Multiple Mutations of the Human Cytochrome P450IID6 Gene (CYP2D6) in Poor Metabolizers of Debrisoquine

STUDY OF THE FUNCTIONAL SIGNIFICANCE OF INDIVIDUAL MUTATIONS BY EXPRESSION OF CHIMERIC GENES

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The debrisoquine/sparteine-type polymorphism is a clinically important inherited variation of drug metabolism characterized by two phenotypes, the extensive metabolizer and the poor metabolizer (PM). Five to 10 percent of individuals in Caucasian populations are of the PM phenotype and have deficient metabolism of debrisoquine and over 25 other drugs. Our previous studies have revealed absence of cytochrome P450IID6 protein and aberrant splicing of IID6 premRNA in livers of PMs. Moreover, two mutant alleles of the P450IID6 gene locus (CYP2D6) were identified by restriction fragment length analysis to be associated with the PM phenotype. However, the mutations of the CYP2D6 gene causing absent P450IID6 protein have not been defined.

Here we report the cloning and sequencing of two types of mutant alleles of CYP2D6 isolated from genomic libraries of three PM individuals. One allele (29-A) was characterized by a single nucleotide deletion in the 5th exon with consequent frameshift and was observed in one individual only. The other type of mutant allele (29-B) was present in all three PM individuals and its sequence contained multiple mutations, notably four base changes causing amino acid changes in exons 1, 2 and 9, and a point mutation at the consensus sequence of the splice site of the 3rd intron. To understand the significance of the individual mutations, chimeric genes were constructed between the wild-type IID gene and the mutant 29-B allele or site-specific merit genes were constructed between the wild-type and the functionally deficient IID protein and the mutation at the splice site in absent IID protein, whereas the mutations in exons 2 and 9 were of no consequence for IID6 function. Only the mutation at the splice site thus explains the absence of P450IID6 protein in livers of PM individuals and appears to be a common cause of polymorphic drug oxidation.

determined variations in drug metabolism (1, 2). It causes deficient metabolism of debrisoquine, sparteine, bufuralol, dextromethorphan, and numerous other drugs in so-called poor metabolizer (PM) individuals. The PM phenotype is inherited as an autosomal recessive trait and occurs with a frequency of 5-10% in most populations studied (3).

Our earlier investigations have shown that defective metabolism of drugs is due to the absence of cytochrome P450IID6 in the liver of PM individuals with the PM phenotype (4). Moreover, cDNA analysis of RNA from two PM livers provided evidence for incorrectly spliced pre-mRNAs as a possible cause for absent IID6 protein (45). The gene for P450IID6, designated CYP2D6 (6), has been localized to chromosome 22 (7). A presumed pseudogene CYP2D7 and a definite pseudogene CYP2D8 are localized 5' of the CYP2D6 locus (8). In further studies, two mutant alleles of the CYP2D6 gene were identified by restriction fragment length analysis of genomic DNA after hybridization with the IID6 cDNA (9). These mutant alleles are reflected by 44- and 11.5-kb fragments after digestion with the XbaI endonuclease and, when present together, are linked to the PM phenotype. However, the presence of the two mutant alleles allows prediction of the phenotype in only 25% of PMs. Additional gene-inactivating mutations therefore must be present in 75% of PM individuals in which no or only one mutant allele can be identified by restriction fragment length polymorphism (RFLP). Both the RFLPs and the cDNA analysis of RNA from PM livers therefore suggest that multiple mutations can cause the PM phenotype. However, neither the DNA mutations causing the incorrect splicing of IID6 pre-mRNA nor the mutations responsible for the RFLP, nor other mutations of the CYP2D6 gene which can explain the absence of P450IID6 protein, have been identified.

To characterize the mutations causing absent P450IID6 protein in PMs, clones containing the CYP2D6 genes were isolated from genomic libraries constructed from leukocyte DNA of three PM individuals, all belonging to the group in which no mutant allele can be detected by RFLP analysis. Two types of mutant alleles were isolated, their exons and exon-intron junctions were sequenced and the entire gene was inserted into the pCMV vector and transiently expressed in cell culture. The functional significance of the different mu-

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1 The abbreviations used are: PM, poor metabolizer; P450IID6, cytochrome P450IID6; RFLP, restriction fragment length polymorphism; DMEM, Dulbecco's modified Eagle's medium; bp, base pairs; kb, kilobase pairs.

2 The cytochrome P-450 enzyme which is deficient in PMs of debrisoquine has previously been called P450bufl (4), P450bdi (5), and P450ldi (7). P450IID6 is the designation proposed in the most recent update on P-450 nomenclature (6) and is used throughout this paper.
Debrisoquine Polymorphism

Characterization of PM Individuals

The leukocyte DNA of three individuals of PM phenotype was selected for this study from our previous collection of DNA samples for population and family studies (9). The three subjects indexed as PM1 (ZICL), PM2 (KABI), and PM3 (B07) were identified as PMs by phenotyping with either debrisoquine (10) or sparteine (11). The leukocyte DNA of each of the three PM individuals. Since Southern blot analysis of their genomic DNA had the same EcoRI pattern as EMS it was assumed that their 16-, 9.4-, and 8.5-kb fragments contain the same CYP2D genes as DNA of the wild-type or homozygous extensive metabolizer. DNA was completely digested with EcoRI and inserted into the vector λgtWES (Bethesda Research Laboratories; Ref. 12). This vector can process 2-15-kb inserts. Because the 16-kb fragment is too long to be accepted by λgtWES, these libraries contain only the 9.4- and 8.5-kb fragments, corresponding to the CYP2D6 and CYP2D8 genes. The libraries were screened with two probes to ensure the identification of clones representing the CYP2D6 gene. Both probes were labeled with 32P by nick translation. The first screening was done with the full length IIDG-cDNA (5), which recognizes both the CYP2D6 and CYP2D8 clones. We therefore used an additional probe, a SacI 0.4-kb fragment (bp -717 to -305) prepared from the CYP2D6 gene, but not the CYP2D8 gene. As the library only contains CYP2D8 genes, the second screening with this probe thus selects the CYP2D6 genomic DNA of a homozygous extensive metabolizer. This fragment recognizes the 5′-flanking region of both the CYP2D6 and CYP2D7 gene, but not the CYP2D8 gene. As the library only contains CYP2D6 and CYP2D8 genes, the second screening with this probe thus selects for CYP2D6. The EcoRI fragments of the positive clones were digested with various restriction enzymes to smaller DNA fragments; these were subcloned into pUC19 and sequenced by the double strand dideoxy chain termination method (13, 14), using universal and reverse primers as well as 18 synthesized oligonucleotides (20-mers) corresponding to the 5′ and 3′ part of each of the 9 exons and the intron-exon junctions.

Fig. 1. Schematic description of the construction of a eukaryotic expression vector containing the wild-type and chimeric CYP2D6 genes. The procedure and the vector are detailed under “Materials and Methods.” R, EcoRI; B, BamHI; Hc, HincII; Bg, BglII; A, AccI; K, KpnI; S, SmaI; H, HindIII. The chimeric CYP2D6 genes were inserted into the wild-type cDNA (Fig. 5). All constructs were sequenced to exclude polymerase chain reaction artifacts.

Construction of Expression Clones

The construction of the full length expression clones is summarized in Fig. 1. CYP2D6 Wild-type—An AccI-KpnI fragment from the 3′ part of CYP2D6 wild-type gene was blunt-ended by treatment with T4 DNA polymerase (BRL) and subcloned into pUC19 by using the HindIII site in the correct orientation. The HindII fragment of the same gene was subcloned into the Smal site of the Bluescript vector (Promega) in the correct orientation. The EcoRI-BamHI fragment of the former clone was then replaced with that of the latter clone to construct a full length gene in pUC19. The resulting gene was excised by EcoRI and HindIII and inserted into the pCMV expression vector (15, 16), using the same restriction sites.

CYP2D6 Mutated Genes (29-B Allele)—The EcoRI-KpnI fragment of the mutant 29-B gene was subcloned into pUC19. This clone was digested by HindIII and SalI, blunt-ended by T4 DNA polymerase, and ligated again to eliminate the AccI site in the vector. The HindIII site in the vector was maintained during this procedure. The HindII fragment of the mutated 29-B gene was then subcloned into the Smal site of another pUC19 in the correct orientation and the EcoRI-BamHI fragment of the former clone was then replaced with that of this clone. The engineered full length gene in pUC19 was further subcloned into pCMV using EcoRI and HindIII sites.

Chimeric Genes

Chimeric genes were assembled in pUC19 using combinations of the three parts (EcoRI-BamHI, 1.8 kb; BamHI-AccI, 0.8 kb; AccI-HindIII, 1.8 kb) of the constructed full length gene clones (Fig. 4A). The total length of the chimeric genes thus was 4.4 kb. The chimeric genes were inserted as described above into pCMV using EcoRI and HindIII restriction sites.

eDNAs with Point Mutations

A full length human CYP2D6-cDNA was constructed by subcloning a 400-bp EcoRI-Smal fragment containing the first 140 bp of the coding sequence and 260 bp of the 5′-untranslated region of a CYP2D6 wild-type genomic clone (8) into a rat-human hybrid cDNA that was deleted of the corresponding part by cutting it with the same restriction enzyme. The same strategy was used to construct a cDNA containing only mutation 1 (MI, 188 C to T) by using a genomic clone of a 29-B allele. Mutation 2 (MII, 1062 C to A) and mutation 3 (MIII, 1072 A to G) were introduced into the wild-type cDNA by the polymerase chain reaction according to Kammann et al. (17), using two mutagenic primers (GGGTACCATGCCTGGCC for MII, TCTTCATGCGCGCTCTGA for MIII). A unique XhoII site was used to subclone the polymerase chain reaction generated fragments into the wild-type cDNA (Fig. 5). All constructs were sequenced to exclude polymerase chain reaction artifacts.
DNA Transfection of COS-1 Cells

Expression clones were transfected into COS-1 cells (18) by the DEAE-dextran method (19, 20) with slight modifications. Sixteen hours before transfection, COS-1 cells were passaged from a confluent 100-mm culture dish to four dishes in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum. Transfection was performed by incubation of the cells for 2 h with DEAE-dextran (200 µg/ml; Pharmacia) and DNA (20 µg/plate) in serum-free DMEM, followed by an incubation for 3 h in DMEM containing 10% fetal calf serum and chloroquine (52 µg/ml; Sigma). The cells were harvested for analysis of IID6 protein and function after 68 h of incubation in DMEM with 10% fetal calf serum. For an assessment of IID6 function in intact cultured cells, (+)-bufuralol (200 µM) was added to the cultures for the last 24 h, and 1'-hydroxybufuralol analyzed in the medium (21).

RNA Blot Analysis

Twenty micrograms of total RNA, which was isolated (22) from the transfected COS-1 cells, was size-fractionated by electrophoresis in 1.0% agarose-formaldehyde gels (23). The full length CYP2D6 cDNA, which was radiolabeled by the random priming method (24), was used as the probe. Transfer of the RNA to a nylon membrane (GeneScreen Plus; Du Pont-New England Nuclear) and hybridization with the radiolabeled probe were performed under the conditions recommended by Du Pont.

Immunoblot Analysis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of COS-1 cell homogenate (protein 50–100 µg/lane) was performed in a 10% polyacrylamide gel, and the proteins were transferred to nitrocellulose and then exposed to the monoclonal antibody 114/2 and subsequently to rabbit anti-mouse IgG. The bound IgG was visualized by autoradiography after incubation with 125I-protein A. Details of the technique and the specificity of monoclonal antibody 114/2 in the recognition of P450IID6 have recently been described (21).

Assay of Bufuralol 1'-Hydroxylation

Bufuralol 1'-hydroxylation assays were done as described for microsomal fractions (4) in the presence of NADPH and O2. COS-1 cells were harvested in phosphate-buffered saline, the suspension centrifuged at 1000 g for 3 min, the pellet resuspended in sodium phosphate buffer, pH 7.4, sonicated three times for 10 s at 4 °C, and the sonate performed with 350 µg of protein. Substrate concentration of (+)-bufuralol was 500 µM.

RESULTS

Cloning and Sequencing of Mutant CYP2D6 Genes

Two positive clones were isolated from the genomic libraries of each of the three PM individuals (Fig. 2). Four of these six clones were fully sequenced in all exons and intron-exon junctions. The remaining two clones were only partially sequenced as detailed below.

PM1 and PM2—One clone of the two isolated from each PM library was first sequenced in all exons and intron-exon junctions. It became clear that these two clones had identical mutations as well as an additional BamHI restriction site when compared with the wild-type CYP2D6 gene (Figs. 2 and 3). They were designated 29-B. The second clone from each PM was sequenced only in two areas where mutations had been identified, namely the 3'-intron-exon junction of the 3rd intron and exon 2. The same mutations were again detected as well as the additional BamHI site present in both alleles in PM1 and PM2, already evident in the genomic Southern blot analysis. It is of course unknown whether or not the two clones are derived from the same allele.

The mutations of the 29-B allele are summarized in Fig. 3. They include two silent mutations (1080 C to G, 1749 G to C), four amino acid changes (188 C to T resulting in 34 Pro to Ser, 1062 C to A resulting in 91 Leu to Met, 1072 A to G resulting in 94 His to Arg, 4288 G to C resulting in 486 Ser to Thr), and one nucleotide change (1934 G to A) at the 3' end of the 3rd intron. The G to A change at the acceptor site consensus sequence presumably results in incorrect splicing, but the 29-B allele contains multiple additional mutations and may have more in the unsequenced introns. The sequence positions correspond to the recently published CYP2D6 sequence (8).

PM3—With the knowledge of the additional BamHI site in the clones from PM1 and PM2, PM3 was selected to be studied because the poor metabolizer individual in his genomic Southern blot was heterozygous for this BamHI site. Both clones from PM3 were fully sequenced. One allele was identical to the mutant allele 29-B, except for the silent mutation (1749 G to C) in exon 3 and it therefore was designated 29-B'. The other allele, which had no additional BamHI site, had one nucleotide deletion (2637 A) in the 5th exon resulting in a frame shift. This allele was designated 29-A (Figs. 2 and 3).

Expression of the CYP2D6 Wild-Type Gene, the Mutated 29-B Allele, and Chimeras of Both Genes

To evaluate the functional consequences of each mutation and of unsequenced introns, we constructed full length gene expression clones of CYP2D6 wild-type and the mutated 29-B allele (Fig. 2). No difference was found in the 5'-flanking
sequences (bp −77 to start codon) of the 29-B and CYP2D6 genes and no additional translation start signal (ATG) was present in this area. After construction of these clones, we divided the two genes into three parts and exchanged these parts in order to construct the four chimeric genes shown in Fig. 4A. The middle fragment of 29-B was completely sequenced and a mutation (2185 A to G) was detected in intron 4 in addition to the two previously identified mutations in this segment, namely the one silent mutation in the 3rd exon and the G to A mutation of the last nucleotide of the 3rd intron (Fig. 3).

**Fig. 4.** Expression of chimeric gene constructs from wild-type CYP2D6 and its mutant 29-B allele in COS-1 cells. A, description of the three parts of the wild-type (W) gene which were exchanged with the corresponding parts of the 29-B allele (B) with mutations. ●, the mutations causing amino acid changes in exons 1, 2, and 5; ○, the mutation in the splice site consensus sequence of the 3rd intron. B, Northern blot (mRNA), Western blot (Protein), and bufuralol 1'-hydroxylation of COS-1 cell extracts 66 h after transfection with the DNA constructs 1–4, the intact wild-type (CYP2D6), and the mutant (29-B) gene. Control, mock-transfected cells.

**Chimeric Genes 1 and 2.—**Because of the suspected importance of the mutation in the splice-site consensus sequence at the 3' end of the 3rd intron, we first constructed chimeric genes which would allow us to test the consequences of this mutation. The chimeric gene 1 includes the middle part of the mutated 29-B allele, and the 5' and 3' part of the wild-type gene (Fig. 4A). In construct 2, on the other hand, the middle part was derived from the wild-type gene, and the 5' and 3' part from the mutated 29-B allele. On expression, chimeric gene 1 resulted in no recognizable protein and no enzymatic activity could be demonstrated in transfected COS-1 cells. Interestingly, mRNA was recognized by the IID6 cDNA in Northern blots and had the same apparent size for both constructs.

**Chimeric Genes 3 and 4.—**To evaluate the effect of the amino acid changes in the 5' and 3' part of the 29-B allele, we constructed clones 3 and 4 which include the 5' and 3' parts of the 29-B gene, each combined with the other two parts of the wild-type gene. Clone 3 produced an immunoreactive protein, but no activity, as did chimeric gene 2 described above. Therefore the three amino acid changes in the 5' part of the gene together or alone are capable of destroying the function of this protein. However, chimeric gene 4 conferred on expression the same or even higher activity as the wild-type gene. This indicates that the amino acid change (486 Ser to Thr) caused by the mutation in the 9th exon is not important for expression or activity. Western blot analysis of the products of clones 2, 3, and 4 revealed several additional shorter bands, some of which were also seen in the products of the wild-type gene (lane 2).

**Mutations in Exons 1 and 2—**The three mutations causing amino acid changes in exons 1 and 2, which were expressed in combination in chimeric gene 3, were reproduced separately by site-specific mutation of the IID6-cDNA and expressed in COS-1 cells (Fig. 5). All three mutated cDNAs resulted in immunoreactive protein, but only the mutation in exon 1 (188 C to T, 34 Pro to Ser) abolished the activity of the expressed protein, the activity being as low as in mock-transfected control cells.

The expression experiment described in Fig. 4 was performed at least 3 times (chimeras 3 and 4) and up to 6 times (chimeras 1 and 2). The same results in regard to relative activities and proteins on Western blots were observed. Two experiments with the cDNAs in which the mutations of exon 1 and 2 were introduced (Fig. 5) also revealed reproducibility of the reported findings.

**DISCUSSION**

In the present report we describe multiple mutations of the human CYP2D6 gene in two types of mutant alleles isolated from PMs of debrisoquine. A causal relationship between one of these mutations, a point mutation in the splice site consensus sequence, and the previously shown absence of cytochrome
P450IID6 in livers of PMs (4, 5) is strongly suggested. This was possible by functional expression in cell culture of chimeric genes in which parts of the mutant gene were combined with parts of the wild-type gene. Our studies provide an explanation at the DNA level for the previously postulated mechanism of aberrant splicing of P450IID6 premRNA (5) and define two additional mutant alleles of the CYP2D6 gene associated with the PM phenotype.

The two types of mutant CYP2D6 alleles described here, designated 29-A and 29-B, were isolated from genomic DNA libraries of three PM individuals. They appear to occur at different frequencies. The 29-A allele, which contains a single frameshift mutation in exon 5 (Fig. 3), was identified as one of two alleles in only one individual, whereas the 29-B allele was found in all three individuals. These data suggest that the 29-B allele may be a common cause of deficient metabolism of debrisoquine, because ~75% of PM individuals have at least one 29-kb fragment of XbaI Southern blots (9). Obviously, studies in a larger number of phenotyped individuals are necessary to evaluate this point.

The two previously identified mutant alleles characterized by XbaI 44- and 11.5-kb fragments occurred with allele frequencies of 0.31 and 0.14, respectively, in PM individuals (9). The mutations in these alleles which lead to the PM phenotype are not known yet. The two new alleles described here bring the total number of mutant alleles to at least four. We have accumulated preliminary evidence that these four alleles account for the great majority of variant CYP2D6 genes associated with poor metabolism of debrisoquine. Moreover, this study provides first insights into the molecular mechanism at the DNA level of the debrisoquine polymorphism.

The single mutation in exon 5 of the mutant allele 29-A, the deletion of one nucleotide, causes a reading frame disruption and therefore, if present in the homozygous state, readily would explain the absence of IID6 protein and function in poor metabolizers by premature termination of protein synthesis.

The allele 29-B contained four mutations causing amino acid changes. Only the mutations in the middle part of the mutant 29-B allele (chimera 1) on expression resulted in a total absence of the IID6 protein and consequently its function, as measured by virtually absent bufuralol 1'-hydroxylation. A total of three mutations were identified in this segment, namely the 1749 G to C in exon 3, the additional 2185 A to G in intron 4 detected when sequencing the whole intron, and the 1934 G to A at the 3' end of intron 3. We suspect that the G to A change at the last nucleotide of intron 3 found in all 29-B alleles is the dominant cause for absent protein and function, because the consensus acceptor site sequence AG is conserved to 100% in numerous genes of human and other species examined (25, 26). Point mutagenesis experiments support the concept that the "AG" consensus acceptor site sequence is a prerequisite for a normal splicing mechanism (27). Interestingly, there was no difference in the size of the mRNA in the Northern blots analysis of the COS cells in which this mutation was expressed (Fig. 4B), which would point to retention of an intron or truncation of the mRNA, but it is possible that too small a number of nucleotides was deleted or retained by aberrant splicing to be detected by this technique. In any case, the present observations support our previous proposal that aberrant splicing may be a cause of absent IID6 protein in livers of PMs of debrisoquine (5). In these studies, Northern blot analysis with RNA from livers of PMs suggested the presence of additional RNA bands. The origin of these additional bands was suspected to come from retained introns 5 and 6, because cDNA sequences from some of these PM livers contained these intronic sequences. The present data therefore may suggest that a defect in the splice site consensus sequence for the third intron may lead to retention of other introns, a hypothesis presently under investigation.

The three amino acid changes in the 5' part of the 29-B allele were tested separately by site-specific mutations in regard to their consequences for protein synthesis, stability, or function. These mutations, in combination (chimera 3) led to a total loss of P450IID6 function on expression, although immunoreactive protein of the right molecular weight was formed. The experiments summarized in Fig. 5 reveal that the mutation in exon 1 is predominantly responsible for this change in function. The amino acid changes in exons 2 (Fig. 5) and 9 (chimera 4, Fig. 4B) had no significant effect on either IID6 protein or function. In fact the activity and amount of protein in four experiments with chimera 4 always appeared higher when compared with the expression of the wild-type gene, but the semiquantitative nature of these experiments precludes further interpretation. Thus, these mutations have no apparent significance for the PM trait. Together the above experiments demonstrate that the 29-B allele has at least two mutations (the G to A splice site mutation in intron 3 and the C to T point mutation in exon 1), either of which can abolish the function of P450IID6.

Several additional protein bands of faster mobility and presumably smaller size were observed on expression of the chimeric genes, but the same bands were also present in COS cells in which the normal wild-type CYP2D6 was expressed (Fig. 4). Expression of the full length IID6 cDNA in COS-1 cells under the same conditions always produced only a single protein band recognized by the same monoclonal antibody as the one used in the present study (Fig. 5). These additional bands also were not observed in livers of PMs so far. Thus, the expression of the entire gene and of the cDNA produces different results at least in the COS-1 cell system used. The additional bands could reflect lability of the protein or alternate splicing mechanisms and have not yet been investigated further.

In trying to explain the highly polymorphic nature of the CYP2D locus noted on restriction analysis of genomic DNA in a larger population (9) and further documented here, one observation of the present study is of particular interest. With exception of the mutation in the splice site and in exon 1, the 29-B allele contains the same mutations and the additional BamHI restriction site as the recently reported CYP2D7 gene, which is located 5' of CYP2D6 and suspected to represent a gene duplication of CYP2D6, having 97% amino acid similarity to CYP2D6 (8). It is not yet entirely clear whether CYP2D7 is transcribed into a mRNA and produces a protein as it has only a single reading frame disrupting insertion in its 1st exon (8). The shared mutations between CYP2D7 and mutated CYP2D6 genes, as reflected by the 29-B allele, may be a consequence of homologous recombination or gene conversion events. Gene conversion between CYP2D7 and a pseudogene CYP2D8, which is located 5' of CYP2D6 and suspected to represent a gene duplication of CYP2D6, having 97% amino acid similarity to CYP2D6 (8). It is not yet entirely clear whether CYP2D7 is transcribed into a mRNA and produces a protein as it has only a single reading frame disrupting insertion in its 1st exon (8). The shared mutations between CYP2D7 and mutated CYP2D6 genes, as reflected by the 29-B allele, may be a consequence of homologous recombination or gene conversion events. Gene conversion between CYP2D7 and a pseudogene CYP2D8, which is located 5' of CYP2D6 and suspected to represent a gene duplication of CYP2D6, having 97% amino acid similarity to CYP2D6 (8).
Debrisoquine Polymorphism

In the course of the accumulation of mutations, splicing errors presumably will appear sooner or later. Alternative splicing mechanisms or aberrant splicing has been reported for other cytochrome P-450 enzymes (28, 29). It is conceivable that more splicing errors will be found in drug metabolizing P-450 genes, because they are not under selective pressure. Even normally functioning genes of this group of enzymes may have fragile splicing mechanisms and structures. We believe that the polymorphic CYP2D6 gene might be a gene predisposed to extinction. Both the mechanisms of gene conversion and of aberrant splicing seem to play an important role in these events. Many inactivated or P-450 pseudogenes will probably be found in the human genome.

On the other hand, these polymorphic genes play an important role as causes of interindividual variation in drug metabolism and in the occurrence of side effects and therapeutic failures. Moreover, they serve as genetic markers for numerous diseases. The elucidation of these mutations therefore has clinical importance and the definition of the mutations of the IID6 gene will allow the development of simple tests for the detection of the respective genotype.

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