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Development of DNA Probes to Investigate Genetic Variation of Alcohol Metabolizing Enzymes

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INTRODUCTION

Alcohol dehydrogenase (ADH) is responsible for the oxidation of a wide variety of primary, secondary, and aromatic alcohols, but is most noted as being the enzyme primarily responsible for oxidizing ethanol to acetaldehyde. Human ADH exists as a set of at least 15 different isoenzymes that differ in electrophoretic mobility, substrate affinities, and inhibition characteristics (Smith et al. 1973; Li 1977; Pares and Vallee 1981). All isoenzymes are similar in that they are dimeric, each monomer having a molecular weight of approximately 40,000 daltons. The various isoenzymes are categorized into three classes. Class I contains a large group of isoenzymes possessing various combinations of α -, β -, and γ -ADH subunits coded by three gene loci: ADH1, ADH2, and ADH3, respectively. Class II contains the π -ADH isoenzyme (Bosron et al. 1979; Li and Magnes 1975). Class III contains the χ -ADH isoenzyme, which oxidizes high molecular weight alcohols, but not ethanol (Pares and Vallee 1981). Class I and II are primarily liver-specific enzymes, although small amounts of activity of these isoenzymes occur in intestine, kidney, and lung (Smith et al. 1971). Class III ADH is a constitutive enzyme and is found in all cell types. Studies on the gene products of human class I ADH genes have revealed genetic polymorphisms.

Common genetic variation occurs at the ADH2 and the ADH3 gene loci. One of the variant forms of ADH2 is known to give rise to an altered ADH which differs in its kinetic properties and is implicated in differences in alcohol tolerance. The exact role of genetic variation in relation to propensity to develop alcoholism have been difficult to assess since this enzyme is primarily a liver-specific enzyme and is not readily analyzable in accessible tissues such as blood cells or serum.

We have initiated a research study aimed at isolating ADH and aldehyde dehydrogenase (ALDH) genes so that molecular probes may be derived to analyze genetic variation in the ADH and ALDH genes and their flanking regions. The results presented here relate to the derivation of DNA probes for the class I ADH genes.

Availability of partial amino acid sequence data for the β -ADH isozyme, coded by the ADH2 gene, enabled us to select a sequence of five amino acids with relatively low code degeneracy, near the carboxy terminal region of the ADH polypeptide. An oligonucleotide probe corresponding to this sequence was commercially obtained. The probe consisted of a mixture of 16 different oligomers, one of which is perfectly complementary to ADH mRNA (figure 1). The radiolabeled oligonucleotide probe was used to screen colonies derived from the adult human liver cDNA library of Orkin (Woods et al. 1982).

Ala Asp	Phe	Met	Ala	Amino acids 332-336
5'-GCN GA ^U _C	UU ^U _C	Aug	GCN-3'	mRNA sequence
3'-CGN ^A _G CT ^A	AA ^A _G	TAC	CG -5'	Synthetic oligonucleotide sequence

FIGURE 1

SEQUENCE OF OLIGONUCLEOTIDE PROBE (14 mer) FOR HUMAN ADH

One clone isolated from the cDNA library and designated pADH12 was found to contain an insert of 1100 base pairs. A restriction map of this clone was derived by further digesting the isolated cDNA insert by various enzymes. Restriction fragments were then cloned into the M13 bacteriophage and sequenced (Duester et al. 1984). Analysis of the DNA sequence of the insert in the pADH12 clone revealed that it contained a 593 base pair 3' untranslated region in addition to a 273 base pair translated region coding for 91 amino acids. The DNA sequence in this region predicted exactly the amino acid sequence of the terminal 91 amino acids in β -ADH as derived by Böhler and coworkers (1984).

This pADH12 cDNA clone has been used to derive genomic ADH clones. The Maniatis library of human DNA in lambda phage was screened with pADH12. Information from peptide mapping studies (Strydom and Vallee 1982) and partial sequence information on the ADH3 polypeptide γ (Bröhler et al. 1984) indicate that the coding regions of the three class I ADH genes are closely homologous. Using radiolabeled pADH12 as a probe under conditions of low stringency, it should be possible to isolate from libraries of human genomic or cDNA, clones corresponding to all three class I ADH genes. One of the genomic clones, which was isolated from the Maniatis library using pADH12, has been found to contain a terminal exon, which is similar but not identical to that in pADH12 in addition to an adjacent intron and 3' untranslated region (Duester 1984). The base modifications present in the exon of the ADH53 clone correspond to those which one would expect to find in the ADH1 gene, based on the amino acid differences between β - and α -ADH polypeptide as determined by Jörnvall (personal communication, 1984).

INVESTIGATION OF ADH GENETIC VARIATION USING THE pADH12 AND THE ADH53 PROBES

Prior to using the ADH53 clone in studies of human DNA polymorphism, we ascertained that this clone did not contain any repetitive DNA sequences. Human DNA was isolated from peripheral blood leukocytes or from cultured lymphoblastoid cells. Human DNA was digested with a number of different restriction endonucleases, and individual DNA digests were subjected to agarose gel electrophoresis and Southern transfer to nitrocellulose filters (Southern 1972). Filters were hybridized with radiolabeled ADH gene probes and then washed at different stringencies prior to autoradiography. The restriction endonuclease MSP 1 has thus far proved most useful for detecting DNA polymorphism in the ADH genes (figure 2).



FIGURE 2

MSP1 FRAGMENTS OF HUMAN GENOMIC DNA REVEALED BY HYBRIDIZATION TO ADH53 PROBE

Using the ADH53 probe and a low-stringency wash of the filters (1 x SSC, at 55 C for 40 minutes), four or five MSP 1 fragments are seen. These fragments are 12.5, 10.0, 8.6, 6.8, and 3.8 Kb in size (see figure 2 and figure 3). Occasional individuals have also been found to have a 4.2 Kb fragment. Following a high-stringency wash (0.1 x SSC/65 C/30 minutes) only the 10.0, the 8.6, and the 3.8 Kb fragments remain, suggesting that these fragments are most analogous to the ADH53 probe, which probably represents the ADH1 gene. Using the pADH12 probe, 4 fragments may be seen. These are the 12.5, 10.0, 8.6, and 6.8 Kb fragments. However, the 6.8 Kb fragment is frequently very weak with pADH12. Following a high-stringency wash, the 8.6 Kb fragment is the only fragment still visualized, suggesting that this fragment is most analogous to the ADH2 gene (see figure 3).

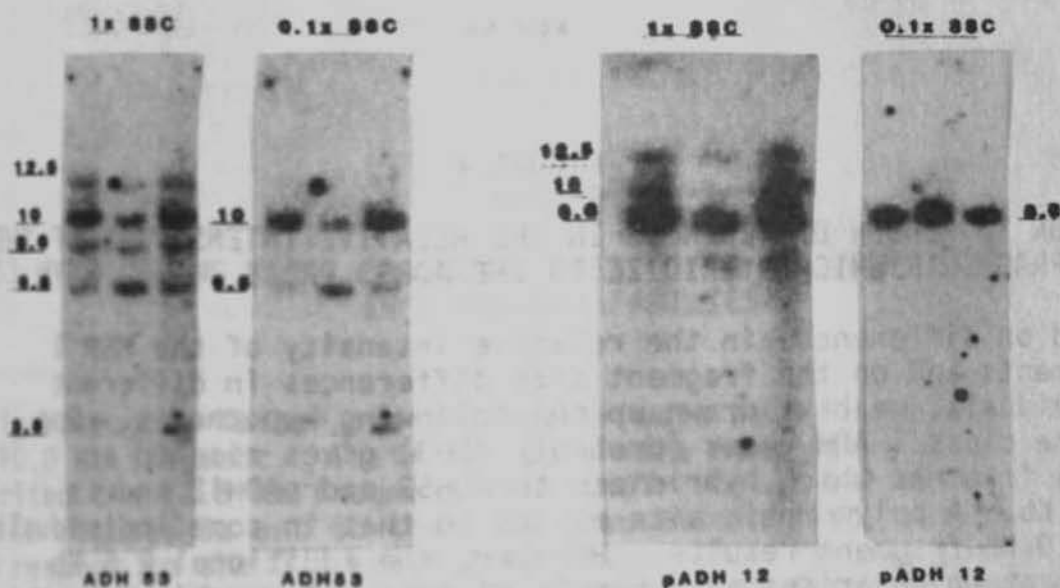


FIGURE 3

CHANGES IN PATTERNS OF DNA FRAGMENTS WHICH HYBRIDIZE TO ADH53 AND ADH12 UNDER CONDITIONS OF LOW AND HIGH STRINGENCY

In occasional individuals, an 11 Kb band may be visualized. This fragment is apparently more analogous to ADH2, based on the observation that it is usually more intense with pADH12 than with ADH53. The 12.5 Kb MSP 1 fragment hybridizes equally well with pADH12 and ADH53 at low stringency. At high stringency, it is not present with either probe. This finding suggests that the 12.5 Kb fragment may be derived from a third class I ADH gene, possibly the ADH3 gene which codes for the γ polypeptide. Individual variation has been observed with respect to the number of MSP 1 fragments. In some individuals, the 12.5 Kb fragment is absent, while in other individuals the 10.0 Kb fragment is absent (see figure 2). Individual variation has also been observed with respect to the relative intensity of the different fragments (see figure 4).

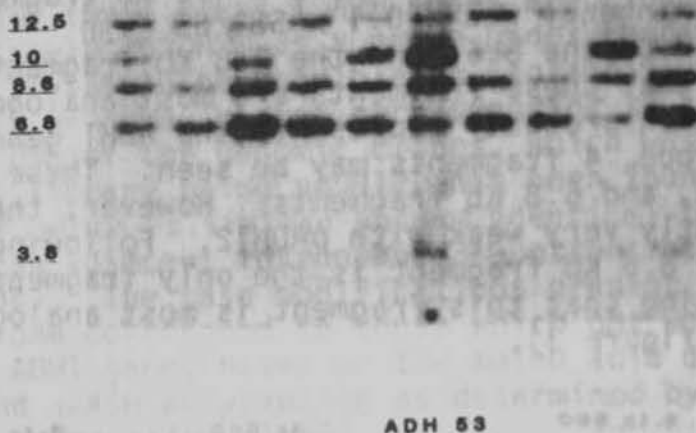
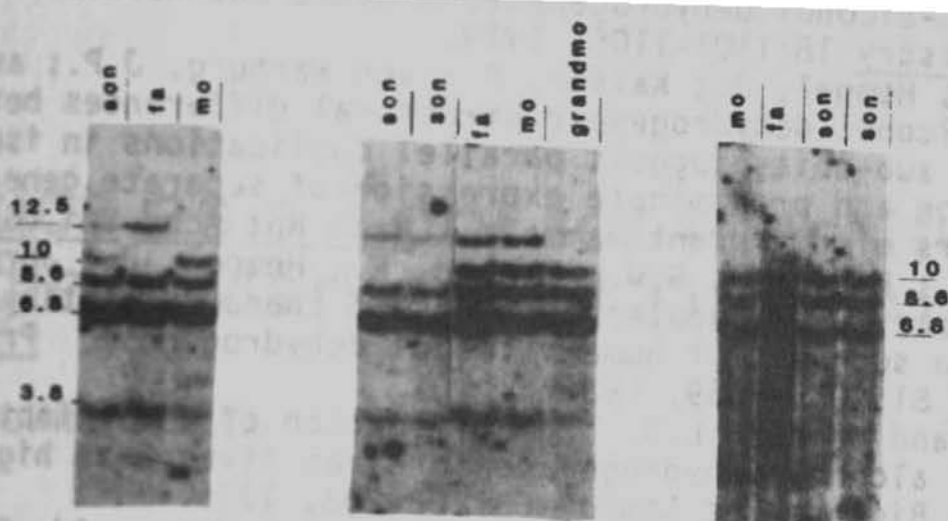


FIGURE 4

PERSON TO PERSON DIFFERENCES IN THE RELATIVE INTENSITIES OF THE DNA FRAGMENTS WHICH HYBRIDIZE TO THE ADH53 PROBE

Based on differences in the relative intensity of the MSP 1 fragments and on the fragment size differences in different individuals, we have drawn up the following hypothesis. One of the class I ADH genes (probably ADH3) gives rise to an MSP 1 fragment which hybridizes to ADH53 and pADH12 and is 12.5 Kb. A polymorphic site exists so that in some individuals a 10.0 Kb fragment results. However, the additional 2.5 Kb fragment which arises as a result of this polymorphism may not be visualized with ADH53 or pADH12. A second ADH gene (probably ADH1) gives rise to a 6.8 Kb fragment. In certain individuals an MSP 1 site is absent so that a 10.0 Kb fragment results. Individuals who are doubly heterozygous at the two loci described above will therefore exhibit a very intense 10.0 Kb fragment.

Examination of MSP 1 digestion patterns in a number of nuclear families has provided evidence of heritability of certain fragments. In figure 5a, the mother is homozygous for the 10.0 Kb fragment, while the father is homozygous for the 12.5 Kb fragment. Their son is an obligate heterozygote, having inherited the 12.5 Kb fragment from his father and the 10.0 Kb fragment from his mother.



ADH 53

FIGURE 5

ADH53 HYBRIDIZING MSP 1 FRAGMENTS PRESENT IN DNA FROM INDIVIDUALS IN 3 NUCLEAR FAMILIES

In figure 5c the father and the mother were both homozygous for the 10.0 Kb fragment. Their two sons were homozygous since each inherited the 12.5 Kb fragment from their mother and father. Note that in figures 5a and 5b there is also evidence of polymorphism with respect to the 3.8 and 4.2 Kb fragments. The exact relationship of this variation and the 12.5 to 10.0 Kb fragment variation is not clear at present. We have thus far examined DNA from 101 individuals, including 80 Caucasoids, 10 Orientals, and 11 Negroids. Polymorphism has been demonstrated in each population group.

In the analytical system described above, the presence of an MSP 1 fragment 10.0 Kb in size could result from polymorphism of either the ADH1 or the ADH3 gene. In an attempt to further resolve this polymorphism, we are currently carrying out double digestions of human DNA with EcoRI and MSP 1. In addition, studies are being carried out to derive gene-specific probes and/or gene-specific hybridization conditions. Further studies are also required to define the origin and significance of the 11 Kb band which occurs in occasional individuals and which is apparently more intense with the pADH12 (i.e., the ADH2) gene probe.

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