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Genetics

Inherited amplification of an active gene in the cytochrome P450 *CYP2D* locus as a cause of ultrarapid metabolism of debrisoquine

(gene amplification/sparteine/bufuralol/pharmacogenetics/drug therapy)

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ABSTRACT **Deficient hydroxylation of debrisoquine is an autosomal recessive trait that affects ≈7% of the Caucasian population. These individuals (poor metabolizers) carry deficient *CYP2D6* gene variants and have an impaired metabolism of several commonly used drugs. The opposite phenomenon also exists, and certain individuals metabolize the drugs very rapidly, resulting in subtherapeutic plasma concentrations at normal doses. In the present study, we have investigated the molecular genetic basis for ultrarapid metabolism of debrisoquine. Restriction fragment length polymorphism analysis of the *CYP2D* locus in two families with very rapid metabolism of debrisoquine [metabolic ratio (MR) for debrisoquine = 0.01–0.1] revealed the variant *CYP2D6* gene *CYP2D6L*. *Eco*RI RFLP and *Xba* I pulsed-field gel electrophoresis analyses showed that this gene had been amplified 12-fold in three members (father and his two children) of one of the families, and two copies were present among members of the other family. The *CYP2D6L* gene had an open reading frame and carried two mutations causing amino acid substitutions: one in exon 6, yielding an Arg-296 → Cys exchange and one in exon 9 causing Ser-486 → Thr. The MR of subjects carrying one copy of the *CYP2D6L* gene did not significantly differ from that of those with the wild-type gene, indicating that the structural alterations were not of importance for the catalytic properties of the gene product. Examination of the MR among subjects carrying wild-type *CYP2D6*, *CYP2D6L*, or deficient alleles revealed a relationship between the number of active genes and MR. The data show the principle of inherited amplification of an active gene. Furthermore, the finding of a specific haplotype with two or more active *CYP2D6* genes allows genotyping for ultrarapid drug metabolizers. This genotyping could be of predictive value for individualized and more efficient drug therapy.**

Gene amplification was first described in 1978 as a mechanism of acquired resistance in cultured mammalian cells (1, 2). Since then, most examples of gene amplification have been related to cancer biology, in particular to oncogene amplification. The extent of oncogene amplification appears to be highest in the most malignant tumors (3, 4). Gene amplification is usually responsible for the high frequency of resistance to certain drugs commonly observed upon *in vitro* selection in many mammalian cell lines (5). However, DNA amplification is rare in normal human cells (6), and thus only a limited number of examples of DNA amplification in such cells have been reported. Srivastava *et al.* (7) found that the Ha-*ras* gene is amplified 4-fold in cultured normal human fibroblasts during quiescence and concluded that a succession of clones with a high *ras* copy number occurred during serial passage. In addition, Turner *et al.* (8) showed that inactivation of the hypoxanthine guanine phosphoryribosyltransferase gene in normal human lymphocytes, selected with 6-thioguanine, was accompanied by amplification.

Inherited gene amplification has apparently been described only once: Prody *et al.* (9) found that parts of a particular "silent" choline esterase gene were highly amplified in a farmer and his son, who were members of a family exposed to organophosphorous insecticides for several generations. The initial amplification event probably occurred and was selected for during spermatogenesis or oogenesis and was then inherited.

Cytochrome P450 enzymes are involved in the oxidative metabolism of both endogenous and exogenous molecules. Among the physiological substrates are steroids, fatty acids, prostaglandins, leukotrienes, and biogenic amines. Exogenous compounds include plant toxins, drugs, and carcinogens. In mammals, >100 individual genes belonging to 12 different gene families have been identified. During evolution particular P450s in a given species may have been required for survival and therefore selected (cf. ref. 10). Mutant alleles of some P450 genes appear to have been inbred in some populations, constituting the basis for marked inherited variation and interethnic differences in drug metabolism (10–12).

Genetic deficiency of cytochrome P4502D6 (CYP2D6), which is inherited as an autosomal recessive trait, causes the debrisoquine/sparteine polymorphism. Approximately 7% of the Caucasian population are poor metabolizers—i.e., they are deficient in the metabolism of the antihypertensive probe drug debrisoquine and about 25 other commonly prescribed drugs such as neuroleptics, antidepressants, and lipophilic β-adrenergic blockers (11), whereas the remaining are classified as extensive metabolizers. The *CYP2D6* gene is part of a cluster on chromosome 22, which includes two to three related pseudogenes (13, 14). Four allelic variants of the *CYP2D6* gene have hitherto been identified. The most common defective alleles consist of variant genes with point mutations, having a splicing defect (*CYP2D6B*), a frame-shift mutation (*CYP2D6A*) (15), or a deletion of the entire *CYP2D6* gene (*CYP2D6D*) (16). About 95% of poor metabolizers in the Swedish population could be identified by allele-specific PCR analysis of genomic DNA designed for these variant genes (17).

The rate of CYP2D6-dependent hydroxylase activity can be studied *in vivo* by measuring the ratio between the urinary recovery of debrisoquine and that of 4-hydroxydebrisoquine after a single oral dose of debrisoquine. This ratio (debrisoquine metabolic ratio, MR) varies in the population from ≈0.01 to >100, and the extensive metabolizer phenotype is defined as people having MR < 12.6 (11, 17, 18). A small percentage of the Swedish population is extremely rapid metabolizers of debrisoquine with MRs of <0.2 (18). Case

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Abbreviations: MR, metabolic ratio; RFLP, restriction fragment length pattern; wt, wild type.

reports strongly suggest that such individuals may require very high doses of drugs metabolized by CYP2D6 for a therapeutic effect (19, 20). We have investigated the molecular genetic basis for this rapid drug metabolism. In the current report we present the occurrence of a 12-fold-amplified active variant of the *CYP2D6* gene in a family with MRs ≤ 0.02. The amplified variant *CYP2D6* gene appears to be present in two copies in another family with very low MRs for debrisoquine.

MATERIALS AND METHODS

Subjects for Genetic Analysis. DNA samples used in the present investigation were obtained from participants of a previous study of the polymorphic hydroxylation of debrisoquine and desmethylimipramine (17). Data regarding their phenotype for hydroxylation of both drugs as well as their *CYP2D6* genotype with respect to *Xba* I restriction fragment length polymorphism (RFLP) and occurrence of the defective *CYP2D6A* and *CYP2D6B* alleles have been reported (17).

Genomic RFLP Analysis. DNA was isolated from peripheral leukocytes by a guanidinium isothiocyanate method. After digestion with *Xba* I or *Eco*RI, agarose electrophoresis was done (0.55% or 0.85% agarose) at 0.5 V/cm for 10 days or 1 V/cm for 48 hr. Southern blotting was done by the use of GeneScreen*Plus* filters (DuPont) and hybridization with [α-^{32}P]dCTP-labeled CYP2D6 cDNA, provided by U. A. Meyer (Biozentrum, Basel, Switzerland) and F. J. Gonzalez (National Cancer Institute, National Institutes of Health, Bethesda, MD). Autoradiograms were analyzed with a laser densitometer, Personal Densitometer with ImageQuant version 3.2 software (Molecular Dynamics).

Pulsed Field Gel Electrophoresis. Very high-molecular-weight DNA was isolated according to the method described by Sambrook *et al.* (21). After digestion with *Xba* I, the DNA fragments were separated on 1.2% agarose in 0.5× TBE (Tris/borate/EDTA) buffer using a Gene Navigator system (Pharmacia LKB). The gel was run for 10 hr at 300 V, and the pulse time was 10 sec. λ DNA concatemers were used as markers.

Isolation of the *CYP2D* Genes. Leukocyte DNA was completely digested with *Eco*RI and ligated to λEMBL4 arms for construction of a gene library. The DNA was packaged using Gigapack II Gold (Stratagene). The DNA library was screened with ^{32}P-labeled CYP2D6 cDNA. Phages from positive clones were analyzed for *CYP2D6* allele-specific PCR amplification (22) and restriction maps after digestion with *Eco*RI, *Eco*RI plus *Bam*HI and *Eco*RI plus *Kpn* I, respectively.

Sequencing Analysis. Phage DNA was isolated from clones corresponding to the *CYP2D* genes and was digested with *Eco*RI and *Kpn* I. The 5.8-kb fragments, containing all 9 exons and 1531 bp of the upstream region, were subcloned into the pUC19 vector (Pharmacia). The *Eco*RI–*Kpn* I fragments were partly sequenced by the double-stranded dideoxynucleotide chain-termination method using Sequenase version 2.0 T7 DNA polymerase (United States Biochemical). Synthesized oligonucleotides (17-mers), universal primers, and reverse primers were used.

PCR-Based Allele-Specific Analysis. *CYP2D6A* and *CYP2D6B* were analyzed as described (17, 22). Mutations in both exon 1 ($C^{188} \rightarrow T$) and exon 9 ($G^{4268} \rightarrow C$), which cause amino acid substitutions and are present in active *CYP2D6* alleles among the Chinese population, were determined by allele-specific PCR amplification and subsequent analysis of mutations using primers described elsewhere (I.J., Q. Y. Yue, L.B., F.S., M.I.-S., unpublished work). In addition, an alternative primer 10B, 5′-GTGGTGGGGCATCCTCAGG-3′, was used for amplification of allelic CYP2D6 DNA containing the sequence between bp 302 and 333 in intron 1, which is identical to the pseudogene *CYP2D7P* (cf. ref. 23). A mutation ($C^{2938} \rightarrow T$) in exon 6 was analyzed by allele-specific amplification of exons 5 and 6 (22), followed by specific amplification using primer 19, 5′-GCCACCACTATGCG-3′ for the wt gene and primer 20, 5′-GCCACCACTATGCA-3′ for the mutant variant.

RESULTS

Analysis of the *CYP2D* Locus from Ultrarapid Metabolizers of Debrisoquine. Among the participants of a previous study of the polymorphic metabolism of desmethylimipramine (17), we identified five subjects belonging to two different families (I and II), with MRs for debrisoquine of <0.1 (Table 1). Genomic DNA from family I was subjected to *Xba* I RFLP. The two sons were heterozygous with a *Xba* I 42/29-kb genotype, whereas the mother had one 42-kb and one 11.5-kb haplotype (the latter not visible in Fig. 1*A*). A 11.5-kb fragment indicates deletion of the active *CYP2D6* gene (*CYP2D6D*) (16), a 29-kb fragment indicates the presence of the normal locus with two pseudogenes (*CYP2D8P* and *CYP2D7P*) and one copy of the *CYP2D6* gene (13), whereas a 42-kb fragment suggests a gene insertion. *Eco*RI RFLP of genomic DNA from family I revealed the presence of four DNA fragments of 15.1-, 12.1-, 9.4-, and 8.8-kb size (Fig. 1*B*), indicative of the presence of four genes in the locus. The 15.1- and 8.8-kb fragments correspond to the pseudogenes *CYP2D7P* and *CYP2D8P*, respectively, whereas the *Eco*RI 9.4-kb fragment corresponds to the *CYP2D6* gene (23). It can be concluded that the 9.4-kb fragment does not represent either the *CYP2D6A* or *CYP2D6B* defective variants, because PCR analysis of genomic DNA was negative for these mutations (Table 1). The 12.1-kb

Table 1. *CYP2D6* genotype and phenotype of families I and II

	Debrisoquine MR	Allele-specific PCR analysis*	*Xba* I fragments, kb	*Eco*RI fragments, kb
Family I				
Father	0.52	wt/*L*	29/29	15.1, 9.4, 8.8
Mother	0.12	*L*/−	42/11.5	15.1, 13.0, 12.1, 9.4, 8.8
Son 1	0.07	*L*/wt	42/29	15.1, 12.1, 9.4, 8.8
Son 2	0.05	*L*/wt	42/29	15.1, 12.1, 9.4, 8.8
Family II				
Father	0.02	*L*/*B*	175/44	15.1, 13.7, 12.1, 9.4, 8.8
Mother	102	*B*/*B*	29/29	15.1, 9.4, 8.8
Daughter	0.02	*L*/*B*	175/29	15.1, 12.1, 9.4, 8.8
Son	0.01	*L*/*B*	175/29	15.1, 12.1, 9.4, 8.8

Phenotype data are from Dahl *et al.* (17).

*PCR analysis was designed for analysis of wt *CYP2D6*, the active variant *CYP2D6L*, and the defective alleles *CYP2D6A* (*A*) and *CYP2D6B* (*B*) (compare with Table 2).

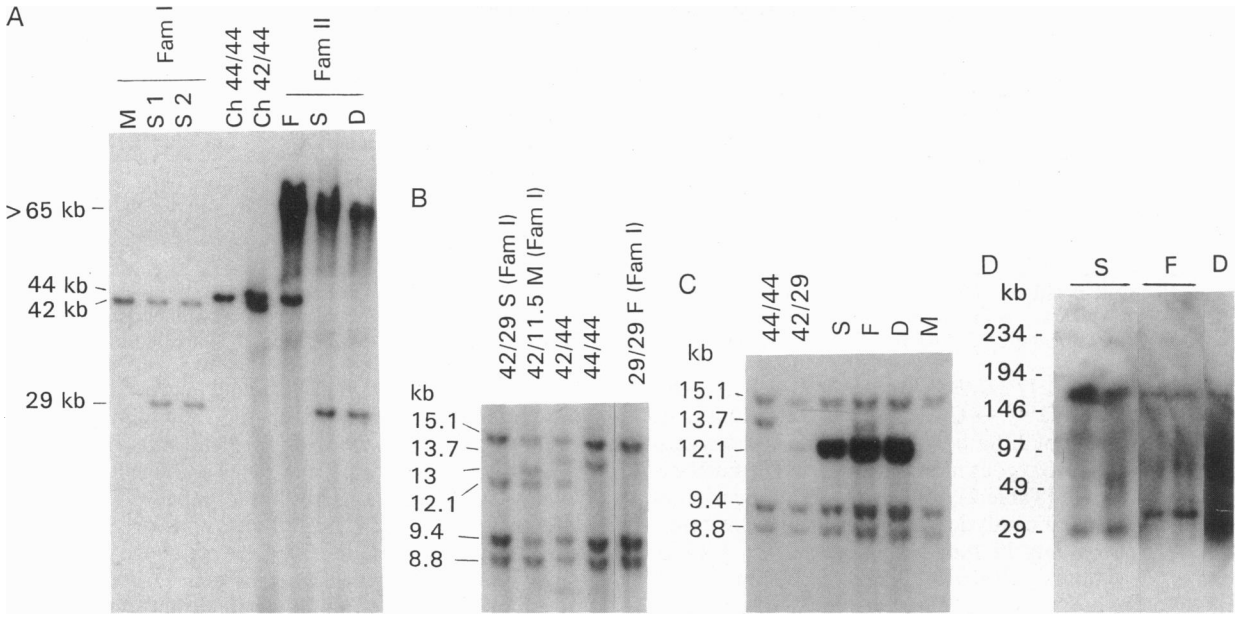

FIG. 1. *Xba* I and *Eco*RI RFLP analysis of the *CYP2D* locus of genomic DNA from families I and II. (*A*) *Xba* I RFLP of genomic DNA from family (Fam) I and family II in comparison with Chinese subjects carrying the *Xba* I 42- and 44-kb haplotypes. The mother (M) of family I is of the *Xba* I 42/11.5-kb haplotype. Under the conditions used, the 11.5-kb fragment is not visible on the gel. The allelic *Xba* I fragments from family II, father (F), son (S), and daughter (D) are too long for accurate size determination because of fragmentation. (*B*) *Eco*RI RFLP of family I, son (S), mother (M), and father (F) in comparison with samples obtained from subjects carrying the *Xba* I 42- and 44-kb haplotypes. The *Eco*RI fragments described (23) correspond to *CYP2D7AP* (15.1 kb), *CYP2D7BP* (13.7 kb), wt *CYP2D6* or *CYP2D6B* (9.4 kb), and *CYP2D8P* (8.8 kb). The 13-kb fragment originates from the *Xba* I 11.5 allele, lacking the entire *CYP2D6* gene, whereas the 12.1-kb fragment originates from the *CYP2D6L* gene (see text). (*C*) *Eco*RI RFLP of DNA from family II compared with DNA from subjects with *Xba* I 44-, 42-, and 29-kb haplotypes. The mother, homozygous for the *CYP2D6B* allele, lacks the 13.7- and 12.1-kb fragments. The 12.1-kb fragment, corresponding to the *CYP2D6L* gene, is present at an intensity similar to that of the pseudogenes in the *Xba* I 42/29 individual but is much stronger in the samples from the son (S), father (F), and daughter (D) of family II. The 13.7-kb band from the father corresponds to the pseudogene *CYP2D7BP*. (*D*) Pulsed-field gel electrophoresis of genomic DNA from the son (S), daughter (D), and father (F) of family II after digestion with *Xba* I. As evident, fragments of 175, 29, and 44 kb, respectively, could be identified. The 175-kb fragment corresponds to 12 copies of the *CYP2D6L* gene, one additional *CYP2D6* gene, probably of the *CYP2D6L* type, and the *CYP2D7P* gene present between the *Xba* I restriction sites (compare text and ref. 23). The DNA from the daughter, prepared by the guanidinium isothiocyanate method, has been partially degraded.

fragment represents a variant of the *CYP2D6* gene not described earlier and was further characterized (see below).

Among members of family II, the father, son, and daughter exhibited urinary debrisoquine/4-OH-debrisoquine ratios of ≤0.02 (Table 1) (17). *Xba* I RFLP analysis (Fig. 1*A*) showed the presence of DNA fragments with lengths of >65 kb from all three individuals. In addition, *Xba* I fragments of 44-kb (father) and 29-kb length (son and daughter) were seen. Allele-specific PCR analysis revealed that all three were heterozygous with one defective allele each (*CYP2D6B*) (Table 1). *Eco*RI RFLP analysis indicated the presence of the 12.1-kb fragment in all three individuals (Fig. 1*C*) but with a very high intensity compared with the fragments corresponding to the *CYP2D6*, *CYP2D7P*, and *CYP2D8P* genes. To analyze the number of gene copies represented by the 12.1-kb fragment, *Eco*RI-digested DNA samples (0.25–5 μg) were subjected to quantitative Southern blot analysis, using the *CYP2D6* band (9.4 kb) as internal standard. The number of gene copies thus determined was ≈12 (father, 14; son, 11; and daughter, 12).

The length of the *Xba* I fragment found from these individuals was further determined by pulsed field gel electrophoresis of high-molecular-weight DNA. Fig. 1*D* shows that the length was ≈175 kb in the genomic DNA obtained from both the father and his two children. This length corresponds to 12 extra copies of the *CYP2D* gene, one normal *CYP2D6* gene, and the pseudogene *CYP2D7P*.

Characterization and Sequence Analysis of the Amplified Gene. The *CYP2D* gene within the 12.1-kb *Eco*RI fragment was characterized. A genomic library was constructed from the son of family II. The phage DNA from 10 *CYP2D6*-positive clones was digested with *Eco*RI, and the length of the inserted DNA was determined. All isolated clones exhibited the 12.1-kb DNA insert and, thus, represented the amplified gene and, in addition, were positive for *CYP2D6*, as determined by *CYP2D6*-specific PCR amplification (cf. ref. 22). The 12.1-kb fragment was digested with *Eco*RI plus *Bam*HI, and the length of the fragments that hybridized to CYP2D6 cDNA were 3.2, 2.2, and 1.9 kb. Similar analysis with *Eco*RI plus *Kpn* I yielded a 5.8-kb fragment. This fragmentation pattern is inconsistent with those of the pseudogenes *CYP2D7P*, *CYP2D7AP*, *CYP2D7BP*, and *CYP2D8P* but is identical to that described for *CYP2D6B* (23) and the active Chinese variant $CYP2D6Ch_1$ (I.J., Q. Y. Yue, L.B., F.S., M.I.-S., unpublished work), which both contain an extra *Bam*HI cleavage site in intron 1.

The nine exons of the amplified *CYP2D* gene were sequenced. In comparison to wt *CYP2D6*, three mutations were found (Table 2). Two caused amino acid substitutions: one in exon 6, Arg-296 → Cys, and one in exon 9, Ser-486 → Thr. The gene was termed *CYP2D6L*. The introns were only partially sequenced. However, in intron 1, the sequence between G^{302} and A^{333} was identical to that of the pseudogene *CYP2D7P*, indicating the occurrence of a gene conversion event.

Distribution of the *CYP2D6L* Gene Among Swedes. The distribution of the *CYP2D6L* allele among efficient metabolizers homozygous for the *Xba* I 29-kb haplotype, lacking the *-A* and *-B* mutations ($n = 71$) (17) was determined by analysis of (*i*) the C^{188} → T (Pro-34 → Ser) mutation found among Chinese alleles (I.J., Q. Y. Yue, L.B., F.S., M.I.-S., unpublished work), (*ii*) the gene conversion of the fragment G^{302}–

Table 2. Relationship between debrisoquine MR and number of wt *CYP2D6* or *CYP2D6L* alleles among efficient metabolizers homozygous for the *Xba* I 29-kb haplotype

Allele		Mutation				
Type	Frequency,* %	Exon no.	Base substitution	Amino acid exchange	Genotype	Debrisoquine MR, median (*n*)
wt	55.6				wt/wt	0.36 (21)
L	34.7	3	$G^{1726} \rightarrow C$	None	*L*/wt	0.43 (34)
		6	$C^{2938} \rightarrow T$	Arg-296 → Cys	*L*/*L*	0.42 (5)
		9	$G^{4268} \rightarrow C$	Ser-486 → Thr		

The subjects have been previously phenotyped (17).
*Allele frequency is based on results obtained from unrelated individuals only (n = 36) and includes a comparison with subjects carrying *CYP2D6J* and *CYP2D6Ch* alleles (I.J., Q. Y. Yue, L.B., F.S., and M.I.-S., unpublished work).

A^{333} in intron 1 from *CYP2D7P*, (*iii*) the $C^{2938} \rightarrow T$ (Arg-296 → Cys), and (*iv*) the $G^{4268} \rightarrow C$ (Ser-486 → Thr) mutations. The median MR did not differ between subjects homozygous for the wt allele, heterozygous individuals (*L*/wt) or homozygous for the -*L* allele (Table 2), indicating the expression of an enzyme with similar catalytic properties in all three cases.

Allelic *CYP2D6* Genes in Individuals of the *Xba* I 42-kb Haplotype. A genomic λEMBL4 library was constructed from the mother of family I (as described for family II). Ten CYP2D6 cDNA-positive plaques were analyzed by *CYP2D6*-specific PCR amplification. Seven were positive for *CYP2D6*, and phage DNAs were isolated. All yielded a 12.1-kb fragment after digestion with *Eco*RI. Restriction cleavage with *Bam*HI yielded fragments of 3.2, 2.2, and 1.9 kb. The clones were positive for both the $C^{2938} \rightarrow T$ and $G^{4268} \rightarrow C$ mutations but were negative for the $C^{188} \rightarrow T$ mutation. This result indicates their identity with the *CYP2D6L* gene amplified in family II.

The genomic DNA from the mother of family I, whose *Xba* I 11.5/42-kb haplotype indicates a *CYP2D6* deletion in one allele, was positive for both the $C^{2938} \rightarrow T$ and $G^{4268} \rightarrow C$ mutations but were negative for the -*A*, -*B*, and $C^{188} \rightarrow T$ mutations. The data thus suggest that the locus is composed of *CYP2D8P* (*Eco*RI 8.8 kb), *CYP2D7P* (*Eco*RI 15.1 kb), and two *CYP2D6L* genes yielding *Eco*RI fragments of 9.4 kb (*CYP2D6L*$_1$) and 12.1 kb (*CYP2D6L*$_2$) (see Figs. 1*B* and 2). The presence of two active *CYP2D6L* genes is also supported by the mother's low MR for debrisoquine.

Debrisoquine MR Among Subjects with Different Numbers of Active *CYP2D6* Genes. No difference in MR was found between individuals with the *Xba* I 29-kb haplotype carrying either the wt *CYP2D6* or the *CYP2D6L* allele (compare with above). This result indicates that the low MR among the members of families I and II is mainly caused by the presence of several active genes expressed individually at a similar level. Accordingly, we examined the relationship between MR and the number of active genes among all genotyped and phenotyped Swedish individuals (compare ref. 17) having the defective alleles, wt *CYP2D6* and/or *CYP2D6L* alleles. As shown in Fig. 3, a relationship between these parameters was observed for up to three active genes. For the amplified allele, the MR was 4-fold lower than that of the alleles with three genes, which is smaller than the proportional difference in the number of genes. This difference is probably the result of lack of substrate saturation of the enzyme because the amount of substrate remaining is insignificant (1/100th of the product concentration). Another factor to consider is the difficulty of accurately determining MR values ≤0.01, as the debrisoquine concentration in such samples is extremely low.

DISCUSSION

Our results demonstrate an inherited 12-fold amplification of the active *CYP2D6L* gene in a family (II), whose members exhibit the lowest described MRs for debrisoquine among 1011 Swedes (18). The mother is a poor metabolizer, MR = 102, with both alleles deficient with respect to functional *CYP2D6* genes. This result indicates a dominant inheritance of the amplified gene from the father to both children. The same type of gene (*CYP2D6L*) appears to be present in two copies in the other family (I), whose members also exhibit very rapid metabolism for debrisoquine. This result might suggest the presence of a sequence in the flanking regions of this particular gene that facilitates duplication and/or amplification. *CYP2D6L* was also found among individuals of the *Xba* I 29-kb haplotype who have only one active gene. Its

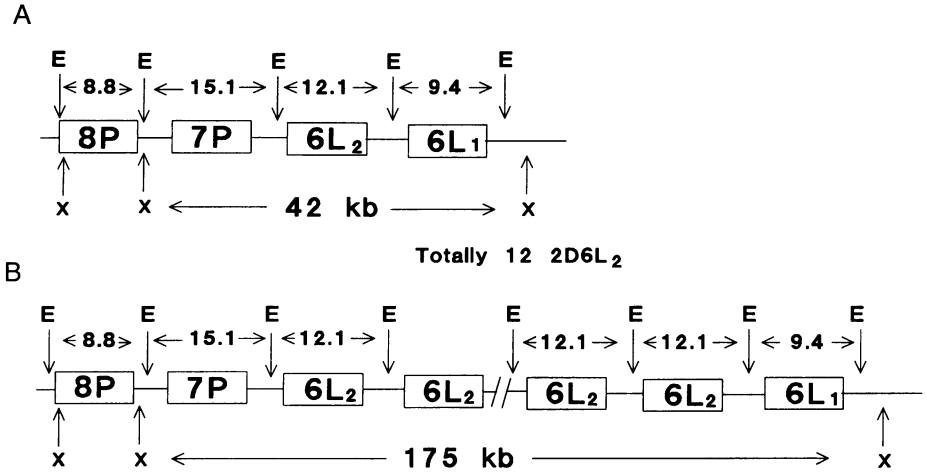

FIG. 2. Schematic illustration of the *CYP2D* locus in individuals of the *Xba* I 42-kb (*A*) and 175-kb (*B*) haplotypes. E and X represent cleavage sites with *Eco*RI and *Xba* I, respectively. Relative positions of the genes have been determined by construction of restriction enzyme maps (E.L., unpublished results). For further explanations, see text. 8P, *CYP2D8P*; 7P, *CYP2D7P*; 6L$_2$, *CYP2D6L*$_2$; 6L$_1$, *CYP2D6L*$_1$.

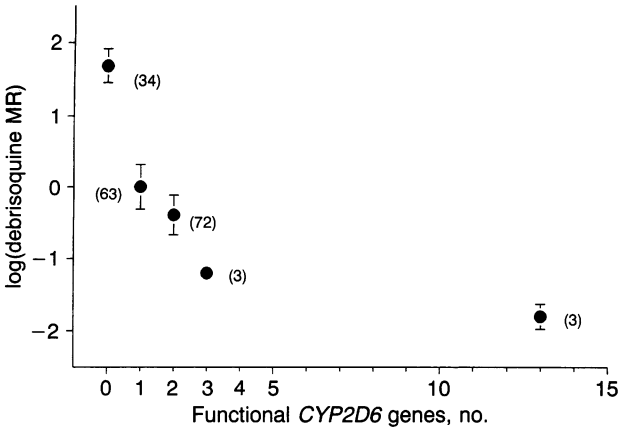

FIG. 3. Relationship between number of active *CYP2D6* genes and MR for debrisoquine. All phenotyped subjects (17) carrying the wt *CYP2D6*, *CYP2D6L*, *CYP2D6A*, *CYP2D6B*, or *CYP2D6D* alleles or combinations thereof were considered. Number of individuals is indicated within parentheses. The SD of the debrisoquine MR for subjects with three genes is too small for visualization.

presence did not influence the MR, indicating the expression of an enzyme with similar catalytic activity in both cases. The correlation between the number of active wt *CYP2D6* or *CYP2D6L* genes and the MR indicates that the cause of increased metabolic capacity among individuals carrying multiple gene copies is restricted to the amount of enzyme expressed.

The inherited amplification of the *CYP2D6L* gene described here is, in many aspects, different from the previously reported case, in which a 100-fold amplification of an acetylcholinesterase gene coding for serum butyrylcholinesterase was shown (9): (*i*) In the present case, the entire gene had apparently been amplified, as indicated by the same size of the *CYP2D6*-hybridizing *Eco*RI fragment (12.1 kb) obtained from both family I and family II (Fig. 1). For the butyrylcholinesterase gene, the central part of the gene was amplified more extensively than the external regions. This finding is consistent with the "onion skin" model described for several amplification units, serving as an early intermediate in the amplification process (3, 24). Thus, different mechanisms of amplification appear to operate in each of the two cases. (*ii*) Furthermore, on an enzymatic level, the subjects carrying the amplified butyrylcholinesterase gene did not exhibit higher serum butyrylcholinesterase activities, indicating that the amplified gene was not expressed. For *CYP2D6L*, it is apparent that the gene is functional on the basis of structural criteria and the consequences for debrisoquine metabolism *in vivo* (compare with above). (*iii*) At the probable time of butyrylcholinesterase amplification, the grandparents were exposed to high levels of parathion, which could have triggered gene amplification. Alternatively, the ability for gene amplification was inherited. In our case, the cause for amplification is unknown. The possible trigger for gene amplification might have been a defective gene in a particular oocyte or sperm cell, and amplification of the *CYP2D6* gene might have compensated for the physiological function exerted by the defective gene. Alternatively, the *CYP2D6* gene may not have any important physiological function, and therefore no pressure has been placed on removal of the extra genes.

The subjects with two or more copies of the *CYP2D6L* gene may not reach therapeutic plasma concentrations of drugs metabolized by CYP2D6 at normally prescribed doses. We have previously described two cases in which doses of nortriptyline and clomipramine 3- to 4-fold higher than normal were required to achieve therapeutic concentrations (19, 20). The concentration of the main active metabolite of nortriptyline was 3-fold higher than normal in one of these subjects (19), implicating an essential role of the *CYP2D6* genotype for the activation of certain drugs. We have recently found that these two individuals exhibit the *Xba* I 42-kb haplotype (20), which according to our present findings is indicative of two active *CYP2D6L* genes. In the case of the *CYP2D6L* gene being amplified 12-fold, the problem of reaching therapeutic doses will be further accentuated.

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