other two Class I ADH genes ADH1 and ADH3 are also located on chromosome 4. Interestingly, studies on the ADH5 gene encoding Class III ADH indicate that this gene is also located on human chromosome 4 (S. Hiroshige and M. Smith, unpublished data). There is as yet no information on the chromosomal location of the ADH4 gene encoding Class II ADH. Additional regional mapping and genomic cloning studies will be required to determine if all the Class I, II, and III ADH genes form a gene family, or whether only certain members therein, such as the three Class I genes, form a gene family.

An important finding here is that the β ADH cDNA is useful probe for detecting a restriction fragment length polymorphism on human chromosome 4. Interestingly, the two Caucasian DNA samples exhibited a common hybridization pattern, whereas the Oriental sample was of a different pattern. More individuals, both Caucasian and Oriental, will of course need to be examined, but it is interesting to speculate that the restriction fragment length polymorphism observed here might correlate to either the β1/β2 or γ1/γ2 polymorphisms that were discussed above. This question can be approached using DNA probes specific for particular ADH genes, such as DNA fragments that contain only 3' untranslated regions or intron sequences that do not exhibit cross-hybridization to other ADH genes. In conclusion, DNA fragments derived from human ADH cDNA and genomic clones constitute useful probes for detecting polymorphisms on chromosome 4, and may facilitate genetic studies of various alcohol-related syndromes.

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