The Human Debrisoquine 4-Hydroxylase (CYP2D) Locus: Sequence and Identification of the Polymorphic CYP2D6 Gene, a Related Gene, and a Pseudogene

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Summary

The debrisoquine-4-hydroxylase polymorphism is a genetic variation in oxidative drug metabolism characterized by two phenotypes, the extensive metabolizer (EM) and poor metabolizer (PM). Of the Caucasian populations of Europe and North America, 5%-10% are of the PM phenotype and are unable to metabolize debrisoquine and numerous other drugs. The defect is caused by several mutant alleles of the CYP2D6 gene, two of which are detected in about 70% of PMs. We have constructed a genomic library from lymphocyte DNA of an EM positively identified by pedigree analysis to be homozygous for the normal CYP2D6 allele. The normal CYP2D6 gene was isolated; was completely sequenced, including 1,531 and 3,522 bp of 5' and 3' flanking DNA, respectively; and was found to contain nine exons within 4,378 bp. Two other genes, designated CYP2D7 and CYP2D8P, were also cloned and sequenced. CYP2D8P contains several gene-disrupting insertions, deletions, and termination codons within its exons, indicating that this is a pseudogene. CYP2D7, which is just downstream of CYP2D8P, is apparently normal, except for the presence, in the first exon, of an insertion that disrupts the reading frame. A hypothesis is presented that the presence of a pseudogene within the CYP2D subfamily transfers detrimental mutations via gene conversions into the CYP2D6 gene, thus accounting for the high frequency of mutations observed in the CYP2D6 gene in humans.

Introduction

The cytochrome P450 gene superfamily consists of nine gene families in mammals (Nebert et al. 1989). Five of the families code for enzymes expressed in specialized steroidogenic tissues that catalyze steps in steroid biosynthesis. The remaining four families of P450 are expressed in liver and to some extent in extrahepatic tissues, and these enzymes catalyze the oxidation of fatty acids, steroids, and numerous chemicals, including drugs and carcinogens. The hepatic P450 system is responsible for the metabolism and elimination of most foreign chemicals that are ingested. Typically, the

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P450s will oxidize a hydrophobic chemical to introduce functional groups that can be substrates for various conjugating enzymes. These latter enzymes introduce chemical moieties that render the substrate more hydrophilic so that it can be excreted via urine or bile. Many P450s can also metabolize compounds of diverse structures, and this unique feature allows the organism to process and eliminate scores of chemicals. It is generally thought that many P450s evolve to metabolize plant toxins (Nebert and Gonzalez 1985; Nelson and Strobel 1987; Gonzalez 1988). Thus, P450s may have been required to detoxify poisonous chemicals in plants. As plants or habitats changed, a particular P450 may not have been required for survival and its presence was no longer selected for. This may be the basis for drug oxidation polymorphisms detected in rodents and man (Gonzalez 1988).

The debrisoquine 4-hydroxylase genetic deficiency

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is the most widely studied human drug oxidation defect. Debrisoquine, an adrenergic blocking agent previously used to control hypertension, was found to be inefficiently metabolized by a significant number of individuals. This lack of metabolism resulted in exaggerated response during clinical administration of the drug. Individuals who cannot metabolize debrisoquine are termed poor metabolizers (PMs), while those capable of metabolism are called extensive metabolizers (EMs). PMs and EMs can be identified by administering subclinical doses of debrisoquine or other drugs and monitoring accumulation of both the parent compound and the hydroxylated metabolite in the urine. By use of urine metabolite analysis, the deficiency in debrisoquine metabolism was found to be inherited as an autosomal recessive trait and to affect 5%-10% of the Caucasian population of Europe and North America (Idle and Smith 1979; Eichelbaum 1986). Numerous other drugs and chemicals have been shown to be subjected to this genetic defect.

P450 IID1 responsible for debrisoquine oxidation has been purified from rats (Larrey et al. 1984; Gonzalez et al. 1987) and man (Distlerath et al. 1985; Gut et al. 1986). The rat (Gonzalez et al. 1987) and human (Gonzalez et al. 1988b) IID1 cDNAs have also been cloned and sequenced. The human IID1 cDNA was used to determine that mutant IID1 (CYP2D6) genes account for the drug oxidation defect (Gonzalez et al. 1988a). Two mutant CYP2D6 alleles were found that produce incorrectly spliced transcripts. RFLP haplotype analysis was used to identify two mutant alleles in lymphocyte DNA in individuals who cannot metabolize debrisoquine (Skoda et al. 1988). In about 70% of PM individuals at least one mutant allele can be detected by RFLP analysis.

In the present report we have cloned and sequenced the CYP2D6 gene and two related genes—designated CYP2D7 and CYP2D8P—at the CYP2D locus. The three CYP2D genes display 92%–97% nucleotide similarities with each other across their introns and exons. The CYP2D8P gene was found to be a pseudogene, while the CYP2D7 gene was found to have in its first exon an insertion that disrupts the reading frame of the protein. The role of the CYP2D7 and CYP2D8P genes in the generation of mutant CYP2D6 alleles is discussed.

Material and Methods

Material

λEMBL3 DNA was obtained from Promega Biotec.

 λ DASH and Gigapak GoldTM were purchased from Stratagene. Sequenase was from United States Biochemicals and α^{35} S-dATP (500 Ci/mmol) was purchased from New England Nuclear. *Sma*I-digested and phosphatase-treated M13 mp10 was purchased from Amersham.

Isolation and Sequencing of the CYP2D Genes

Two human gene libraries were constructed. One library was constructed from a liver with extensive in vitro metabolism of debrisoquine. DNA from this liver was homozygous for the polymorphic XbaI 29-kbp fragment (XbaI pattern A of Skoda et al. [1988]), as is the case with most EMs. The DNA was partially digested with MboI, size-fractionated on an NaCl gradient (5%–25%), and ligated into λ EMBL3 arms according to a method described by Kaiser and Murray (1985). The DNA was packaged in vitro using Gigapak Gold™, and packaged phage were plated using Escherichia coli strain K802. A second library was constructed using λ DASH and DNA from lymphocytes of an EM of debrisoquine who was phenotyped in vivo. This individual was determined to carry two normal CYP2D alleles by the segregation of BamHI RFLPs in his family (fig. 1). Complete HindIII digestion of lymphocyte DNA was carried out, and fragments greater than 15 kbp were isolated and ligated into λ DASH that had been digested with XbaI and HindIII. The DNA was processed as described above. Phage were screened at a density of 30,000 pfu/150-mm dish by plaque hybridization using nick-translated IID1 cDNA probe. The specific clones were plaque purified, restriction maps of their insert DNAs were obtained, and the individual BamHI fragments from the inserts were subcloned into pUC9. Insert DNA was isolated by gel purification, selfligated, and shotgun cloned into M13 mp10 according to a method described elsewhere (Deininger 1983). DNA was sequenced using the dideoxy chain-termination method (Sanger et al. 1977), except that Sequenase was substituted for DNA polymerase Klenow fragment. Sequence data were analyzed using the Beckman Microgenie[®] program.

Other Procedures

The transcription start site of CYP2D was determined by primer extension analysis and S1 nuclease protection assays. In brief, poly(A) RNA was isolated from a liver containing high levels of CYP2D6 mRNA (Gonzalez et al. 1988*a*). A primer for primer extension (20mer, +17 to +36; fig. 4) and a probe for S1 mapping (60-mer, -24 to +36, fig. 4) were synthesized using an Applied Biosystems 380B DNA synthesizer. The oli-



Figure 1 Identification of a homozygous EM by analysis of the segregation of *Bam*HI RFLPs. Each individual within the Za pedigree is placed above the corresponding lane. $\Box = EM$ phenotype; \Box or Q = EM phenotype obligate heterozygote by pedigree analysis; $\blacksquare = PM$ phenotype. The 6-kbp fragment together with the 2.3and the faint 2.1- and 1.9-kbp fragments represent the mutant allele in CYP2D6 in this family because II₁ is homozygous for these fragments. These fragments behave allelic to the 7.9- and 4.7-kbp fragments which behave as markers for the normal allele. Individual II₂ is homozygous for the normal 7.9/4.7-kbp allele.

gonucleotides were purified by agarose gel electrophoresis, 5'-end labeled with T4 polynucleotide kinase and $[\gamma^{32}P]$ -ATP, and hybridized to 10 µg poly(A) RNA. The primer was extended using AMV reverse transcriptase. S1 nuclease digestion was performed after the probe was allowed to anneal to Poly(A) RNA. Yeast tRNA was used as control. The reaction products were electrophoresed on an 8% polyacrylamide–50% urea gel. An M13 sequencing ladder was electrophoresed concurrently to determine the size of the extended products. Southern blotting was performed according to a method described by McBride et al. (1986) by using BioTrace RP[®] nylon membrane (Gelman Sciences, Inc.) and the human IID1 cDNA as a probe (Gonzalez et al. 1988*b*).

Results and Discussion

Isolation of the CYP2D Genes

In the first attempt to isolate the CYP2D6 gene, a library was constructed in λ EMBL3 by using DNA isolated from a human liver. This liver had bufuralol 1'hydroxylase activity representative of an extensive metabolizer and was homozygous for the Xbal 29-kbp fragment (XbaI pattern A of Skoda et al. [1988]). Two overlapping phage clones (λ 2D-A and λ 2D-B) were isolated as shown in figure 2 and were completely sequenced. These contained two tandemly arranged genes that each had nine exons. After careful comparison of their exonic sequences with the CYP2D6 cDNA (Gonzalez et al. 1988b), they were found to be distinct from the CYP2D6 gene and hence were designated CYP2D7 and CYP2D8P. The exonic sequence of CYP2D7 and CYP2D8P bore 97% and 92% similarities to the CYP2D6 cDNA, respectively. The restriction maps of these phage DNAs were constructed and compared with the Southern blotting data of Skoda et al. (1988), and unique fragments were identified that were not represented by our restriction map of the cloned CYP2D7 and CYP2D8P genes in figure 2; for example, only two of the three fragments found with EcoRI can be explained by the single EcoRI site located between CYP2D8P and CYP2D7. On the basis of these data, a third gene was suspected to be present in EM individuals, and a second library was prepared from lymphocyte DNA taken from an EM who was positively identified by pedigree analysis to carry two normal CYP2D6 alleles (fig. 1). This was important because heterozygotes cannot be determined by the urine analysis phenotyping procedure and because heterozygotes



Restriction map of the CYP2D locus. A partial restriction map and the position of the exons in the CYP2D6, CYP2D7, and CYP2D8P genes derived from the sequence data in figs. 4 and 5 are displayed. The individual λ clones used to determine the sequence of the genes are shown below the consensus map. A thick line indicates regions of the clones that were completely sequenced. The exons and the *EcoRI*, *Bam*HI, *HindIII*, and *Xbal* restriction-enzyme sites are denoted by black rectangles E, B, H, and X, respectively. Figure 2

are expected to account for 35%-43% of the Caucasian population. With this library the CYP2D6 gene was isolated as a 15-kbp HindIII fragment in λ DASH (λ 2D-18/2; fig. 2). A second clone (λ 2D-18/1) was also isolated that contained the CYP2D8P gene. This clone was restriction mapped and not further characterized.

Determination of the Transcription Start Site of CYP2D6

The transcription start site of the CYP2D6 gene was determined by primer extension and S1 mapping. It must be noted that the three CYP2D genes contain virtually identical sequence in their first exons. However, since the CYP2D8P gene is a pseudogene (see below) and since we have not detected significant levels of CYP2D7 in several human liver RNAs, we believe that we are only detecting transcripts derived from the CYP2D6 gene. One distinct extended fragment was observed after reverse transcriptase treatment of the CYP2D6 primer that had been annealed with liver RNAs from two individuals, and the position of this extended fragment matched that of the major S1 protected fragment (fig. 3, fragment of 36 bp). The size of the fragments corresponds to a CYP2D6 gene G residue which was assigned position +1 in figure 4.

Sequence of the CYP2D6 Gene

The complete sequence of the CYP2D6 gene is presented in figure 4, including 1,531 and 3,522 bp of 5' and 3' flanking DNA. The putative transcription start site, determined in figure 3, is designated +1 and is preceded by a TATA box at -24 to -28. No CCAAT box was noted within 200 bp upstream of the start site. The 5' untranslated portion of the mRNA is 88 bases. The CYP2D6 gene contains nine exons and spans 4,378 bp from the polymerase start site to the polyadenylation site. Nine exons, including coding first and last exons are typical of other CYP2 family genes (Gonzalez 1988).

Sequences of the CYP2D7 and CYP2D8P Genes

The sequences of the CYP2D7 and CYP2D8P genes are displayed in figure 5. The two genes are aligned tandemly head to tail and are separated by 4,665 bp of intragenic DNA. The CYP2D7 gene is located downstream of the CYP2D8P gene. CYP2D7 is apparently a normal gene, except for the presence of a single T insertion at position +226 in the first exon. This insertion disrupts the protein reading frame, indicating that the CYP2D7 gene would produce an mRNA incapable of translating a functional P450. Screening of eight human liver RNAs with an oligonucleotide specific to the





Figure 3 Primer extension and S1 mapping analyses of the CYP2D6 gene. The primer (20-mer, +17 to +36; fig. 3) for primer extension and S1 probe (60-mer, -24 to +36; fig. 3) for S1 mapping were labeled with [³²P], annealed with 10 µg human liver poly(A) RNAs from two individuals, and either extended with reverse transcriptase or treated with S1 nuclease, respectively. Yeast tRNA was used as control. The reaction products were electrophoresed concurrently with a ladder of DNA sequence derived from M13 mp18. mRNA1 and mRNA2 were isolated from different human liver samples. The numbers at the right represent the fragment sizes in base pairs.

putative CYP2D7 mRNA failed to detect an RNA on a Northern blot (authors' unpublished data). If it is assumed that the CYP2D7 gene could have the same start site as CYP2D6 (as shown by (+1) in fig. 5), a TATA box is found at -24 to -28. Among the first 774 bp immediately upstream from the putative cap site, nucleo-

| -1500 | |
|--|--------|
| GAATTCAAGACCAGCCTGGACAACTTGGAAGAACCCGGTCTCTACAAAAAATACAAAATTAGCTGGGATTGGGTGCGGTGGCTCATGCCTATAATCCCAGCACTTTGGGAGCCTGAGGTG - 1400 - 1300 | |
| GGTGGATCACCTGAAGTCAGGAGTTCAAGACTAGCCTGGCCAACATGGTGAAACCCTATCTCTACTGAAAATACAAAAAGCTAGACGTGGTGGCACACACCTGTAATCCCAGCTACTTAG | |
| GAGGCTGAGGCAGGAGAATTGCTTGAAGCCTAGAGGTGAAGGTTGTAGTGAGCCGAGATTGCATCATTGCACAATGGAGGGGAGCCACCAGCCTGGGCAACAAGAGGAAATCTCCGTCTC | |
| CAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA | |
| | |
| GAAAGCAGTGG1GGAGGACGACCCTCAGGCAGCCCGGGAGGATGTTGTCACAGGCTGGGGGCAAGGGCCTTCCGGCTACCAACTGGGAGCTCTGGGAACAGCCCTGTTGCAAACAAGAAGC | |
| | |
| ATGTGTGTGTGTGTGTGTGTGTGTGAGAGAGAGAGAGAG | |
| CAAGTGTGAACAAGTGGACAAGTGTCTGGGAGTGGACAAGAGATCTGTGCACCATCAGGTGTGTGCATAGCGTCTGTGCATGTCAAGAGTGCAAGGTGAAGGGACCAGGCCCATG | |
| ATGCCACTCATCATCAGGAGCTCTAAGGCCCCAGGTAAGTGCCAGTGACAGATAAGGGTGCTGAAGGTCACTCTGGAGTGGGCAGGTGGGGGAAGGGCAAGGGCAAGGCCATGTTCTGGA | |
| GGAGGGGTTGTGACTACATTAGGGTGTATGAGCCTAGCTGGGAGGTGGATGGCCGGGGTCCACTGAAACCCTGGTTATCCCAGAAGGCTTTGCAGGCTTCAGGAGCTTGGAGTGGGGGAGAG | |
| GGGGTGACTTCTCCGACCAGGCCCCTCCACCGGCCTACCCTGGGTAAGGGCCTGGAGCAGGAAGCAGGGGCAAGAACCTCTGGAGCAGCCACTACCCGCCCTGGCCTGACTCTGCCACTG | |
| GCAGCACAGTCAACACAGCAGGTTCACTCACAGCAGAGGGGCAAAGGCCATCATCAGCTCCCTTTATAAGGGAAGGGTCACGCGCTCGGTGT GCTGAGAGTGTCCTGCCTGGTCCTCTGTG | |
| CCTGGTGGGGTGGGGGTGCCAGGTGTGTCCAGAGGAGGCCCATTTGGTAGTGAGGCAGGTATGGGGCTAGAAGCACTGGTGCCCCTGGCCGTGATAGTGGCCATCTTCCTGCTCGTGG M G L E A L V P L A V I V A I F L L L V | EXON 1 |
| 200 ACCTGATGCACCGGCGCCAACGCTGGGCTGCACGCTACCCACGAGGCCCCCTGCCACTGCCGGGCTGGGCAACCTGCTGCATGTGGACTTCCAGAACACACCATACTGCTTCGACCAG G D L M H R R Q R W A A R Y P P G P L P L P G L G N L L H V D F Q N T P Y C F D Q | |
| TGAGGGAGGAGGTCCTGGAGGGCGGCAGAGGTGCTGAGGCTCCCCCTACCAGAAGCAAACATGGATGG | |
| | |
| CCCATTGGGCAACATATGTTATGGAGTACAAAGTCCCTTCTGCTGACACCAGAAGGAAAGGCCTTGGGAATGGAAGATGAGTTAGTCCTGAGTGCCGTTTAAATCACGAAATCGAGGATG | |
| | |
| GTGACTCTTGCAAGGTCATACCTGGGTGACGCATCCAAACTGAGTTCCTCCATCACAGAAGGTGTGACCCCCACCGCCCCACGATCAGGAGGCTGGGTCTCCTCCTTCCACCTGCTC | |
| ACTCCTGGTAGCCCCGGGGGTCGTCCAAGGTTCAAATAGGACTAGGACCTGTAGTCTGGGGTGATCCTGGCTTGACAAGAGGCCCTGACCCTCCCT | FXON 2 |
| 1000 GACGTGTTCAGCCTGCAGCTGGCCGGGACGCCGGTGGTCGTCGTGCGCGGCGGGCG | |
| ACCCAGATCCTGGGTTCGGGCCGCGTTCCCAAG GCAAGCAGCGGTGGGGGACAGAGACAGATTTCCGTGGGACCCGGGTGGGT | |
| | |
| 1400 AGACCTGGCAGGAGCCCCAATGGGTGAGCGTGGCGCATTTCCCAGCTGGAATCCGGTGTCGAAGTGGGGGGCGGGGGACCGCACCTGTGCTGTGCTGTGGGGGGGG | |
| 1500 GGGTCTTCCCTGAGTGCAAAGGCGGTCAGGGTGGGCAGAGACGAGGTGGGGCAAAGCCTGCCCCAGCCAAGGGAGCAAGGTGGATGCACAAAGAGTGGGCCCTGTGACCAGCTGGACAGA | |
| 1600 GCCAGGGACTGCGGGAGACCAGGGGGGGGGAGCATAGGGTTGGAGTGGGGGGGG | EXON 3 |
| 1800 CTATGGGCCCGCGTGGCGCGAGCAGGAGGCGCTTCTCCGTGTCCACCTTGGCCAACTTGGGCCTGGGCAAGAAGTCGCTGGGCGGGGGGCGGCGGCGGCGCCTGCCT | |
| 1900 CTTCGCCAACCACTCCG GTGGGTGATGGGCAGAAGGGGCACAAAGCGGGAACTGGGAAGGCGGGGGACGGGGGACGCCCTTACCCCGCATCTCCCACCCCCCA GACGCCCCTTTCGCC F A N H S G R P F R | EXON 4 |
| 2000 CCAACGGTCTCTTGGACAAAGCCGTGAGCAACGTGATGGACCGACGCGCCTCCGAGTACGACGACCGCCTCCTCAGGACCGACC | |

tide sequences of CYP2D6 and CYP2D7 genes are 97% similar. Therefore, in spite of the finding of no RNA in a limited survey, it is entirely possible that CYP2D7 may be expressed in some livers. The presence of a premature termination codon at bases 2587 to 2589 in exon 5 of CYP2D7 that was introduced by the T insertion in exon 1 would probably render the mRNA unstable (Daar and Maquat 1988). We do not know

the frequency of this mutant CYP2D7 allele in the human population at this time.

The CYP2D8P gene resides 4,666 bp upstream of the CYP2D7 gene, spans 5,267 bp of DNA, and contains nine exons (fig. 5). Upstream DNA of 1,303 bp was also sequenced. When compared with the CYP2D6 gene, this gene contains multiple deletions and insertions in its exonic sequence, resulting in a fully disrupted

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| 2100 | |
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| AGGAGGAGTCGGGCTTTCTGCGCGAG GTGCGGAGCGAGAGACCGAGGAGTCTCTGCAGGGCGAGCTCCCGAGAGGTGCCGGGGCTGGACTGGGGCCTCGGAAGAGCAGGATTTGCATAGA | |
| | |
| | |
| GCCAGGCCCTGGGTCTACCTGGAGATGGCTGGGGCCTGAGACTTGTCCAGGTGAACGCAGAGCACAGGAGGGATTGAGACCCCGTTCTGTCTG | EXON 5 |
| 2600 CTCCTGCATATCCCAGCGCTGGCTGGCAAGGTCCTACGCTTCCAAAAGGCTTTCCTGACCCAGCTGGATGAGCTGCTAACTGAGCACAGGATGACCTGGGACCCAGCCCAGCCCCCCGA L L H I P A L A G K V L R F Q K A F L T Q L D E L L T E H R M T W D P A Q P P R | |
| GACCTGACTGAGGCCTTCCTGGCAGAGATGGAGAAG GTGAGAGTGGCTGCCACGGTGGGGGGGGGAAGGGTGGTGGGTTGAGCGTCCCAGGAGGAATGAGGGGAGGCTGGGCAAAAGGTTGG | |
| 2800 ACCAGTGCATCACCCGGCGAGCCGCATCTGGGCTGACAGGTGCAGAATTGGAGGTCATTTGGGGGGCTACCCCGTTCTGTCCCGAGTATGCTCTCGGCCCTGCTCAG A C C N D | EXON 6 |
| TGAGAGCAGCTTCAATGATGAGAACCTGCGCATAGTGGTGGCTGACCTGTTCTCTGCCGGGATGGTGACCACCTCGACCACGCTGGCCTGGGGCCTCCTGCTCATGATCCTACATCCGGA ESSFNDENLRIVVADLFSAGMVTTSTTLAWGLLLMILHPD 3100 | |
| TGTGCAGC GTGAGCCCATCTGGGAAACAGTGCAGGGGGCCGAGGGAGG | |
| GTAAGCCTGACCTCCTCCAACATAGGAGGGCAAGAAGGAGTGTCAGGGGCCGGACCCCCTGGGTGCCGACCCATTGTGGGGACGCATGTCTGTC | EXON 7 |
| CGTGATAGGGCAGGTGCGGCGACCAGGATGGGTGACCAGGCTCACATGCCCTACACCACTGCCGTGATTCATGAGGTGCAGCGCTTTGGGGACATCGTCCCCTGGGTGTGACCCATAT VIGQVRRPEMGDQAHMPYTTAVIHEVQRFGDIVPLGVTHM 3400 | |
| GACATCCCGTGACATCGAAGTACAGGGCTTCCGCATCCCTAAG GTAGGCCTGGCGCCCTCCTCACCCCAGCTCAGCACCAGCACCGGTGATAGCCCCAGCATGGCTACTGCCAGGTGGG T S R D I E V Q G F R I P K | |
| | |
| GGGAGACAAACCAGGACCTGCCAGAATGTTGGAGGACCCAACGCCTGCAGGGAGAGGGGGGGG | |
| TGGAGCTTCTCGGGCGCAGGACTAGTTGACAGAGTCCAGCTGTGTGCCCAGGCAGTGTGTGT | |
| CTGCATCTCCTGCCCAG GGAACGACACTCATCACCAACCTGCCATCGGTGCTGAAGGATGAGGCCGTCTGGGAGAAGCCCTTCCGCTTCCACCCCGAACACTTCCTGGATGCCCAGGGCC G T T L I T N L S S V L K D E A V W E K P F R F H P E H F L D A G G 4000 | EXON 8 |
| ACTITIGEAAAGCCGGAGGCCTTCCTGCCTTTCTCAGCAG GTGCCTGTGGGGAGCCCGGCTCCCTGTCCCTTCCGTGGAGTCTTGCAGGGGTATCACCCAGGAGCCAGGCTCACTGACGC H F V K P E A F L P F S A | |
| CCCTCCCCCCCCACAG GCCGCCGGCATGCGCCCCGGGGGGGGCCCCCTGGCCCGCATGGAGCCCCTCCTCTTCTCCCCTCCTGCCCCGCACTGGGCCCCACTGGACAGCC G R R A C L G E P L A R M E L F L F F T S L L Q H F S F S V P T G Q P | EXON 9 |
| CCGGCCCACCATGGTGTCTTTGCTTTCCTGGTGAGCCCATCCCCCTATGAGCTTTGTGCTGTGCCCGCTAGAATGGGGTACCTAGTCCCCAGCCTGCTCCCTAGCCAGAGGCTCT R P S H H G V F A F L V S P S P Y E L C A V P R * | |
| AATGTACAATAAAGCAATGTGGTAGTTCC AACTCGGGTCCCCTGCTCACGCCCTCGTTGGGATCATCCTCCTCAGGGCAACCCCACCCCTGCCTCATTCCTGCTTACCCCACCGCCTGGC | |
| CGCATTTGAGACAGGGGTACGTTGAGGCTGAGCAGATGTCAGTTACCCTTGCCCCATAATCCCATGTCCCCCACTGACCCAACTCGCCCAGATTGGTGACAAGGACTACATTGTCC | |
| TGGCATGTGGGGAAGGGGCCAGAATGGGCTGACTAGAGGTGTCAGTCA | |
| | |
| CAGGGCTCAGTCGGCAGGTGCCAGAACGTTCCCTGGGAAGGCCCCATGGAAGCCCAGGACTGAGCCACCACCCTCAGCCTCGTCACCTCACCACAGGACTGGCTACCTCTCTGGGCCCCTC | |
| AGGGATGCTGCTGTACAGACCCCTGACCAGTGACGAGTTCGCACTCAGGGCCAGGCTGGCGCTGGAGGAGGACACTTGTTTGGCTCCAACCCTAGGTACCATCCTCCCAGTAGGGATCAG 700 800 | |
| GCAGGGCCCACAGGCCTGCCCTAGGGACAGGAGTCAACCTTGGACCCATAAGGCACTGGGGCGGGC | |
| CTTGCTCTGCCATTACCCCGTGTGACCCCGGGCCCACCCTTCCCCACCCTTCCCCACCCGGGCTTCTGTTTCCTTCTGCCAACGAGAAGGCTGCTTCACCTGCCCGAGTCCTGTCTTC 1000 | |
| | |

protein open reading frame. The mutations in CYP2D8P are shown in table 1. Because of the large number of insertions and deletions, these data would suggest that the CYP2D8P gene has been inactive for a longer period of time, suggesting that it is a bona fide pseudogene.

Comparison of the CYP2D Genes

To compare the three CYP2D genes, dot-matrix anal-

ysis (fig. 6) and direct nucleotide alignment comparisons (table 2) were carried out. The CYP2D6 gene shares high nucleotide similarity with both CYP2D7 and CYP2D8P. The nucleotide similarities between CYP2D6 and CYP2D7 are, in general, greater across both introns and exons than are those between CYP2D6 and CYP2D8P(table 2). Further, the nucleotide similarities between CYP2D6 and CYP2D7 extend several

1100 GCCCAAGAGTTTCTAATGAGCATATGATTACCTGAGTCCTGGGCAGACCTTCTTAGGGAACAGCCTGGGACAGAGAACCACACAGACACTCTGAGGAGCCACCCTGAGGCCTCTTTGCCAG AGGACCCTACAGCCTCCCTGGCAGCAGTTCCGCCAGCATTTCTGTAAATGCCCTCATGCCAGGGTGCGGCCCGGCTGTCAGCACGAGAGGGGCGTTGGTCTGTCCCCTGGCACCGAGTAG 1300 GTCAGAAGGGTGGCCAGGGCCCCCTTGGGCCCCCTCCAGAGACAATCCACTGTGGTCACACGGCTCGGTGGCAGGAAGTGCTGTTCCTGCAGCTGTGGGGACAGGGAGTGTGGATGAAGCC 1500 AGGCTGGGTTTGTCTGAAGACGGAGGCCCCGAAAGGTGGCAGCCTGGCCTATAGCAGCAGCAACTCTTGGAATTTATTGGAAAGATTTTCTTCACGGTTCTGAGTCTTGGGGGGTGTTAGAG 1600 GCTCAGAACCAGTCCAGCCAGAGCTCTGTCATGGGCACGTAGACCCGGTCCCAGGGCCTTTGCTCTTTGCTGTCCTCAGAGGCCTCTGCAAAGTAGAAACAGGCAGCCTTGTGAGTCCCC TTCTGTGTTGCACAGACAGGGAAACAGGCTCTGTCCACAAAAGTCGGTGGGGGCCAGGATGAGGCCCAGTCTGTTCACACATGGCTCCTCTCAGCTCTCGCACAGACGTCCTCGCCC 2200 AGACGTTCAGTCGAAGCCCCCGGGCTGTCCTTACCTCCCAGTCTGGGGTACCTGCCACCTCCTGCTCAGCAGGAATGGGGCTAGGTGCTTCCTCCCCTGGGGACTTCACCTGCCTCCCCTC CTGGGATAAGACGGCAGCCTCCTCCTTGGGGGCAGCAGCAGCATTCAGTCCTCCAGGTCTCCTGGGGGTCGTGACCTGCAGGAGGAATAAGAGGGCAGACTGGGCAGAAAGGCCTTCAGAGCA 2500 CCTCATCCTCCTGTTCTCACACTGGGGTGTCACAGTCCTGGGAAGTTCTTCCTTTTCAGTTGAGCTGTGGTAACCTTGTGAGTTTCCTGGAGGGGGGCCTGCCACTACCCTTGGGAACTCCC 2800 GGGAGGTGGGAGGGGTGACACAACCCTGACACCCACACTATGAGTGATGAGTGATGCCCCCGACTGGCCCCATCCTTTCCAGGTGCAGTCCCCCTTACTGTGTCTGCCAGGGTGCCAG 3000 CAAGGAGAGAGAGAACAGGCTGAGGTGAGAGCTACTGTCAACACCTAAAACCTAAAAAATCTATAATTGGGCTGGGCAGGGTGGCTCACGCCTGTAATCCCAGCACTTTGGGAGGCCGAGATG 3100 GGTGGATCACCTGAGGTCAGATGTTCGAGACCAGCCTGGCCAACATGGTGAAACCCCCGTCTCTACTAAAAATACAAAAAATTAGCTGGGCGTGGTGGTGGGGGGCCTGTAATCCCAGCTAC TATAATTGATATCTTTAGAAAGATAAAACTTTGCATTCATGAAATAAGAATAGGAGGGTCTAAAATAAAAATGTCCAAACACCCCACCACCACTAATTCTTGACAAAAATAAGTCTGGGT 3500 GCCTTAGCTCATGCCTGTAATCCCAGCATTTTGGGAGGCTAAGGCAGGAGGATTGTTTGAGCCTAGGAATTC

Figure 4 Sequence of the CYP2D6 gene. The nucleotide sequence of an EcoRI fragment encompassing the CYP2D6 gene was determined. The transcription start site was designated +1 on the basis of the data in fig. 3. The position where the CYP2D6 gene is polyadenylated was determined from the cDNA clone. Upstream and downstream DNA are numbered beginning with -1 and +1 before the transcription start site and after the polyadenylation site, respectively. The amino acids are designated by the single-letter code.

hundred base pairs both upstream and downstream, whereas the similarities of nucleotide sequence between *CYP2D6* and *CYP2D8P* extended just upstream of the first exon and almost precisely at the end of the termination codon in their ninth exons. These data suggest

Table I

Mutations in the CYP2D8P Gene

| Exon 1 | 3-base deletion | Between 250 and 251 |
|---------|------------------|-----------------------|
| 2.000 1 | 5 base detetion | between 250 and 251 |
| Exon 4 | C→T} termination | 3000 |
| | C→A} | 3002 |
| | 2-base insertion | 3006-3007 |
| Exon 5 | C→T termination | 3584 |
| Exon 7 | 3-base deletion | Between 4177 and 4178 |
| | C→T termination | 4205 |
| Exon 9 | 1-base insertion | 5056 |

NOTE. - The sequence of the CYP2D8P gene was compared with that of the CYP2D6 gene, and detrimental mutations were identified.

that the gene duplication events that gave rise to CYP2D6 and CYP2D7 involved upstream and downstream intragenic DNA, whereas that giving rise to CYP2D8P involved only the structural gene and a small portion of upstream DNA, including the TATA box. Since the transcription regulatory elements in the CYP2D6 gene have not been defined, it is unknown whether CYP2D8P would have the appropriate upstream DNA to be accurately transcribed and regulated.

Another interesting finding is the presence of a large insertion of DNA in the first intron of CYP2D8P (figs. 5 and 6). This insertion represents three tandem R.dre.1 (Alu) sequences and is flanked by the direct repeats 5'-GAAATCA-3'. These R.dre.1 repeats of about 300 bp display 68%-75% nucleotide similarities with each other. The 900-bp insertion must have occurred subsequent to the formation of the three CYP2D genes.

A comparison of the amino acid sequences of the three CYP2D genes is shown in figure 7. The alignments were made on the basis of the deduced amino acid sequence of CYP2D6, and the percent similarities

Table 2

Comparisons of the Exon and Intron Lengths and Percent Nucleotide Similarities between CYP2D6, CYP2D7, and CYP2D8P Genes

| | | Length ^a (bp) | | | % Similarity | (|
|---------------------|--------|-----------------------------|--------------------|---------|--------------|----------|
| | CYP2D6 | CYP2D7 | CYP2D8P | CYP 6/7 | CYP 7/8P | CYP 6/8P |
| Upstream | 774 | 777 | | 97 | | |
| - | | 186 | 183 | | 92 | |
| | 189 | | 186 | | | 89 |
| Exon 1 | 268 | 269 | 265 | 97 | 94 | 93 |
| Intron 1 | 703 | 701 | 1,620 ^b | 98 | 90 | 89 |
| Exon 2 | 172 | 172 | 172 | 95 | 94 | 91 |
| Intron 2 | 550 | 528 | 546 | 74 | 78 | 77 |
| Exon 3 | 153 | 553 | 153 | 98 | 93 | 92 |
| Intron 3 | 88 | 88 | 88 | 98 | 91 | 93 |
| Exon 4 | 161 | 161 | 161 | 98 | 89 | 91 |
| Intron 4 | 433 | 425 | 449 | 94 | 85 | 86 |
| Exon 5 | 177 | 177 | 177 | 99 | 93 | 92 |
| Intron 5 | 190 | 192 | 186 | 97 | 84 | 83 |
| Exon 6 | 142 | 142 | 142 | 94 | 92 | 96 |
| Intron 6 | 207 | 194 | 204 | 82 | 87 | 90 |
| Exon 7 | 188 | 188 | 185 | 98 | 94 | 95 |
| Intron 7 | 454 | 454 | 449 | 98 | 91 | 91 |
| Exon 8 | 142 | 142 | 142 | 99 | 96 | 96 |
| Intron 8 | 98 | 98 | 96 | 100 | 97 | 97 |
| Exon 9 ^c | 252 | 252 | | 94 | | |
| | | 180 | 181 | | 95 | |
| | 180 | | 181 | | | 92 |
| 3' Flanking | 538 | 528 | | 97 | | |

NOTE. - Alignments were performed on the Beckman Microgenie[®] program. Exons or introns that are the same size among genes are underlined.

^a Lengths of upstream and downstream DNAs compared were those of maximal nucleotide similarities. DNA further upstream and downstream did not display significant similarities.

^b Contains a 920-bp insertion.

^c When CYP2D8P exon 9 sequence was compared with CYP2D6 and CYP2D7, alignment finished 1 base after the termination codon.

reflect that of the CYP2D genes' exonic regions. CYP2D6 displayed 97% and 92% gene-deduced amino acid similarities with CYP2D7 and CYP2D8P, respectively. It is interesting that certain segments of sequence between CYP2D7 and CYP2D8P are more similar to each other than to CYP2D6; for example, residues 166–168 are DQA in both CYP2D7 and CYP2D8P and are NHS in CYP2D6 (fig. 7). Residues 478–481 are SRVV in the former two genes but are HGVF in the latter. These data suggest that gene conversions have occurred between CYP2D7 and CYP2D8P subsequent to the recent formation of CYP2D6 and CYP2D7 by gene duplication event. Gene conversions have been seen in several other P450 subfamilies (Gonzalez 1988).

Conclusions

In the present report we have identified three genes that compose the CYP2D gene cluster in man. These genes are located distal to IGL on the long arm of chromosome 22 (q11.2-qter) (Gonzalez et al. 1988b). The

| TCATGCTGCCATATCTTGAATTACAGGCCTCTGGCTGGTAAGGAGGGGCACTCGGGAGGACACTGCCCACATTGCAGGCATGCCTGTCCCTGCCCTTCACACCCCCCATCATGATTCATGAT | |
|--|---------------|
| - (100 | |
| GACTGCTTGGGGAGGGCCTGACACCTCAAAAGGGCCAAGAGTGCATACAGGTAATGTATAAAGGGCCACATGTAACAAGCACCCACC | |
| TCACAGTCCCAAATAGAACAGTGCTGCCATGTAGGACAGGAACATTCATT | |
| TCTCTGTCAGTTGAGTAGCTTCAAAACTTCTGTTGGGCCCAGGTGGCTCATGCCTCTATTTCCAACACTTTGGGAGGCCAAGGCAGGAGGATCACTTGAAGCCAGGAGTTCCAGACCAGCG | |
| TGGGCAACATAGTGAGGACCCATCTCAACAAAAAATTAGCCGAGCGTAATGATGTGCACCTGTAGTTCCAGCTACTCGGGACGCTAAAATAGGATCTCTTGATTGA | |
| GAGTGAGCTATGATCACAGCTCTGCACTCCAGCCTGGGCAACAGAGCAAGATCTTGTCTCTAAAAAATATATAT | |
| CAGATCCAGTCTTCATCCAGACCTGAAAAGACCCAGGCTCCAGCTGCTGCCGCCTCCTGCCCCCCCAGGGCCACCTGCACAGGGAAATTCCAGGGGTGGGT | |
| GGCCTACAGTGCTAGGCAGCCCCTCAGTCAGCTAGACAAAGTTCTCCCATGAATCCTTCCCAGGAAAGTCCTGTTCCAGCCTGGGACAACGTCCCCATGGACCCTCATGGCACTGCTGGCTT | |
| GTCATGTCAGCTATGTTACCTTCCTACTCCCCGTGGTCATCATTACGTTGGGGCATTGACTCACAGCCTTACCACCATGCTCCCAGTACACAGCCCAGCACCCAGTACAATCCATACCTC | |
| | |
| CTGCACCCACTGGCAGCACAGTCAACACAGGAGGTTGGCTCACAGCAGAAGGCAAAGGCCATCATCAGCTCCCTTTATAAGGGAACGGTCACGCGCTCGGTGT GCTGAGAGTGTCCTGCC | |
| TGGTCCTCTGTGCCTGGTGGGGGTGGGGGGTGCCAGGTGTGTCCAGAGGAGCCCAGTTGGTAGTGAGGCAGCCATGGGGCCTGGATGGA | |
| | EXUN I |
| TGCTCCTGGTGGGACCTGATGCAGCAGCACCAACGCTGGACTGCACCGCCACCGCCCGC | |
| | |
| 400 | |
| TTCCTGGGGAGGGCATTTATGCATGGCATGAAAGATGGGATTTTCCAAAGGCCAAGGAAGAGTAGGGCCAAGGGCCTGGAGGTGGAGCTGGACTTGGCAGTGGGCGTGCAAGCCCATTGGG 500 | |
| CAGCATATGTTAGGAGCACAAAGTCCCCTCTGCTGACACCAGAAGGAAAGGCCTTGGGAATGGAAGACGAGTCAGGGTCCTGTGTGCCGTTTAAATCAGGAAATCAGGAAATCAGGCTGTGCGTGGGTG | |
| CTCACGCTATAATCCCAGCACTTAAGGAAGCCAAGGTGGGCGGATCACCTGAGGTCAGGGGGTCCCAGATGAGTCTGGCCAACATGGCAAAAAACCGGTCTCTACTAAACATACAAAAAATG | |
| | |
| | |
| AGE I BAGCACAGI IGI I CACAGE I BAGA I CECAGE I ACTI I BAGAGE I BAG | |
| 900 CTCGGTGACACAGCCAGACAATGTCTAAATAAACGAATAAGAAATCAGGCCGGGGCGGGGCGGCTCACCGGCCTGTAATCCCGGCCCTTTGGAGGCTAAGGCGGGCG | Alu |
| DO CTCGGTGACACAGCCAGACAATGTCTAAATAAACGAATAAGAAATCAGGCCGGGGGGGG | Alu |
| DO CTCGGTGACACAGCCAGCCATGTCTAAAAAAAAAAAAAA | Alu |
| CTCGGTGACACAGTGGTGGCCAGACATGTCTAAATAAACGAATAAGAAATCAGGCCGGGGGGGG | Alu |
| CTCGGTGACACAGCCAGACAATGTCTAAATAAACGAATAAGAAATCAGGCCGGGGGGGG | Alu |
| AGE ISBECTAGE IS | Alu |
| DOC CTCGGTGACACAGCCAGCAATGTCTAAATAAACGAATAAGGAATCAGGCCGGGGCGCGGGGGCTCACGCCTGTAATCCCGGCCCTTTGGAGGCCAAGGCGGGCG | Alu |
| CTCGGTGACACAGCCAGCAATGTCTAAATAAACGAATAAGAAATCAGGCCGGGGGGGG | Alu Exon 2 |
| CTCGGTGACACAGCCAGCAATGTCTAAATAAACGAATAAGAAATCAGGCCGGGGCGCGGGGCCCCGGTCCACGCCTGTAATCCCGGCCCTTTGGAGGCCAAGGCGGGCG | Alu exon 2 |
| CTCGGTGACACAGUACAGUAGUAGUAGUAGUAGUAGUAGUAGUAGUAGUAGUAGUA | Alu exon 2 |
| CTCGGTGACACAGCCAGCAATGCCTAAATAAACGAATAAGAAATCAGGCCGGGGCGCGGGGCCCCGGTCCACGCCTGTAATCCCGGCCCTTTGGAGGCCAAGGCGGGCG | Alu exon 2 |
| CTCGGTGACACAGUTGUTGCACGCUTGACATGUTACTAGAATCCCAGUTACTGGGGCGGGGGGGGGG | Alu exon 2 |
| $ \begin{array}{c} \label{eq:rescaled} \begin{tabular}{lllllllllllllllllllllllllllllllllll$ | Alu exon 2 |

CYP2D8P gene was found to be a pseudogene, while the CYP2D7 gene contained a single reading frame-disrupting insertion in its first exon. The CYP2D6 gene is that associated with polymorphic debrisoquine metabolism. We do not know whether the CYP2D7 gene product is capable of metabolizing debrisoquine. The Southern blot data presented elsewhere (Skoda et al. 1988), together with the sequence data presented here, are consistent with CYP2D6 being located 3' downstream of CYP2D8P and CYP2D7; for example, the *Bcl*I site found -693 bp upstream of the CYP2D8P gene (fig. 4) can account for the single 40-kbp frag-

| GAGTGGGCGGCGGAGGGCGGGGGCCAAGGCCTCCATGACCACGTCCATGTGTCCGTCC | EXON 3 |
|--|---------|
| 2700 TGTCCACCTTGCGCAACTTGGGCCTGGGGCAAGAAGGCCCCCTGGGCGGCGGCGGCGGCGACGAAGGCGGC V S T L R N L G L G K K S L E R W V T E E A A C L C A A F A D Q A | |
| 2800 ACAAAGCGGGAACTGGGAAGGTGGAGGACTGGGAAGGCGACCCCTGACCCGGCATCTCCCGCCCCAG GACGCCCCTTTCACCCCCAACGGCCTCCTGAACAAAGCGGCGAGCAACGTGATC R R P F H P N G L L N K A A S N V I | EXON 4 |
| 2900 GCCTCCCTCACCTGCGGGTGCCGCTTCGAGTACGACGACCCTCGCTTCCTCAGGCTACTGGACCTAGGCTCAGGAGGAGGAGGAGGAGGCGGGGCTTTCTGGAGGAGAT A S L T C G C R F E Y D D P R F L R L L D L A Q K G L K E E L G F L (U) E | |
| 3100 GGACCGCAGGGTCTCTGCAGGGCCGAGCTCCTGAGAGGTGCCCGGGACTGCAGCCGGACCTCCAAGGAGCAGGGTTTGCATAGAGTGGTTTGGGAAAGGACATTCCAGAAGAGCTCACTGCT | |
| AGAGGAAGGGCCTTGAGGAGGAGGAGGAGACATCTCAGATACGGTCGTGGGAGAGGTGTGCCCCGGGTCAGGGGGGCACCAAGAAAGGCCAAGGACCCTGTGCCTCTGTCCACATTGGAGATTT | |
| 3300 TGATTTTTAGGTTTCTCCTCTGGCAGCCCAGGGCAAGGAGAGGAGGAGGGGGGAGGGCCGGGAGGGGAGGGGGG | |
| GCAGCTGGGGCCTGAGACTGGTCCAGGTGAACGCAGAGCACAGGAGGGATTGAGACCCCGTTCTGTGTCAGCTGTAG ATGCTGAATGTTGTCCCCCTCCTCCTGCGCATCCCAGGGCTGG | |
| 3500 3600 3600 3600 3600 3600 3600 3600 | EXUN 3 |
| AGKVLRSQKAFLTQLDELLTEHRMIWDPA(U) PPRDLTEAFL 3700 | |
| CAGAGAAGGAGAGAG GTGAGAGTGGCTGACACGGTAGGGGTGGTGGGTGGGTGGG | |
| 3800 GGGCTGACAGGTGCAGAATGTGGAGGTCATTTGGGGGGCTTTCCCGTTCTGTCCCCTGAGTACCCTCCAGCCCTGCTCAG GCCAAGGGGAACCCTGAGAGCAGCTTCAATGATGAGAACC | 5.YOU (|
| 3900 AKGNPESSFNDEN | EXUN O |
| TGCGCATGGTGGCTGACCTGTTCTTTGCCGGGATGGTGACCACCTCGATCACGCTGGGCCTGGGGCCTCCTGCTCATGATCCTACGCCCGGATGTGCAGC GTGAGCCCAGCTGGGGCC L R M V V A D L F F A G M V T T S I T L A W G L L L M I L R P D V Q | |
| CAGTGCAGGGGGCAAGGGAGGAAGGGTACAGGTGGGGGGCCCCTGAGCTTAGCTGGGACACCCGGGACTCCAAGCACAGGCTTGGCCAGGTTCCTGTAAGCCTAACCTCCTCCAACACAGG | |
| 4100 4200 4200 4200 4200 4200 4200 4200 | |
| R R V Q Q I D N V I G Q V W (U) P E M 4300 | EXON 7 |
| GTGACCAGGCTCGCATGCCCTGCACCACTGCCGTGATTCACGAGGTGCAGCGCTTTGGGGACATCGTCCCCCTGGGTGTGACCCCATATGACATCCCGTGACATCGAAGTACAGGGCTTCC G D Q A R M P C T T A V I H E V Q R F G D I V P L G V T H M T S R D I E V Q G F | |
| GCATCCCTAAG GTAGGCCTGGCACCCTCCTCACCCCAGCTCAGCACCAGCCCCTGGTGATAGCCCCAGCATGGCCACTGCCAGGTGGGCCCAGTCTAGGAACCCTGGCCACCCAGTCCTC | |
| 4500 | |
| AATGCCACCACATCGACTGTCCCAGCCTGGGTGTGGGGTGCAGAGTATAGGCAGGGCTGGCCTGTCCATCCA | |
| GAGGACCCCAATACCTGTAGGGAGAGGGGTAGCGTGGGCGCTCCCAGGAGGGTGTGACTGCGCCCTGCCGTGGGGTCGGAGAGGGTGCTCTGGAGCTTCTCGGGCACAGGACTAGTTGACA | |
| GAGTCCAGCTGTGTGCCAGGCAGTGTGTGTCCCCTGTGTGTG | EXON 8 |
| | Evon o |
| L S S V L K D E A V W K K P F R F H P E H F L D A Q G H F V K P E A F L P F S A | |
| AG GTGCCTGTGGGGAGCCCGGCTCCTGTCCCCTTCCGTGGAGTCTTGCAGGGGTATCACCCGGGAGCCAGGCTCACTGACGCCCTCCCCCACAG GCCGCCGTGCATGCCTCGGGG | ; |
| 5100 G K K A C L G | EXUN 9 |
| AGCCCCTGGCCCGCATAGAGCTCTTCTCTCTTCTTCACCTCCCTGCTGCAGCACTTCAGCTTCTGGTGCCCACCGGACAGCCCCGGCCCAGCCACTCTCGTGTCGGCTTTCTGGTGA Q P L A R I E L F L F F T S L L Q H F S F S V P T G Q P R P S H S R V V G F L V | |
| CGCCATCCCCTATGAGCTTTGTGCCGCGCCCGCTAGAGTTGCCCCCTGAGCTGGGGACCCTGTTGTACAATAAATTAGTCTAGTGGCTCC CACTTGGTTTCTGTATCCAGTCTGGGCCCC | |
| -4600 TGCCAAGGTCCTGGTTGTGTTGGGTCGTCAGTCACCTGCCTG | |
| -4500 -4400 ACTCTGTCACCCAGGCTGCAGTGCAGTGCGAGTGCAATCTCAGCTCACCTCCGCCTCCAGAGTTCCAGCGATTCTCGTGCCTCCAGCTTCCTGAGTAGCTGCGATTACCGCC | |
| -4300 TACCACCCCCGGCTCATTTTTGTCTTTTTAGTAGTGGTGGGTTTCGCCATGTTGGCCAGTCTGGTTTCAAACTCCTGACTTCACGTGACCACCAGCCTCAGCCTCCCAAAGTGCTGGGAT | |

2600

ment detected in Southern blots if CYP2D6 is downstream of CYP2D7. On the other hand, if CYP2D6 had been located 5' upstream of CYP2D8P, two BclI fragments would have been expected. By similar reasoning with data from other restriction enzymes, we believe the placement of the three CYP2D genes is as shown in figure 2. On the basis of both the high frequency of mutant alleles in the population (35%-43%); Gonzalez et al. 1988*a*) and the apparent lack of selection for the presence of the *CYP2D6* gene product (see below), it appears that the *CYP2D6* gene is beginning to vanish. The occurrences of gene conversions between the pseudogene and the *CYP2D6* gene might hasten the in-

-4200 TACAGGCGTGAGCCACCGAGACCAGCCTCACCTCATTCACTCTGGACGCCTGACTTTACTTGAGATACAGGCATAGTGATTCTCAGCAGGAAACAGCCTGCCCCCACGTCACGCC 4100 CAGAGACCCATCACTGGCTGGCTTGGTGACAAAGTCCATGCGTAAGTCTTGGCTGGGGTGGATATGAATAGGCATATGCCAAGAATCAACCCATTCCCTGGCTAGGGTGGGAGACT GTGTTGTGCCCCCCAGACCACCCCCAGGTTCAGTGATTTCTAGAAGGTCTCACAGCCCCTAGAAAAGCTGTTATTCCCCCTGTTAACAGTTTATTACAGAGAAGGGTACAGATTAAAAGTC -3900 AGCAAAGATGAAAGGCACAGGGACCAGAGTCCAGAATGACCAGGCCAAGGCTGCAGCTCTCTTTTCTGGTGGACTCCTACAGGCAGTGCTTAATTCTCCCCCCAACAGTAAGTGAGGCAGC AGAGAGCCCTGCCAGCCACGGAAGCTCACCTGGGCCTTGGTGTCCATGGTTTTGTTGGGAGTTGGTCATCCTAGGCCTGAGCCCCGCAGCATGGCCGACCTTGGTCATCCTAGGCCTG -3600 AGCCCCCGCAGCATGGCTGACCTCAGTTACTCAGTCTCCAGCCCCTCCTGAAGTCAGATGGATACACGTGACGGCCCCACCCTCGATCACATTGTTGGCATAAACTGTGTTGTACGGTCC 3500 AAGGCCCTAGCTATGTACAAAGACACTATTTCAGGCAGGACATTCCAAGGCCTTAGCAGATATCTCCCCAGCCTCCTGTCAAGAGTCAGTTTGGACCTCTGGTCCAGTGGCTTGCATTGCATTGCA CTTGAGACAGGGTCTTGCTCTGTCACCGAGGCTGGAGTGCAGTGCAGTGCATCACGGGCTCACTGCAGCCTTGACTTCCTGGGTTCAAGCAATCCTCCCATCTCAGCCTCCCTAGTAGCCACTGG GACTGCAGGCACATGCCACCATGCTATGCTAATTTTTGTATTTTTTGTAGAGACGGAGTTTCACCATGTTGGCCAGGCTGCTTTCGAACTCCCTATCTCAGGTCATCTGACTGCCTCAGC -3000 TGTATCCTTGAGAATGATCCATGTGCTGAGGAAGAATGTGTATTCTGCAGACTTTAGACGAAGTGTTCTGTAAGTATCTAGTAGGTCCATTTCTTTGTAGTGCAGATTAAGTCTAAT GTTTTCTTATTGGGTTTCCATCTGGGACACCCGTCCAATGCTGAATGTGGGGTGTTGACGTCTTTAGCTGTTATTGCGTTAACGTCTCTCTTGGGGCTCCAATAACATTTGCTTTAGCGTG TCTCGCTCTGTCACCCAGGCTGGAGTGCAGTGGCGCGATCTTGGCTCACTGCAAGCTTCGCCTCCCAGGTTGACGCCATTCTCCTGCCTCAGCCCCTGAGTAGCTGGGACTACAGGCGC GGGATTACAGGCGTGAGCCACCCCGGCCTCCTCTACAGTTTTGTTTAAAATCTGTTCTGTCTAAGTATTGCTACTCCTGCTCTTTTTGTTTTCCATTGGCATGGAGTATCTT 1800 TGCTTTTTGATTTGAGGTTGCCGTGAGGCTTGGCAAATATTATCTTATAACTTATTTTTAAACGGATGACAACACTGATTGCGTAAACAAAACATAAAGCAAAAAGGAAGACTAATAAAA ACTCTACACTTTAAGTTCATCTTAGTGCTTTTTAACTTTTTGTTGTTGTTCTCTTTTTGTTGTTTTTGAGATAAAGTCTTGCTCTGTTGCCCAGGCTAGAGTGCAGTGGCACGATCTCAGCTC ACTGTAACCTCCACGTTCCAAGGTTCAACCGATTCTCCTGCCTCAGCCTCCTGGGTAGCAGGCGCCCCACCACCATGCCCAGCTAAATTTTTTGTATTTTTAGTAGAGATGGGGTTTCACCA 1200 TCTGTCTTGAAAAGTACTTATTATTTTGATTGGTTCGATCATTTAGTCTAATTAAAAATAAGAGTAGTTTACCACCACAATTACAGTATTATAATACTCTGTTTTTCTGTGTGCCTTACTA GTGTGAGAGAGAATGTGTGCCCTGAGTGTCAGTGTGAGTCTGTGTATGTGTGGAATATTGTCTTTGTGTGGGGTGATTTTCTGCATGTGTAATCGTGTCCCTGCAAGTGTGAACAAGTGGGAC AAGTGTCTGGGAGTGGACAAGAGATCTGTGCACCATCAGGTGTGTGCATAGCGTCTGTGCATGTCAAGAGTGCAAGGTGAAGTGAAGGGACCAGGCCCATGATGCCACTCATCATCAGGA -400 GCTCTAAGGCCCCAGGTAAGTGCCAGTGACAGATAAGGGTGCTGAAGGTCACTCTGGAGTGGGCAGGTGGGGGAAAGGGCAAGGTCATGTTCTGGAGGAGGGGTTGTGACTACAT GGCCTTTCTACCACCCTACCCTGGGTAAGGGCCTGGAGCAGGAAGCAGCGGCAAGGACCTCTGCAGCCGCCATACCCGCCCTGGCCTGACCCTGGCCCACTGGCAGCACAGTCAACAC

troduction of mutations into CYP2D6. Indeed, gene conversions between the CYP21A2 gene and the neighboring CYP21A1 pseudogene are known to contribute to the mutations at the steroid 21-hydroxylase locus (Miller 1988).

The unexpected finding of two additional genes other than CYP2D6 allowed us to reexamine some of the variant cDNA transcripts we previously cloned from livers of PM individuals (Gonzalez et al. 1988*a*). Indeed, it appears that our previously described "variant b" could

| AGCAGGTTGGCTCACAGCAGAGGGCCGAAGGCCATCATCAGCTCCCTTTATAAGGGAAGGGTCACGCGCTCGGTGT GCTGAGAGTGTCCTGCCTGGTCCTGGTGGGGGTGGGGGG | | |
|--|--------|---|
| 1000 TGCCAGGTGTGTCCAGAGGAGCCCAGTTGGTAGTGAGGCAGCCATGGGGCCATGGTGGCCCTGGGCCATGATAGTGGCCATCTTCCTGCTCCTGGTGGACCTGATGCACCGGCA M G L E A L V P L A M I V A I F L L V D L M H R H | EXON 1 | 1 |
| 200 CCAACGCTGGGCTGCACGCTACCCGCCAGGTCCCCTGCCACTGCCGGGCTGGGCAACCTIGCTGCATGTGGACTTCCAGAACACACCATACTGCTTCGACCAG GTGAGGGAGGAGGAGGTCC Q R W A A R Y P P G P L P L P G L G N L L H V D F Q N T P Y C F D Q 300 | | |
| TGGAGGGCGGCAGAGGTCCTGAGGATGCCCCACCACCAGCAAACATGGGTGGTGGGTG | | |
| GAAGGACATTTATACATGGCATGAAGGACTGGATTTTCCAAAGGCCAAGGAAGAGTAGGGCCAAGGGCCTGGAGGTGGAGCTGGACTTGGCAAGCCCATTGGGCAACATA | | |
| TGTTATGGAGTACAAAGTCCCTTCTGCTGACACCAGAAGGAAAGGCCTTGGGAATGGAAGATGGAGGTTAGTCCTGAGTGCCGTTTAAATCACGAAATCGAGGATGAAGGGGGGTGCAGTGAC | | |
| CCGGTTCAAACCTTTTGCACTGTGGGTCCTCGGGCCTCACTGCTCACCGGCATGGACCATCATCTGGGGATGCTAACTGGGGCCTCTCGGCAATTTTGGTGACTCTTGCAAGGTC | | |
| | | |
| GGGTCGTCCAAGGTTCAAATAGGACTAGGACCTGTAGTCTGGGGGGGATCCTGGCTTGACAAGAGGCCCTGACCCTCCCT | EXON 2 | 2 |
| GCTGGCCTGGACGCCGGTGGTCGTCGTCGCTATGGGCCGGCGGCGAGGGCGATGGTGACCCGCGGGCGAGGACACGGCCGACCGCCGGCCG | | |
| | | |
| 1300 CAGAGATAAAGGCCAGCGAGTGGGCTGAGGACAGTGGGCCAGGAAACCACCTGCACGGGGGGAGGTGCGAGTCTGTGGGCTGGGAGGGGGGGG | | |
| GGTGGGCGAGGCTGATGCGTCGAAGTGGCGGGGGGGGGG | | |
| 1500 CGAGGTGGGGCCAAACCCCGGCCCCAGGCAGGGGAGCAATGTGGGTGAGCAAAGAGTGGGGCCCTGTGCCCAGCTGGACCGGGCTAGGGACCTCGCGGGAGACCTTGTGGAGCGCCAGGGTTGG | | |
| AGTGGGTGGCGGAGGGTGGGGCCCAAGGCCTTCATGGCAACGCCCACGTGTCCGTCC | EXON 3 | 5 |
| 1800 STLRNLGLGKACTTGGGCCTGGGCAAGAAGTCGCTGGAGCAGTGGGGTGACCGAGGAGGGCGCCTCGCCTTGGCCGCCTTCGCCGACCAAGCCG GTGGGTGATGGGCAGAAGGGCA STLRNLGLGKKSLEQWVTTEEAACLCAAFADQA | | |
| 1900 CACAGCGGGAACTGGGAAGGCGGGGGGGGGGGGGGGGGG | | |
| 2000 GPPFRPNGLLDKAVSNVI | EXON 4 | • |
| CCICCCICACCIGEGGGGGGGGGGGGGGGGGGGGGGGGGG | | |
| GTCTTTGCAGGGCGAGCTCCTGAGAGGTGCCGGGGCTGGACTGGGGCCTCCGAAGGGCAGGATTTGCGTAGATGGGTTTGGGAAAGGACATTCCAGGAGACCCCACTGTAAGAAGGGCCT | | |
| GGAGGAGGAGGGGGACATCTCAGACATGGTCGTGGGGAGAGGTGTGCCCGGGTCAGGGGGGCACCAGGAGGAGGGCCAAGGACTCTGTACCCCCGTCCACGTTGGAGATTTCGATTTTAGGTCTC | | |
| | | |
| CAGATGAACGCAGAGCACAGGAGGGATTGAGACCCCGTTCTGTCTG | EXON 5 | ; |
| GCTTTCCTGACCCAGCTGGATGAGCTGCTAACTGAGGACAGGATGACCTGGGATGGAGAAG GTGAGGAGGAGGGC A F L T Q L D E L L T E H R M T W D P A Q P P R D L T E A F L A E M E K | | |
| | | |
| TTGGAGGTCATTTGGGGGGCTACCCCGTTCTATCCCCTGAGTATCCTCTCGGCCCTGGCCCTGGCGGGGGGGCCCTGAGAGCAGCTTCATGGGGAACCTGCGCATAGTGGGTGG | EXON 6 | 5 |
| CCTGTTCCTTGCCGGGATGGTGACCACCTTGACCACGCTGGCCTCGGGGCCTCCTGCTCATGATCCTACACCTGGATGTGCAGC GTGAGCCCAGCTGGGGCCCAAGGCAGGGACTGAGGGA L F L A G M V T T L T T L A W G L L M I L H L D V Q | | |
| | | |
| GGGTGCTGACCCATTGTGGGGACGCATGTCTGTCCGG TCCGTGTCCAACAGGAGATCGACGACGATGGGGGACGGCGACCAGGGTGACCAGGCTCACATGCCCTACACC L R V Q Q E I D D V I G Q V R R P E M G D Q A H M P Y T | EXON 7 | , |
| | | |

have been transcribed from the CYP2D7 gene instead of from CYP2D6, since it displayed more nucleotide similarity with the former. Alternatively, gene conversion could have occurred between these two genes, giving rise to a CYP2D6 allele that is more similar to CYP2D7. Either of these possibilities is likely; however, our previously described "variant a" transcript clearly corresponds to the CYP2D6 gene described herein.

Finally, the question arises as to what is the driving

| 3300 ACTGCCGTGATTCACGAGGTGCAGCGCTTTGGGGACATCATCCCCTGAGTGTGACCCATATGACATCCCATGACATCGAAGTACAGGGCTTCCGCATCCCTAAG GTAGGCCTGGCGCCC T A V I H E V Q R F G D I I P L S V T H M T S H D I E V Q G F R I P K | |
|--|--------|
| | |
| | |
| GCCAGTGTGGGTGCCTCTGAGAGGTGTGACTGCGCCCCTGCTGTGGGGGTCGGAGAGGGTACTGTGGAGCTTCTCGGGCGCAGGACTAGTTGACAGAGTCCAGCTGTGTGCCAGGCAGTGTG 3800 | |
| TGTCCCCCGTGTGTTTGGTGGCAGGGGTCCCAGCATCCTAGAGTCCAGTCCCCACTCTCACCCTGCATCTCCTGCCCAG GGAACGACACTCATCACCAACCTGTCATCGGTGCTGAAGGA G T T L I T N L S S V L K D | EXON 8 |
| 3900 TGAGGCCGTCTGGAAGAAGCCCTTCCGCTTCCACCCCGAACACTTCCTGGATGCCCAGGGCCACTTTGTGAAGCCGGAGGCCTTCCTGCCTTTCTCAGCAG GTGCCTGTGGGGAGCCCGG E A V W K K P F R F H P E H F L D A Q G H F V K P E A F L P F S A | |
| CTCCCTGTCCCTTCCGTGGAGTCTTGCAGGGGTATCACCCAGGAGCCAGGCCTACTGACGCCCCTCCCCCACAG GCCGCCGTGGAGTCTTGCAGGGGAGCCCCTGGCCCGCATGGAG G R R A C L G E P L A R M E | EXON 9 |
| CTCTTCCTCTTCTCACCTCCCTGCTGCAGGACATCTCCGTGGCCGCCGGACAGCCCCGGCCCAGCCACTCTCGTGTCGTCAGCTTTCTGGTGACCCCCATCCCCCACGAGCTT L F L F F T S L L Q H F S F S V A A G Q P R P S H S R V V S F L V T P S P Y E L 4300 | |
| TGTGCTGTGCCCCGCTAGAATGGGGTACCTAGTCCCCAGCCTGCTCCCTAGCCAGAGGCTCTAATGTACAATAAAGCAATGTGGTAGTTCC ÅACTCGGGTCCCCTGCTCACGCCCTCGTT C A V P R * | |
| 100 | |
| GGGATCATCCTCCTCAGGGCAACCCCACCCCTGCCTCATTCCTGCTTACCCCACCGCCTGGCCGCATTTGAGACGGGTACGTTGAGGCTGAGCAGATGTCAGTTACCCTTGCCCATAATC 200 | |
| CCATGTCCCCCACTGACCCAACTCTGACTGCCCAGATTGGTGACAAGGACTACATTGTCCTGGCATGTGGGGAAGGGGCCAGAATGGGCTGACTAGAGGTGTCAGCCCTGGATGTG 300 | |
| GTGGAGAGGGCAGGACTCAGCCTGGAGGCCCATATTTCAGGCCTAACTCAGCCCACCCCACATCAGGGACAGCAGTCCTGCCAGCACCATCACAACAGTCACCTCCCTTCATATATGACA 400 500 | |
| CCCCAAAATGGAAGACAAATCATGTCAGGGAGCTATATGCCAGGGCTACCTCCCAGGGCTCAGTCGGCAGGTGCCAGAACATTCCCTGGGAAGGCCCCAGGAAAACCCCAGGACCGAGCCA | |
| CCGCCCTCAGCCTGTCACCTTGTGTCCAAAATTGGTGGGTTCTTGGTCTCACTGACTTCAAGAATGAAGCCGTGGACCCTCACGGTGAGTGTTACAGTTCTTAAAGATGGTGTGTTCAGA | |
| GTTTGTTCCTTCTGATGTTAAGACGTGTTCAGAGTTTCTTCCTTC | |
| TGCACGTACGGAGTTGTTCATTCTTCCTGGTGGGTTTGTGGTCTCACTGGCCTCAGGAGTGAAACTGCAGTCCTTCCAGTGTTACAACTCATAAAGGCAGTGTGGACCCAATGAGGGAGC | |
| AGCAGCAGGCAAGACTTACTGCAAACAGCAAAAGAATGATGGCAACCAGGTTGCCGCTGCTACTTCAGGCAGCCTGCTTTTATTCCCTTATCTGACCCCCACCACACCTCGCTGATTGGC | |
| CCATTTTACAGACAGTGGATTGGTCCACTTACAGAGAGCTGATTGGTGCATTTACAATCCCTGAGCTAGACACAGAGTACTGATTGGTATATTTACAAACCTTGAGCTAGACACAGAGTG | |
| CTGAATGGTGTATTTACAATCCCTTAGCTAGACATAAAGGTTGTCCCCAGTCCCCACTAGATTAGCTAGAATAGAGTAGAAGAGAGCAGCAGTGATTGGTGCGTTTACAAACCTTGAGTTAGAC | |
| ACAGGGTGCTGACTGGTGTGTTTACAAACCTTGAGCTAGACACAGAGTGCTGATTGGTGTATTTACAATCTTTTAGCTAGAAATAAAAGTTCCCCCAAGTCCCCACCAGATTAGCTAGATA | |
| CAGAGTGCTAATTGGTGCATGCACAACCCGGAGCTAGACACAGAGTGCTGATTGGTGCATATACAATCCTCTGGCTAGACATAAAAGTTCTCCCAAGTCCCCACCTGACTCAGAGCCCCAGC | |
| CAGCTTCGCCTAGTGGATCC | |

Figure 5 Sequences of the CYP2D7 and CYP2D8P genes. The sequences of several BamHI fragments derived from the λ clones shown in fig. 1 were determined. The putative transcription start sites designated by (+1) and the putative polyadenylation sites were estimated by comparing the sequence similarities between these genes and CYP2D6. Although we have not detected mRNA corresponding to these genes, we have made these designations for purpose of comparisons with the CYP2D6 sequence. The amino acids were displayed on the basis of the CYP2D6 reading frame in fig. 4, even though these genes have mutations that destroy the normal protein reading frames. The intragenic DNA between CYP2D8P and CYP2D7 genes was numbered beginning at -1 upstream of CYP2D7. Base deletion and insertion are shown by an arrow following the number of bases deleted and by an underline, respectively. (U) represents a stop codon due to base changes. The first termination codon introduced in exon 5 of CYP2D7 because of a single base insertion in exon 1 is overlined. Insertion of Alu repeats seen in first introns of CYP2D8P gene is boxed.

force behind the evolution of these drug-metabolizing enzymes. The suggestions that these enzymes evolved to metabolize plant toxins seem quite plausible (Nebert and Gonzalez 1985; Nelson and Strobel 1987; Gonzalez 1988). The current human diets rely almost totally on cultivation, while early man was a huntergatherer. Since man no longer relies on the evolution of wild plant fauna and can selectively avoid toxic plants, the detoxifying enzymes may no longer be evolving, and without this selective pressure of additional dietary toxins, many of the P450 genes, previously required for survival, might be lost, as evidenced by the occurrence of the debrisoquine polymorphism and other drug oxidation defects in man (Gonzalez 1988).



Figure 6 Dot-matrix comparison of the *CYP2D6* gene sequence with the sequence of *CYP2D7* and *CYP2D8P*. Comparisons were made using the Beckman Microgenie program. A dot is generated whenever a stretch of 15 nucleotides displays greater than 85% similarity. The exons are denoted by rectangles. The open rectangles represent the untranslated regions of the mRNAs.



Figure 7 Comparison of the deduced amino acid sequences of the human CYP2D genes. The amino acid sequences are taken from figs. 4 and 5. Base insertion is shown by an arrow with the number of bases inserted. Base deletion is shown by Λ with the number of bases deleted. A stop codon (U) created by base changes is underlined. Note that the CYP2D7 and CYP2D8P gene reading frames are taken from that of CYP2D6 for comparison purposes. These genes contain premature termination codons.

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