Genes and alcoholism: a preliminary report

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Alcohol abuse is a leading cause of medical and social problems in New Zealand. Alcoholism is not a unitary condition. It affects the sexes to different extents and also has a much greater impact on some ethnic groups than on others. For these and other clinical reasons it is desirable to have simple tests capable of identifying individuals at increased risk of alcoholism.

Conventional approaches using electrophysiology, enzyme assays and clinical chemistry have produced equivocal results but recent advances in molecular biology have provided a new set of analytical tools. These are the DNA sequences for genes involved in hepatic alcohol metabolism. This new knowledge makes it possible to design synthetic oligonucleotides to serve as primers in polymerase chain reaction (PCR) amplification of short sections of the genes which code for alcohol metabolising enzymes. Amplified DNA products can then be analysed for base substitutions known to occur in genes encoding alcohol dehydrogenase (ADH2 variants ADH2-1 and ADH2-2) and mitochondrial aldehyde dehydrogenase. We report here a method using direct DNA sequencing of PCR products to characterize ADH2 alleles in individual DNA samples.

Materials and methods

Blood samples were obtained with the approval of the Wellington Area Health Board and with informed consent from volunteers who also answered a short questionnaire about their ethnic origin. Leucocytes were isolated by centrifugation and DNA was prepared by SDS/proteinase K lysis of cells followed by standard solvent extraction steps. RFLP analysis of 5 µg DNA samples was carried out as according to published procedures using pADH12, pADH74 and pADH76 cDNA probes obtained from the American Type Culture Collection.

Primary PCR catalysed amplification of DNA was carried out using synthetic oligonucleotide primers HE45 and HE46 (Operon Inc, Alameda, CA, USA) for the ADH2 gene. A second round of asymmetric PCR was carried out using a primer ratio of 100:1 (HE46:HE45) to amplify single stranded ADH sequences. The 108 nucleotide product was purified by precipitation with isopropanol and ammonium acetate and was sequenced using a 'Sequenase' DNA sequencing kit according to the suppliers (US Biochemicals Corp.) instructions and using HE45 as sequencing primer. The authenticity of double stranded PCR products from some ADH2-2 homozygotes was checked by MaeIII digestion followed by DNA fragment size analysis using polyacrylamide gel electrophoresis.

Results

For our initial study we selected a RFLP approach using cDNA probes for the ADH gene, hoping that this method might reveal novel DNA sequence variability. Restriction fragment sizes observed were similar to those reported by previous workers but good quality data could not be obtained routinely and the RFLP approach was abandoned.

A strategy was then developed to screen for ADH2 variants based on the polymerase chain reaction. It was decided to directly sequence PCR products rather than to screen them for their ability to hybridise with allele specific oligonucleotides. This decision was necessary in order to be certain that correct PCR products were produced and because no subjects were available for whom the ADH genotype was known in advance. It was further argued that if the genotypes of several subjects could be established directly from their ADH sequences, they might serve as controls for future experiments. Ten caucasians (expect ADH2-1 at high frequency), ten oriental Asians (expect ADH2-2 at high frequency) and ten Maori subjects (allele frequency at ADH2-2 unknown, but dependent on ancestry and population size) were chosen as subjects.

Figure 1 shows sequences from a region of the 108 base pair product obtained by PCR using the HE45 and HE46 primers. The ADH2 DNA sequences from ten subjects, in each of the ethnic groups are in Figure 2. The data show how readily ADH 2-1 and ADH 2-2 homozygotes and the ADH2-1/ADH2-2 heterozygote can be distinguished using this procedure. Homozygous ADH2-1 individuals give a single band in the G lane of the sequencing gel, whereas ADH2-2 homozygotes give a band in the A lane and heterozygotes give bands in both lanes. The sequences are identical to those previously published for ADH2. All of the caucasians surveyed were ADH 2-1 homozygotes and in the Asian samples surveyed, the ADH 2-2 allele was present at a level of 0.60, both of these results were consistent with expectations. The Maori samples surveyed gave an intermediate frequency of 0.25 for the ADH 2-2 allele. Results are shown summarised in Table 1. The small number of subjects whose ADH 2 sequences are reported here are reasonably representative of the ethnic groups in New Zealand (based on a more extensive database presently being accumulated but not reported here), and the values obtained for caucasians and Asians agree well with the literature.

Table 1. — ADH 2 allele frequencies in three New Zealand ethnic groups.

Ethnic group	Number of subjects	Nucleotides sequenced	ADH 2-1 alleles	ADH 2-2 alleles	Frequency of ADH 2-2
caucasian	10	431	20	0	0.00
Asians	10	455	8	12	0.60
Maori	10	555	15	5	0.25

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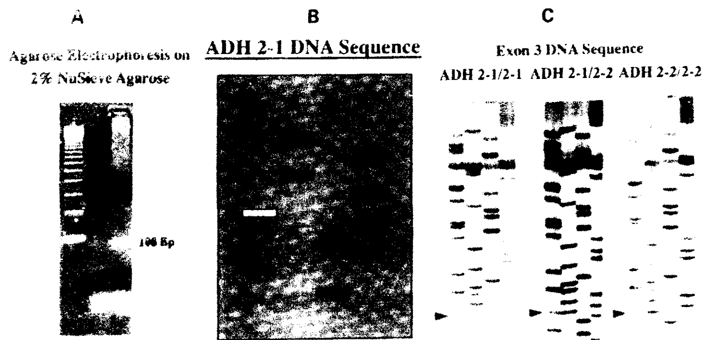

Figure 1.

A: Agarose gel showing the ds DNA amplification product obtained from the experimental protocol shown in Figure 1.
B: The expected DNA sequence around codon 47 in the caucasian ADH2-1 variant reads TGT CGC ACA (bottom to top).
C: Autoradiographs showing DNA sequences of the ADH-2 exon 3 region for three individuals. Data obtained by sequencing the ssDNA product.

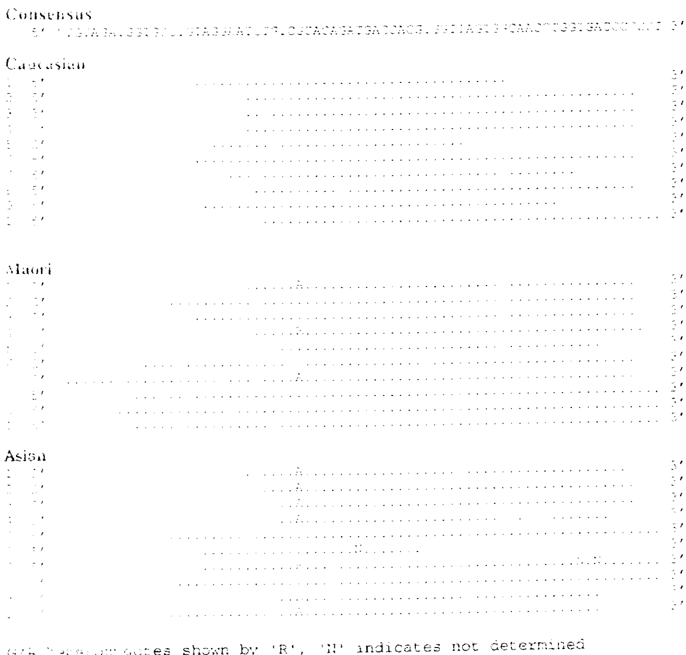

Figure 2 — Nucleotide sequences of the ADH 2 gene from 10 individuals in each of three ethnic groups.

Discussion

We have developed a novel and reliable method for detecting human ADH2-1 and ADH2-2 variants. Our data show the ADH2-2 allele to be relatively common in New Zealand oriental Asians and Maori. The data concerning the presence of ADH-1 variants in the caucasians must be considered provisional because they may possibly carry another rare nucleotide substitution (ADH2-3) elsewhere in the gene. However, it is certain that ADH2-2 alleles are absent in these caucasian subjects.

The discovery that Maori have ADH2-2 alleles is interesting and suggests that they share genetic variation with present day Asians as a result of relatively recent common ancestry. It is now important to discover if the null aldehyde dehydrogenase 2-2 variant is also common in Maori and other Polynesian populations because this allele is believed to exert a protective effect from alcoholism among Asian people. If aldehyde dehydrogenase 2-2 alleles are found in Maori, then the relatively high incidence of alcohol related health problems reported in Maori will seem even more anomalous. Work in progress includes developing methods to screen for other ADH (ADH2-3, ADH3-1, ADH3-2) and aldehyde dehydrogenase 2 variants. It is intended to use these methods to measure allele frequencies in larger population samples from the same ethnic groups and from others including Pacific Island people. At a later stage this work may be extended to include patients in alcohol treatment programs as a step towards establishing clinical tests for individuals at increased risk of alcoholism.

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