INTRODUCTION

Cytochrome P450 2E1 (CYP2E1) is the major component of the microsomal enzyme oxidizing system, which is one of the major pathways of oxidative metabolism of ethanol[1,2] as well as a large number of xenobiotics[3]. CYP2E1 is induced to greater activity by its substrate ethanol, probably via a number of mechanisms, including transcriptional, post-transcriptional and post-translational[4,8].

CYP2E1 activity is expressed in the liver, at sites of maximal alcohol induced damage[6], and in the pancreas, where it is also induced by chronic alcohol consumption[7,8]. These two are the major sites of damage following chronic consumption of ethanol. In addition, it is found in the brain[9], also a site of ethanol induced damage.

The genetic predisposition to both alcoholism and alcohol induced end-organ damage is an area of debate. Alcohol-induced pancreatitis occurs in approximately 5% of alcoholics[10] while no, or minimal, fibrosis is found in 32% of pancreata of alcoholics[11]. Alcoholic cirrhosis occurs in around 10% and hepatitis in 10-35%[12].

The heterogeneity of the response to alcohol implicates genetic factors. Family and twin studies suggest a genetic component to alcoholism[13,14]. Some evidence suggests that the majority of genetic predisposition to psychosis and liver disease may be accounted for by disposition to alcoholism[15].

Recently, an insertion polymorphism in the promoter
region of the gene coding for the enzyme CYP2E1 has been described; sequencing has shown a 96-bp insertion as a series of eight repeats, as opposed to six in the wild type. This corresponds to the restriction fragment length polymorphism, between positions -2270 and -1672. Presence of which is associated with higher CYP2E1 metabolic activity (employing an in vivo chlorazoxazone 6-hydroxylation test) in the presence of recently consumed alcohol or obesity. We have therefore analyzed the frequency of this polymorphism in patients with a history of excessive alcohol consumption, with and without end-organ damage, and normal controls.

The 96-bp insertion, previously described, is a 729-bp fragment employing the PCR based analysis of Fritzsche et al. The wild type allele is 633 bp in length. In addition, a GenBank record also exists for a 48-bp deletion (accession no. J02843), corresponding to 585 bp.

MATERIALS AND METHODS

Subjects
Venous blood samples were drawn from patients giving informed consent and local research ethics committee approval was obtained. We collected samples on 239 Caucasian subjects (ALC) fulfilling the ICD 10 criteria. The age range was 2-45 years; median 10 years. The subjects were sub-divided as follows:

Sixty-seven (36 British and 31 German) subjects without known end-organ disease, AC, collected from clients at alcohol rehabilitation centers.

One hundred and seventy-two with alcohol-related end-organ disease (AEOD). Of which, one hundred and forty-four (39 British and 105 German) patients had alcohol-induced chronic pancreatitis (AICP); all fulfilled the criteria for late- or end-stage AICP, as defined by the Zurich criteria. The twenty-eight patients with alcohol-induced chronic pancreatitis (AICP) had biopsy proven cirrhosis, with jaundice associated with excess alcohol consumption, and without end-organ damage, and normal controls.

Comparing ALC to AC for the presence of the insertion was comparable (P=0.049) (Table 2). The same comparison for genotype of the insertion was comparable (P=0.030) (Table 2).

To delineate whether the difference may have been for alcohol problems per se or end organ disease we initially employed Fisher’s exact test for the presence of the insertion using a three by two contingency table of normal controls, AC and AEOD. This revealed a statistically significant difference (P=0.045), and analysis for insertion genotypes was comparable (P=0.011) (Table 3). Thus, we further analyzed the sub-groups. Comparing AC with NC and comparisons within alcoholic (ALC) subgroups analysis did not reveal any significant differences, as might be expected with such a low frequency in the patient.
DISCUSSION

Polymorphisms in CYP2E1, other than that studied here, have been looked at in previous studies, though some have used small numbers. Their association with alcoholism has been studied: no association was found for the c1/c2 alleles in most studies. A positive association for the D form of the C/D polymorphism was found in Japanese subjects. Although some studies used non-alcoholic controls, in alcohol induced end-organ disease association for these polymorphisms has been found: for ALD and the c2 allele and fatty liver and the c2 allele. However, the positive association with end-organ disease has not been found in a number of studies and two studies found an association with the c1 allele.

The original study describing the polymorphism assessed in this study, showed greater CYP2E1 metabolic activity associated with the 96 bp insertion. In that study chlorazoxazone hydroxylation was higher in the patients with the presence of the polymorphism and who were obese or recent consumers of alcohol; both circumstances when CYP2E1 is induced. The two later descriptions delineate the pattern of 8 repeats of 42-60 bp, as opposed to 6 in the wild type. The first sequencing data showed a run of five repeats (accession no. J02843), which had not been seen in the two further studies; this form would correspond to the smaller band seen in four of our 447 samples (Figure 1B). Hu et al did not find an increased constitutive

Table 1 Full genotype data on all subjects

|                        | Normal controls (n = 208) | ALC (n = 239) | Of ALC | Of AED | Of AEOD |
|------------------------|---------------------------|--------------|--------|--------|--------|
|                        | AC (n = 67) | AEOD (n = 172) |        |        |
| Homozygote wildtype    | 194 | 232 | 63 | 169 | 141 | 28 |
| Heterozygote for insertion | 12 | 4 | 3 | 1 | 1 | 0 |
| Heterozygote for insertion | 0 | 1 | 0 | 1 | 1 | 0 |
| Heterozygote for deletion | 2 | 2 | 1 | 1 | 1 | 0 |

Table 2 Normal control and alcoholic figures and comparisons

|                        | Normal controls n = 208 (%) | ALC n = 239 (%) | Fisher’s exact test |
|------------------------|-----------------------------|----------------|---------------------|
| Presence of insertion polymorphism | 12 (5.8) | 5 (2.1) | P = 0.049 (χ² = 4.110, P = 0.043) |
| Genotype for insertion polymorphism | | | |
| Heterozygote           | 12 (5.8) | 4 (1.7) | P = 0.030 |
| Homozygote             | 0 (0.0) | 1 (0.4) | |

Table 3 Alcoholic subgroup figures and analyses

|                        | Normal controls n = 208 (%) | Alcoholic controls n = 67 (%) | Alcoholic end organ disease n = 172 (%) | Fisher’s exact test |
|------------------------|-----------------------------|-------------------------------|----------------------------------------|---------------------|
| Presence of insertion polymorphism | 12 (5.8) | 3 (4.5) | 2 (1.2) | P = 0.045 |
| Genotype for insertion polymorphism | | | | |
| Heterozygote           | 12 (5.8) | 3 (4.5) | 1 (0.6) | P = 0.011 |
| Homozygote             | 0 (0.0) | 0 (0.0) | 1 (0.6) | |

Figure 1 A: Agarose gel showing wild type homozygotes (633 bp, lanes 1, 3, 5–7) and a heterozygote for the insertion polymorphism (729 bp, lane 4). Run alongside a molecular weight marker (M). B: Agarose gel showing wild type homozygotes (633 bp, lanes 1–3 and 5) and a heterozygote for the deletion polymorphism (565 bp, lane 4). Run alongside a molecular weight marker (M).
expression in luciferase transfection experiments, for the insertion polymorphism, which would agree with McCarver et al.'s findings of increased enzymatic activity only in the induced state (obese subjects and recent alcohol consumers). To our knowledge, this is the first study to look at this polymorphism in patient groups. In previous studies of healthy groups of American Caucasoids, frequencies of 6.9% and 4.2% are seen. Another previous study found the insertion in only 2.1% of healthy Swedish subjects.

At low frequencies these results, on Caucasian subjects, could all be consistent with the frequencies found in our patient, as well as our control, groups; of 5.8% controls (British), 2.2% German ALC and 2% British ALC. However, in the large numbers in our study our results do indicate a statistically significant difference, which remains when isolating the smaller numbers of only British subjects for insertion genotype between NC and ALC (P = 0.03). Population stratification is a confounding factor in all genetic association studies and the possibility of this is recognized, raising the question as to whether our results are biologically as well as statistically significant.

We have shown a significantly lower frequency of this polymorphism, which is associated with increased activity, in the gene coding for the enzyme CYP2E1 when comparing those with alcohol dependence or abuse and normal controls. This could be explained and be analogous to the association found in the functional variations in ADH. It has been shown that high activity forms of ADH (and low activity forms of ALDH) are associated with the protection against alcoholism in a number of studies, as previously reviewed. This is believed to be due to the increased production (or decreased metabolism) of the ethanol metabolite acetaldehyde (and possibly other toxic metabolites), to which associated unpleasant side effects such as flushing are ascribed.

Due to the low frequency in our patient populations it was not possible to delineate whether there was only a relationship to alcohol misuse per se, or a relationship to end-organ disease. If a lower frequency existed in alcoholics, it could then be expected to be increased in those with end-organ disease compared to alcoholic controls. The sole homozygote was a patient with AICP and genotype analysis of those with end-organ disease vs AC approached significance, with P = 0.068. However, to infer a finding from such a result would not be justified.

Further analysis in ethnic populations with a frequency of this polymorphism which is sufficiently common to a component of this is further assessed. We have shown for the first time frequencies of this functional polymorphism in patient groups and there appears to be an association with this high activity polymorphism in the gene coding for cytochrome P450 2E1 and genetic protection against alcohol consumption. However, further studies are required in other populations.

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