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Molecular Genetic Analysis of Human Alcohol Dehydrogenase

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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 γ subunits), and Class III ADH (dimers of X 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β and γ ADH (H. Jornvall, personal communication), and hybridization analysis of human ADH genomic clones (G. Duester, unpublished data) indicate that the Class I ADH genes (ADH1, ADH2, and ADH3) are similar enough to be considered a gene family.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 X 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 ADH31 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 PstI 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 leucocytes, 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 leucocytes, 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 HHW 366 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).

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).

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