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Genetic Polymorphism of Human *CYP2E1*: Characterization of Two Variant Alleles

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SUMMARY

Ethanol-inducible CYP2E1 is an enzyme of major toxicological interest because it metabolizes several precarcinogens, drugs, and solvents to reactive metabolites. CYP2E1 has also been implicated in alcohol liver disease because of its contribution to oxidative stress. Previously, polymorphic alleles with mutations in introns and in the 5′-flanking regulatory region have been described, and their presence has been related to the incidence of alcohol liver disease and lung cancer. In the present investigation, we investigated whether any functional mutations are linked to the above-mentioned rare alleles and also screened for mutations in the open reading frame using single-stranded conformation polymorphism and genomic DNA from almost 200 individuals belonging to either a Chinese, an Italian, or a Swedish population. Two new *CYP2E1* gene variants were found with functional mutations: one (*CYP2E1*2*) in which a G1168A point mutation in exon 2 caused an R76H amino acid substitution, and the other (*CYP2E1*3*) in which a G10059A base substitution in exon 8 yielded a V389I amino acid exchange. The corresponding CYP2E1 cDNAs were constructed, subcloned into the *pCMV4* expression vector, and expressed in COS-1 cells. The cellular levels of CYP2E1 mRNA, CYP2E1 protein, and rate of chlorzoxazone hydroxylation were monitored. The CYP2E1*3 cDNA variant was indistinguishable from the wild-type cDNA on all variables investigated, whereas CYP2E1*2 cDNA, although yielding similar amounts of mRNA, only caused 37% of the protein expression and 36% of the catalytic activity compared with the wild-type cDNA. Complete screening by single-stranded conformation polymorphism of the three populations studied revealed that these variant alleles were rare. We conclude that the human *CYP2E1* gene is functionally surprisingly well conserved compared with other cytochrome P450 enzymes active in drug metabolism, which suggests an important endogenous function in humans.

Ethanol-inducible CYP2E1 has received much attention because of the potentially important toxicological roles of this enzyme. The enzyme is localized mainly in the liver but is also expressed and induced in the brain after ethanol treatment or ischemia (1, 2) and furthermore is distributed in many other tissues (3). The physiological role of this enzyme seems to be connected mainly with the conversion of acetone to gluconeogenetic precursors. Among the more than 70 different substrates specifically metabolized by this enzyme are most organic solvents, paracetamol, and several precarcinogens such as dimethylnitrosamine and ethanol (3, 4). In addition, CYP2E1 causes oxidative stress, and the oxy radicals generated by this enzyme are able to initiate NADPH-dependent lipid peroxidation with the concomitant production of cytotoxic aldehydes. These have been implicated in ethanol-mediated hepatotoxicity (5). Thus, any functional polymorphism of this enzyme might be an important factor in determining the relative risk of alcohol-mediated hepatotoxicity, any form of cancer, or susceptibility for drug toxicity.

With respect to the metabolism of drugs used for clinical purposes, CYP2E1 can be considered to be among the six most important P450s, which also include CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. Among the clinically important drug substrates for CYP2E1 are enflurane, halothane, isoniazid, chlorzoxazone, paracetamol, and theophylline (6). Because CYP2C9, CYP2C19, and CYP2D6 have been shown to exhibit functional polymorphisms (7), it might be assumed that similar inactivating mutations also would occur in the *CYP2E1* gene and that their distribution might be of importance for the efficacy of drug treatment or for interindividual differences in toxicity exerted by paracetamol, for example. In line with this hypothesis, it has been demonstrated that there are important interindividual differences in the expression of human hepatic CYP2E1 (8, 9).

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ABBREVIATIONS: P450, cytochrome P450; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism; SSCP, single-stranded conformation polymorphism.

Analysis and characterization of polymorphisms in the *CYP2E1* gene has hitherto involved mainly RFLP analysis using the restriction endonucleases *Dra*I, *Taq*I, and *Rsa*I. The polymorphic sites localized in introns and in the 5′-flanking region have been monitored in this way (10, 11). Molecular epidemiological studies have been carried out to relate the occurrence of these variant alleles, in particular, the *Dra*I C allele, which has a mutation in intron 6 (allele frequency: Caucasians 10%, Asians 26%) and at −1019 bp (c1/c2) in the 5′-upstream region [allele frequency: Caucasians 4%, Asians 20% (3)], to the incidence of lung cancer, susceptibility for alcohol liver disease (10–14), inducibility of the enzyme by ethanol (15), and *in vivo* activity (15, 16). We evaluated whether these polymorphic sites are associated with any functional mutations in the open reading frame and, furthermore, examined to what extent the human *CYP2E1* gene as such exhibits allelic variations in the open reading frame. For this purpose, we screened genomic DNA for mutations from two Caucasian and one Asian population using SSCP. The results indicate that the human *CYP2E1* gene is highly conserved in these populations compared with other human P450 genes with products active in the metabolism of xenobiotics.

TABLE 1

Locations and sequences of primers used for PCR and DNA sequencing

Primer	Location	Sequence	
P1	−2653–−2636	5′-CCCCAGTCACAGAGAAGA-3′	
P2	−76–−61	5′-GTAGCAAGAGGGCATTGG-3′	
P3	288–305	5′-CAACCACAATTTGTCTGC-3′	
P4	1026–1043	5′-GAGCAACAGCAATACCCG-3′	
P5	1350–1367	5′-TATTTGGCCATCGCCGAC-3′	b
P6	4131–4148	5′-CCTGCCCTGCTCTCCAAG-3′	
P7	4433–4450	5′-ATGCAGTCTAGGAGTCCC-3′	
P8	4672–4689	5′-CTTGGTGAACCTCAGTGG-3′	
P9	5024–5041	5′-CACCCCTCGAAGCTATGT-3′	b
P10	5257–5274	5′-GTTGGTCCAACACACACA-3′	
P11	5572–5589	5′-ACCAGCAAGTGCAGCTCA-3′	
P12	6333–6350	5′-CTGAAAGGAGACAAGCAG-3′	
P13	6532–6549	5′-GTGTCCCTTCAGTCACTT-3′	b
P14	7516–7532	5′-CAGGGTCTCACTCTGTTG-3′	
P15	8034–8051	5′-GGAGGCCAAGGCAGGAGG-3′	b
P16	9294–9311	5′-GGATGATGGGTGGATGCC-3′	
P17	9598–9615	5′-CTGGCAGGAAGGCGATTA-3′	
P18	9968–9985	5′-CAACATTCTTCACTGGGG-3′	
P19	10244–10261	5′-CACATGTGGAGGGGAGAT-3′	b
P20	10996–11013	5′-GCTTCCCCTAGTCTCACT-3′	b
P21	11451–11468	5′-CCTCTCTGTGAGAATCAC-3′	

b indicates that the primer is biotinylated.

Materials and Methods

Isolation of genomic DNA. Blood samples were obtained from healthy Swedish and Chinese control subjects and Italian alcoholic cirrhosis patients as described in previous publications (17–19). Genomic DNA was isolated using a guanidinium-isothiocyanate method or using the phenol extraction method (20). The studies were approved by the ethics committee at Karolinska Institutet.

Construction of genomic libraries. The genomic libraries were made from subjects homozygous for the *Dra*I C and *Dra*I D alleles, respectively. When isolated, the *Dra*I C allele exhibited mutations determined by *Rsa*I and *Taq*I RFLP but was of the c1 genotype with respect to the 5′-upstream polymorphic site at −1019 bp (21). Genomic DNA was partially digested by the restriction enzyme *Sau*3AI and ligated to a λEMBL3 vector. Positive plaques were identified by hybridization with ^{32}P-labeled human CYP2E1 cDNA.

SSCP analysis. One microliter of genomic DNA (~0.5 μg/μl) was amplified using the primers listed in Table 1. The PCR was carried out with initial denaturation for 1.5 min at 94°, followed by 35 cycles, each involving denaturation at 94° for 1 min, annealing at 52° for 1 min, and extension at 72° for 1 min. Five microliters of the PCR products was digested by an appropriate restriction enzyme in a total volume of 35 μl to yield 100- to 200-bp fragments. Subsequently, 15 μl of loading buffer containing 95% formamide, 20 mM EDTA, bromphenol blue, and xylene cyanol FF was added. Just before loading, the samples were heated to 95° for 5 min. Conformation polymorphisms of single-stranded DNA fragments were then analyzed using nondenaturing 8.7% polyacrylamide gels with 10% glycerol in a Hoeffer (San Francisco, CA) standard vertical SE 500-slab gel unit. The gels were run in 2× Tris/borate/EDTA buffer (0.18 M Tris base, 0.18 M boric acid, and 0.004 M EDTA), at 4° for 4 hr at 270 V, and the DNA fragments were visualized by silver staining.

DNA sequencing. DNA was amplified by PCR with primers, as shown in Table 1. One of the primers was biotinylated, and the PCR reaction was conducted as described above. Forty microliters of PCR products was mixed with Dynabeads M-280 Streptavidin (Dynal AS; Oslo, Norway), which was designed as a matrix for simple and efficient separations of biotinylated compounds. DNA strands were separated with 0.1 M NaOH using the Dynal Magnetic Particle Concentrator. Sequencing of DNA was carried out by the dideoxy chain-termination method using Sequenase version 2.0 T7 DNA polymerase (United States Biochemical, Cleveland, OH) and α-^{35}S-labeled dATP (Amersham, Buckinghamshire, UK).

Site-directed mutagenesis. Mutant CYP2E1 cDNAs containing the G1168A or G10059A (numbering based on genomic DNA) mutations were generated with the USE mutagenesis kit (Pharmacia Biotech, Uppsala, Sweden), using a human CYP2E1 wild-type cDNA subcloned into the pBluescript KS$^+$ vector (Stratagene, La Jolla, CA), and mutagenic primers 5′-GCTCGCAGCACATGGTGGT-3′ and 5′-GGCACAGTCATAGTGCCAA-3′, respectively. The three cDNAs were subsequently subcloned into the *pCMV4* expression vector (22) using the restriction sites *Hind*III and *Xba*I.

Expression of mutated cDNAs. COS-1 cells were transfected with the *pCMV4* constructs as previously described (18). After incubation for 72 hr, the cells were harvested in 100 mM sodium phosphate buffer, pH 7.4.

Quantification of CYP2E1 mRNA and apoprotein levels. Total RNA was prepared from transfected cells using the TriPure Isolation Reagent (Boehringer Mannheim, Mannheim, Germany). Northern blot analysis was performed as previously described (23). Cells for Western blot and chlorzoxazone 6-hydroxylation analysis were sonicated for 20 × 1 sec and centrifuged at 10,000 × *g* for 10 min at 4°. Supernatant corresponding to 10 μg of protein was subjected to sodium dodecyl sulfate gel electrophoresis using 8.7% polyacrylamide gels, and the proteins were subsequently transferred to a Hybond C nitrocellulose filter (Amersham) and incubated with anti-rat CYP2E1 serum (24) and with horseradish-peroxidase–linked protein A (BioRad, Hercules, CA). The enhanced chemiluminescense method (Amersham) was used to visualize the proteins, and quantification was carried out using a personal densitometer (Molecular Dynamics, Sunnyvale, CA).

Chlorzoxazone 6-hydroxylation assay. Cell supernatant (10,000 × *g*) corresponding to 300 μg of protein was incubated at 37° in 100 mM sodium phosphate buffer, pH 7.4, with 1 mM chlorzoxazone, 1 mM NADPH, and 28 pmol of rat P450 reductase [purified as described by Ingelman-Sundberg and Glaumann (25)] in a total volume of 500 μl for 180 min. The addition of reductase makes CYP2E1 the rate-determining factor, because the endogenous reductase levels in the COS cells are small. The reaction was terminated by the addition of 50 μl of 43% *orto*-phosphoric acid, and 1 μg of acetaminophen was added as an internal standard. Extraction of samples with dichloromethane and analysis of the amount of 6-OH-

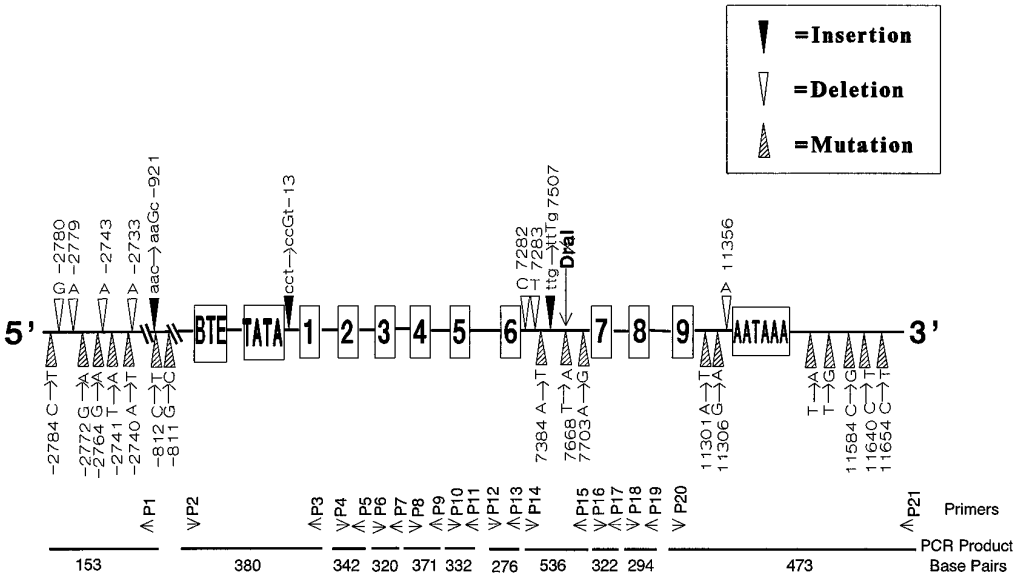

Fig. 1. Schematic description of the results of sequence analysis of the *Dra*I C allele of the human *CYP2E1* gene. The areas sequenced were all exons and exon-intron junctions, bp −2788 to −2634, −1009 to −809, −76 to −1, 7272 to 7892, and 11261 to 11710. DNA fragments of the exons amplified by PCR using the specific primers (P1–P21) and the location of amplified fragments are shown.

chlorzoxazone formed was carried out with an high pressure liquid chromatography system using an electrochemical detector as described elsewhere (2). Linearity of the reaction for 180 min was established in all cases.

Results

Two genomic libraries were constructed from two Caucasian subjects homozygous for the *Dra*I C and *Dra*I D haplotypes, respectively. The C allele of the c1 genotype with respect to the polymorphism at −1019 bp (26) was fully sequenced in its open reading frame and in parts of the 3′- and 5′-flanking regions. Several mutations compared with the *Dra*I D allele (27) were seen in the flanking regions (Fig. 1), among them an insertion (guanine) at position −13 in the promoter region adjacent to the TATA-box. Some mutations in the 3′-flanking region were in putative motifs determining mRNA stability, such as polyadenylation signal (Fig. 1). Furthermore, many mutations were seen in areas sequenced in intron 6, as well as in the more remote 5′-upstream flanking regions. However, no mutations were found in any of the exons.

To investigate any polymorphic alleles with functional mutations, SSCP analysis was carried out on all exons and exon-intron junctions using the genomic DNA of 198 subjects: 78 Swedish control subjects, 78 Chinese control subjects, and 42 Italian patients suffering from alcohol-induced liver cirrhosis. The exons were amplified by PCR using the primers listed in Table 1, and the products were digested by appropriate restriction endonucleases to yield 100- to 200-bp fragments. In two of the 78 Chinese subjects, the SSCP analysis revealed a mobility difference of the PCR products from exon 2 obtained after digestion with *Rsa*I (Fig. 2A). Direct sequencing revealed a G1168A point mutation (Fig. 2B), which caused an R76H amino acid substitution (Fig. 2C). The mutation is localized at a polymorphic restriction *Hha*I site (GC*G*C to GC*A*C) (Fig. 2C), and the allele was designated *CYP2E1*2*.[1]

Using the same approach, we also found that 1 of the 42 Italian alcoholic cirrhosis patients showed a unique pattern in the SSCP analysis of exon 8 (Fig. 3A). Sequence analysis revealed a G10059A mutation (Fig. 3B), which caused a V389I amino acid substitution (Fig. 3C). This allele was designated *CYP2E1*3*.[1] Using SSCP and sequence analysis, we identified a point mutation, $G^{-35}T$, only 2 bp downstream of the basal transcription element in the 5′-flanking region (data not shown).

To evaluate the functional importance of the mutations in the *CYP2E1*2* and *CYP2E1*3* alleles, site-directed mutagenesis was used to introduce these mutations into the wild-type CYP2E1 cDNA. The cDNAs were inserted into the *pCMV4* expression vector and subsequently expressed in COS-1 cells. For comparison, cells transfected with the vector alone were used as negative controls. The amount of CYP2E1 mRNA was detected by Northern blot, and the CYP2E1 apoprotein levels were quantified by Western blot. The CYP2E1-dependent catalytic activities were determined by measuring NADPH-dependent hydroxylation of chlorzoxazone in 10,000 × *g* supernatants of the transfected cells.

All constructs except the *pCMV4* vector yielded CYP2E1 mRNA and immunodetectable CYP2E1 apoprotein. No major differences in mRNA levels were noticed in cells transfected with either of the three different cDNAs (Fig. 4, A and B). The level of CYP2E1 apoprotein in cells transfected with CYP2E1*2 cDNA was approximately 37% of that obtained in cells transfected with CYP2E1*1 cDNA (Fig. 4C). When 6-hydroxylation of chlorzoxazone was measured in the cell homogenates, a similar decrease was seen in activities as monitored on the protein level. By contrast, cells transfected with CYP2E1*3 cDNA had apoprotein levels and rates of chlorzoxazone 6-hydroxylation similar to that of cells transfected with CYP2E1*1 cDNA (Fig. 4D).

The presence of these mutations in the populations inves-

[1] A new nomenclature system has recently been proposed for *CYP2D6* alleles (40). This system is based on general recommendations for allele nomenclature, and the different alleles are denoted *CYP2D6**n. Each allele is assigned a specific number. In accordance with this, we propose a similar system for designation of the *CYP2E1* alleles with the following numbers: *CYP2E1*1,* wild-type sequence; *CYP2E1*2,* G1168A R76H; *CYP2E1*3* G10059A V389I.

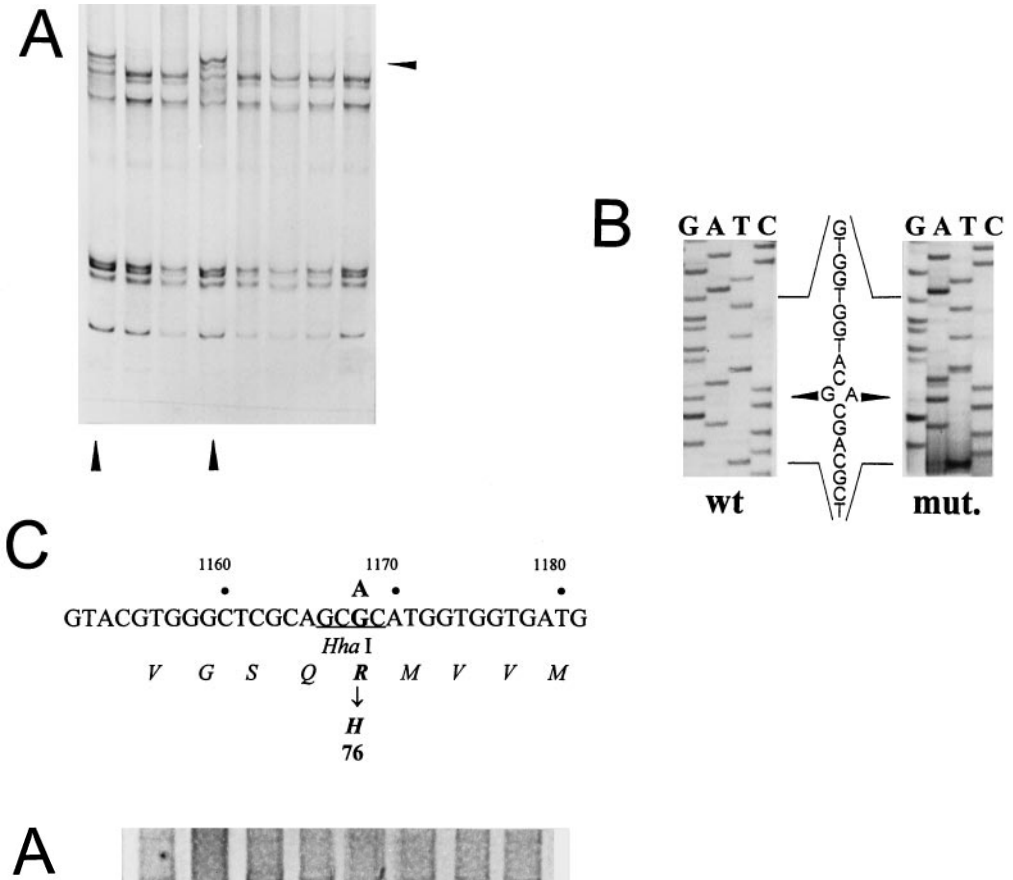

Fig. 2. Identification of a functional mutation in exon 2 of the human *CYP2E1* gene. A, The SSCP analysis was performed using nondenaturing 8.7% polyacrylamide gels. *Arrow* (*bottom*), individuals who carry the mutation; *arrow* (*side*), extra bands seen in these samples. B, Direct PCR sequencing of exon 2 from wild-type and mutant individuals. *Arrow,* G1168A point mutation. C, Nucleotide sequence around the mutation in exon 2. *Bold letter,* point mutation for the codon R76H; *underlining, Hha*I site.

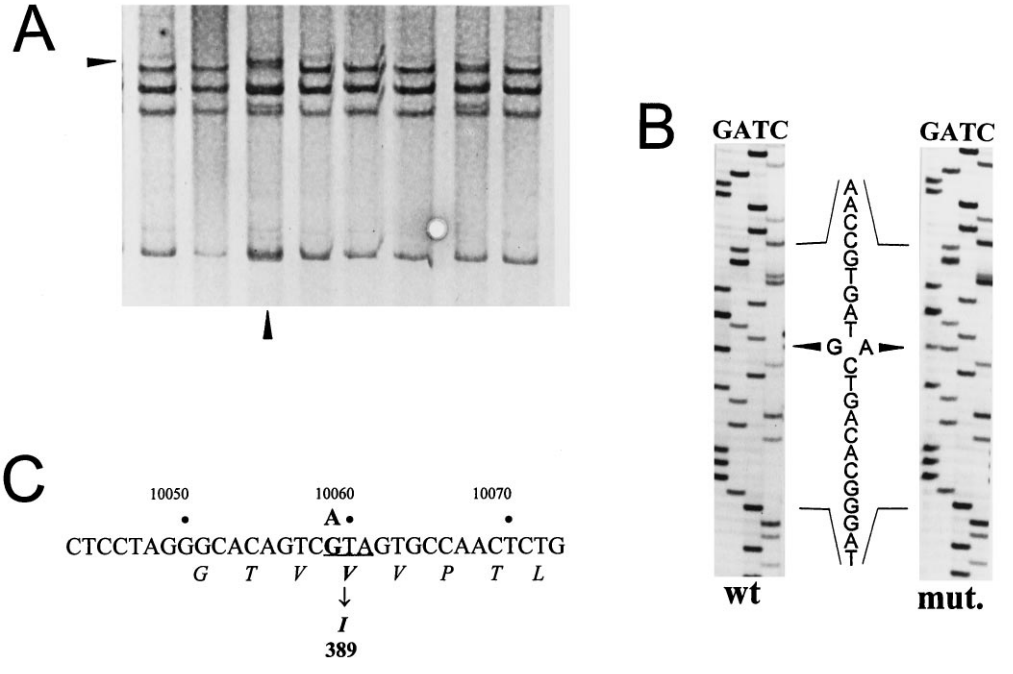

Fig. 3. Identification of a functional mutation of exon 8 of the human *CYP2E1* gene. A, SSCP analysis was performed using nondenaturing 8.7% polyacrylamide gels. *Arrow* (*bottom*), individuals who carry the mutation; *arrow* (*side*), extra bands seen in these samples. B, Direct PCR sequencing of exon 8 from wild-type and mutant individuals. *Arrow,* G10059A point mutation. C, Nucleotide sequence around the mutation in exon 8. *Bold letter,* point mutation for the codon V389I.

tigated was screened by SSCP. Two *CYP2E1*2* alleles were found in the Chinese population and one *CYP2E1*3* allele in the Italian population, whereas neither was present in the Swedish population studied (Table 2). This shows the high conservation of the open reading frame of the human *CYP2E1* gene and indicates an important physiological function of this enzyme *in vivo*. No relationship between the distribution of these variant alleles and the previously described C/D and c1/c2 polymorphisms was found.

Discussion

Much interest has been focused on the relationship between polymorphism of the human *CYP2E1* gene as studied by RFLP and the incidence of lung cancer as well as the occurrence of alcoholic liver disease. In particular, two polymorphic loci, one in intron 6 (*Dra*I C/D) and one in the 5′-flanking upstream region (c1/c2) have received much attention. To evaluate any relationship between these polymor-

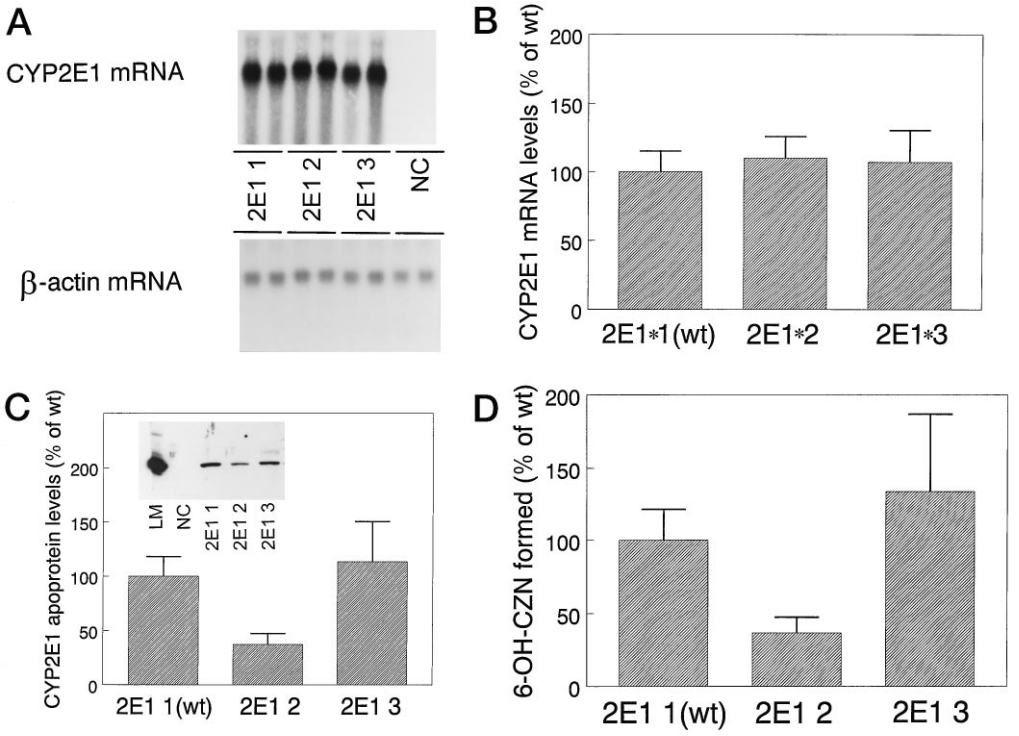

Fig. 4. Expression of wild-type and mutated CYP2E1 cDNAs in COS-1 cells. A and B, Northern blot analysis. CYP2E1 mNRA and β-actin mRNA were measured in cells transfected with the different mutants, and the ratio between CYP2E1 mRNA and β-actin mRNA levels were calculated. Twenty-five micrograms of total RNA was used for analysis. C, Western blot analysis of CYP2E1 showing densitometric quantification of CYP2E1 apoprotein levels in the cell homogenates. Levels are expressed as a percentage of the levels in the cells transfected with CYP2E1*1 cDNA. *Inset,* a representative Western blot from 10,000 × *g* supernatant corresponding to 5 μg of protein per lane and rat liver microsomes as a control. *LM*, liver microsomes; *NC*, negative control, vector only. D, Catalytic activities. Chlorzoxazone 6-hydroxylation activity was measured in 10,000 × *g* supernatants corresponding to 300 μg of protein. The reaction was linear with time for at least 180 min. The minute amount of 6-OH-chlorzoxazone formed in the cells transfected with the *pCMV4* vector alone has been subtracted. The activity is expressed as a percentage of the activity seen in cells transfected with CYP2E1*1 cDNA. The results are data (mean ± standard deviation) from four (B) or six (C and D) independent experiments performed in duplicate. Plasmids from at least two independent plasmid preparations were used for transfection.

TABLE 2

Distribution of variant *CYP2E1* alleles in the three different populations studied

Study population	Base substitution	
	G1168A (*CYP2E1*2*)	G10059A (*CYP2E1*3*)
	No. of alleles	
Chinese control subjects (n = 78)	2	0
Swedish control subjects (n = 78)	0	0
Italian cirrhosis patients (n = 42)	0	1

phic alleles and any functional mutations and, furthermore, to investigate the occurrence of any mutations in the open reading frame, conditions for SSCP analysis were worked out to include all the exons and intron-exon junctions. Screening of a total of 198 individuals revealed that the coding part of the gene is well conserved among individuals and that only three alleles were found to cause amino acid exchanges. One of them, which had the R76H substitution (*CYP2E1*2*), caused less apoprotein levels and catalytic activity in the expression system, whereas no effect was seen on the mRNA level. The very similar decreases of both the protein and catalytic activity of this CYP2E1 variant suggests that the mutation does not affect the catalytic activity of the enzyme but that less protein is formed, perhaps because of a decreased translation efficiency or less stable protein. The expression system used does not, however, allow us to extrapolate this finding to the human *in vivo* situation. Our finding of a detrimental effect of the R76H substitution has to be confirmed *in vivo* using phenotype analysis with the chlorzoxazone of humans carrying the *CYP2E1*2* allele in comparison to subjects homozygous for the *CYP2E1*1* allele under otherwise identical conditions.

The sensitivity for the detection of mutations by SSCP techniques using polyacrylamide gels in the absence or presence of glycerol has been found to be 90% and 70%, respectively (28). Some exons were run in the absence of glycerol, and additional bands were then obtained, but sequencing revealed no exon mutations.[2] Statistically, we may have missed one additional mutation in one or two of the samples, although this would not change the conclusions drawn from the study.

Because no crystal structure of CYP2E1 or any other mammalian P450 is available, alignments and homology-building models are used to make predictions concerning the localization of amino acids in the three-dimensional structure of the enzyme. None of the amino acid substitutions are located in the substrate recognition sites predicted by Gotoh (29) and are thus not expected to be directly involved in substrate binding. Hasemann *et al.* (30) made a structural alignment of three bacterial P450 structures and used this to align some of the mammalian P450 on the amino acid level. Using this

[2] Y. Hu, unpublished observations.

model, it is apparent that both amino acid exchanges are located in the β1-sheet, which implies that they are closely related in space. However, until detailed models or crystal structures are available, the precise role of these residues will remain unknown. Interestingly, Arg^{76} is conserved in all species investigated thus far [namely, human (31), macaque (32), rat (31), mouse (33), rabbit (34), and hamster (35)], which indicates an important function for this residue. This is in agreement with our data, which show altered expression of functional enzyme in the mutated variant. Val^{389} is also well conserved, but in the macaque, isoleucine has replaced valine, which is the same amino acid exchange found in the *CYP2E1*3* allele. In line with an assumption that such an exchange would not affect the function of CYP2E1, our expression data confirm the lack of functional importance of this amino acid exchange.

Our sequence analysis indicates that the flanking regions of the human *CYP2E1* gene are highly polymorphic. In specific areas, up to 5% of all bases were different between the *Dra*I C allele and the wild-type allele (see Fig. 1). An increased rate of transcription of the *CYP2E1* gene has been shown to occur in rats at high ethanol concentrations (36), although the primary level for regulation of CYP2E1 in this species by ethanol is at the post-translational level (37, 38). In a preliminary study, Lucas *et al.* (15) found that human subjects differ with respect to inducibility of the CYP2E1 enzyme by ethanol. The extent of inducibility was lower among subjects of the c2 and *Dra*I C genotypes. In other studies, however, the c2 allele has been shown to be more effectively expressed than the c1 allele in transfected HepG2 cells (26). Nevertheless, accumulating data suggest the occurrence of two populations of humans that are of high and low CYP2E1 ethanol inducibility,[3] and it is plausible that the cause of these differences can be found at some additional polymorphic sites in the flanking regions of the *CYP2E1* gene, a hypothesis supported by the present finding of a high number of mutations in the *Dra*I C allele. Further studies are needed to resolve this question.

It is interesting to note the high number of mutations in the flanking regions of the *Dra*I C allele compared with the wild-type (*Dra*I D) allele. By contrast, the almost complete absence of functional mutations in the open reading frame in both Caucasian and Asian populations indicates a selection for preservation of alleles encoding enzymes with the conserved amino acid sequence. Furthermore, the only functionally important mutation found did not abolish the activity of the enzyme, which further stresses the importance of the gene product. Thus, it is conceivable that CYP2E1 is of high physiological importance for humans. One might assume that a major factor in the past has been its ability to participate in the minor acetone-involving gluconeogenetic pathway (3) and that mutated *CYP2E1* genes have not been beneficial for survival. However, no alteration in physiological function by homozygous inactivation of this gene was observed in well-fed mice (39). Because these mice have not been stressed dietarily, the caloric restriction selection theory for preservation of a functionally intact *CYP2E1* gene in humans is not contradictory to the findings in the knock-out mice.

[3] I. Dupont, D. Lucas, P. Clot, M. Seccia, C. Menez, and E. Albano, submitted for publication.

In conclusion, our data show that the open reading frame of the human *CYP2E1* gene is surprisingly well conserved both in Asians and in Caucasians. This might suggest important endogenous functions and could indicate a pronounced endogenous role of this P450 in humans under certain conditions.

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