FEBS Letters 392 (1996) 30–34 FEBS 17386

Ultrarapid metabolizers of debrisoquine: Characterization and PCR-based detection of alleles with duplication of the *CYP2D6* gene

Roger Løvlie[a], Ann K. Daly[b], Anders Molven[a], Jeffrey R. Idle[b,c], Vidar M. Steen[a,*]

[a]*Dr. Einar Martens' Research Group for Biological Psychiatry, Center for Molecular Medicine, Haukeland University Hospital, N-5021 Bergen, Norway*

[b]*Pharmacogenetics Research Unit, Department of Pharmacological Sciences, University of Newcastle, Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH, UK*

[c]*Institute of Cancer Research and Molecular Biology, Norwegian University of Science and Technology, N-7005 Trondheim, Norway*

Received 4 July 1996

Abstract **Up to 7% of Caucasians may demonstrate ultrarapid metabolism of debrisoquine due to inheritance of alleles with duplicated functional *CYP2D6* genes. Here we describe the genomic organization of the duplicated *CYP2D6* genes in the 42 kb *XbaI* allele. We postulate that this duplication originates from a homologous, unequal cross-over event which involved two 29 kb *XbaI* wild-type alleles, and had break points within a 2.8 kb direct repeat (CYP-REP) flanking the *CYP2D6* gene. Moreover, we have designed two different PCR assays for detection of alleles with duplicated *CYP2D6* genes. Both assays correctly identified 29 out of 29 subjects positive for the 42 kb *XbaI* allele. No false negative or false positive reactions were observed.**

Key words: *CYP2D6* genotyping; Debrisoquine 4-hydroxylase; Gene duplication; Long-PCR; Ultrarapid metabolizer

1. Introduction

The cytochrome P450 enzyme debrisoquine 4-hydroxylase, encoded by the *CYP2D6* gene on chromosome 22, metabolizes many different classes of commonly used drugs such as antidepressants, neuroleptics and cardiovascular agents [1]. CYP2D6 enzyme activity can be measured in vivo after oral intake of a single dose of the probe drugs debrisoquine or sparteine with subsequent determination of the ratio between the urinary recovery of the drug and the metabolite. In Caucasian populations, there is a large variation in the metabolic ratio (MR) of the probe drugs, leading to the classification of three different phenotypes: poor, extensive and ultrarapid metabolizers.

5–10% of Caucasians are classified as poor metabolizers (PMs) of debrisoquine due to inheritance of two mutant *CYP2D6* null alleles [2,3]. With some CYP2D6 drug substrates, PM subjects may develop toxic plasma concentrations and adverse reactions at the standard recommended dose due to impaired metabolism. Most of the non-functional *CYP2D6* alleles have been described and characterized, and more than 95% of PM subjects can easily be detected by the use of PCR assays for the *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*5*, *CYP2D6*6* and *CYP2D6*7* null alleles [4–11].

Up to 7% of Caucasians are ultrarapid metabolizers (UMs) of debrisoquine due to inheritance of alleles with duplication of functional *CYP2D6* genes [12–14]. The mechanism by which this duplication has occurred is not known. When subjected to standard doses of CYP2D6 substrates, UMs may suffer from therapeutic failure because of the very rapid metabolic conversion of the drugs [15,16]. Phenotyping with probe drugs may be used to identify both PM and UM subjects. However, the procedure is time-consuming and expensive, and phenotype determination can be confounded by concomitant use of other drugs which affect CYP2D6 activity.

Restriction fragment length polymorphism (RFLP) analysis after digestion with *XbaI* has been a widely used method to describe different *CYP2D* alleles. The three most frequent *XbaI* fragments are 29, 44 and 13 kb (11.5 kb), respectively [17]. The common 29 kb allele contains the *CYP2D6* gene (wild-type or mutant) and the two pseudogenes *CYP2D8* and *CYP2D7* [18]. The 44 kb *XbaI* allele is usually associated with the *CYP2D6*4* mutation, and comprises the *CYP2D6* gene and the three pseudogenes *CYP2D8*, *CYP2D7AP* and *CYP2D7BP* [19]. The 13 kb *CYP2D6*5* allele is generated by a deletion of the entire *CYP2D6* gene, and contains only the pseudogenes *CYP2D8* and *CYP2D7* [6,20]. In addition, a 42 kb *XbaI* allele with two copies of the *CYP2D6* gene as well as one copy of each of the pseudogenes *CYP2D8* and *CYP2D7* has been described [12–14]. The duplicated *CYP2D6* genes are usually functional, and the 42 kb allele has therefore been associated with the UM phenotype.

We have recently demonstrated the presence of two large direct repeats (CYP-REP) flanking the *CYP2D6* locus [11,20]. The break points of the *CYP2D6*5* gene deletion allele are present within the 2.8 kb CYP-REP regions, indicating that the deletion has occurred by homologous, unequal recombination [20]. We also proposed that alleles with duplication of *CYP2D6* could be explained as a reciprocal of the deletion event, involving the same CYP-REP units.

Here we characterize the UM-related alleles with *CYP2D6* duplication, and describe different specific and reliable PCR assays which can be used to identify UM subjects.

*Corresponding author. Fax: (47) (55) 97 51 41.

Abbreviations: CYP, cytochrome P450; MR, metabolic ratio; PM, poor metabolizer; RFLP, restriction fragment length polymorphism; UM, ultrarapid metabolizer.

2. Materials and methods

2.1. Nomenclature

Throughout this report we use the new recommended nomenclature

PII S0014-5793(96)00779-X

[21] for the different human *CYP2D6* alleles, e.g. *CYP2D6*1* (instead of *CYP2D6wt*), *CYP2D6*2* (*CYP2D6L*), *CYP2D6*3* (*CYP2D6A*), *CYP2D6*4* (*CYP2D6B*), *CYP2D6*5* (*CYP2D6D*), *CYP2D6*6* (*CYP2D6T*), *CYP2D6*7* (*CYP2D6E*) and *CYP2D6*16* (*CYP2D6D2*).

2.2. Subjects

The samples used in this study were from individuals of Caucasian, black American and North African origin and have been described in more detail previously [5,22].

2.3. Debrisoquine phenotyping and CYP2D6 genotyping

Debrisoquine phenotyping was carried out as described previously [5]. DNA was isolated by standard procedures from leukocytes (EDTA-anticoagulated blood). The subjects had previously been genotyped by *Xba*I RFLP analysis to detect the different *CYP2D6 Xba*I alleles and by PCR methods to determine the PM-associated *CYP2D6* mutations *CYP2D6*3*, *CYP2D6*4* and *CYP2D6*5* and, in some cases *CYP2D6*6* and *CYP2D6*7* [5,10,11,23]. The presence of the 42 kb *Xba*I allele was confirmed by RFLP analysis with *Eco*RI. The *CYP2D6*2* allele was identified by a modified PCR-based restriction digestion assay as described previously [24].

2.4. Long-PCR-based methods

All experiments were performed with standard thick-walled PCR tubes (GeneAmp PCR reaction tubes, Perkin Elmer, Foster City, CA, USA) on a Perkin Elmer DNA Thermal Cycler (model TC1 or 480) using the GeneAmp XL PCR kit (Perkin Elmer) which contains r*Tth* DNA polymerase in combination with $Vent_R$ DNA polymerase. The 3.3×XL PCR reaction buffer (supplied by Perkin Elmer) contained Tricine, K(OAc), glycerol and DMSO (exact concentrations not given by the manufacturer). PCR were performed according to the manufacturer's instructions in 100 μl reaction volumes containing 1×XL PCR reaction buffer, 500 ng genomic DNA, 200 μM of each dNTP, 0.3 μM of each primer and 2 U of r*Tth*/$Vent_R$ DNA polymerase. The following primers were used: cyp-17 (forward), 5′-TCCCCCACTGACCCAACTCT-3′; cyp-32 (reverse), 5′-CACGTGCAGGGCACCTAGAT-3′; cyp-42 (reverse), 5′-CCGGATTCCAGCTGGGAAATG-3′; cyp-205 (forward), 5′-CCCAGCCACCATGGTGTCTTT-3′ and cyp-207 (forward), 5′-CCCTCAGCCTCGTCACCTCAC-3′. The conditions for amplification with the primer pairs cyp-205/cyp-42, cyp-207/cyp-32 and cyp-17/cyp-32 were as follows: an initial denaturing step of 93°C for 1 min, followed by 30–35 cycles of 93°C for 1 min, 67°C for 30 s and 68°C for 2–12 min, and a final elongation step of 72°C for 10 min. The resulting PCR products were separated and detected in ethidium bromide-containing 1% agarose gels.

2.5. Restriction enzyme analysis

The cyp-205/cyp-42 PCR product was digested with *Bam*HI, *Eco*RI, *Hin*dIII, *Kpn*I and *Xba*I (New England Biolabs Inc., Beverly, MA, USA) according to the manufacturer's instructions. The digests were analysed by electrophoresis in an ethidium bromide-containing 1% agarose gel.

2.6. Automatic DNA sequencing

The cyp-205/cyp-42 PCR product was purified with a Qiagen PCR purification kit (Qiagen Inc., Chatsworth, CA, USA) and the 5′- and 3′-ends of the CYP-REP-DUP unit were sequenced with the primers cyp-12, 5′-CTAGGGTTGGAGCCAAACAAGTGTC and cyp-30, 5′-CTCCCTGGGAGCTAGCAGAC-3′. The AmpliTaq FS Dye Terminator Cycle Sequencing Kit (Applied Biosystems Inc., Foster City, CA, USA) was applied according to the manufacturer's instructions, and all reactions were analyzed on an ABI 373A DNA Sequencer.

3. Results and discussion

3.1. PCR amplification and genomic characterization of the duplicated CYP2D6 genes

We have recently demonstrated the presence of large, direct repeats flanking the *CYP2D6* gene: a 3.4 kb sequence positioned immediately downstream of *CYP2D6* also occurs downstream of *CYP2D7* [11,20]. However, in the latter case, a 1.6 kb DNA element has been inserted, so that the 3.4 kb sequence splits into 0.6 and 2.8 kb repeats [20] (Fig. 1). The 2.8 kb unit, termed CYP-REP, contains an Alu element and a tandem 10 bp direct repeat, sequences postulated to be hotspots for recombination events [20]. We also demonstrated that the CYP-REP units downstream of *CYP2D7* and *CYP2D6*, referred to as CYP-REP-7 and CYP-REP-6, respectively, have been involved in the generation of the *CYP2D6*5* deletion allele, since the break points for this large gene deletion are present within the repeated 2.8 kb regions [20].

Based on these findings, we decided to study the UM-related 42 kb *Xba*I alleles with duplicated *CYP2D6* genes. Most likely, the mechanism of duplication has involved homologous, unequal cross-over, and either the 0.6 or 2.8 kb direct repeats flanking the *CYP2D6* gene could be implicated in this process. Depending on which repeat was involved in the cross-over event, different *CYP2D Xba*I RFLP alleles would be generated. The two possible recombinations are illustrated in Fig. 1. A sequence starting from any position within the

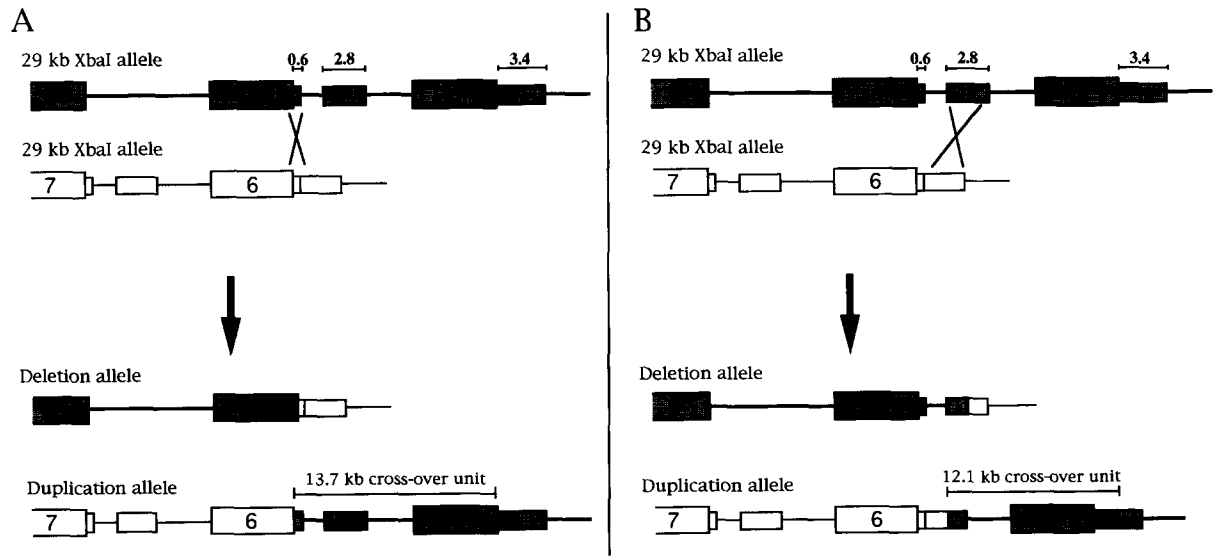

Fig. 1. Postulated mechanisms for generation of alleles with duplicated *CYP2D6* genes. The panels show homologous, unequal cross-over in either the 0.6 kb (A) or 2.8 kb (B) direct repeats flanking the *CYP2D6* gene.

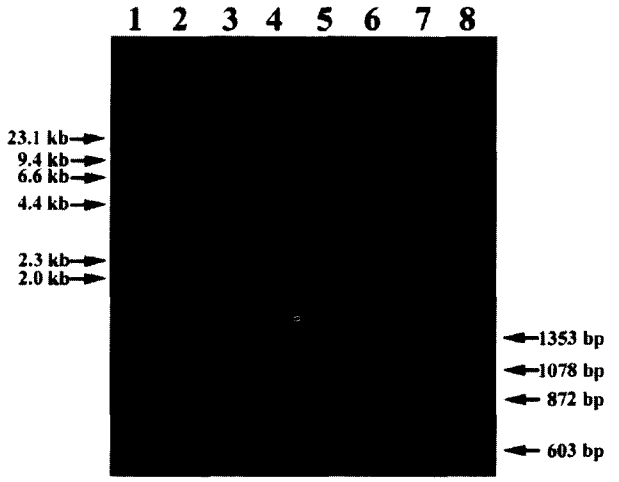

Fig. 2. PCR amplification and restriction enzyme digestion of the *CYP2D6-CYP2D6* intergenic region of a 42 kb *Xba*I allele. The primer pair cyp-205/cyp-42 amplified a 9.3 kb PCR product (lane 2) from the intergenic region between the duplicated genes in a 42 kb *Xba*I allele. Digests of the 9.3 kb fragment by *Bam*HI, *Eco*RI, *Hin*dIII, *Kpn*I and *Xba*I are shown in lanes 3–7, respectively. The sizes of the DNA markers λ/*Hin*dIII (lane 1) and ΦX-174/*Hae*III (lane 8) are indicated.

0.6 kb repeat downstream of *CYP2D7* and extending to the corresponding position in the homologous repeat downstream of *CYP2D6* comprises 13.7 kb. A homologous, unequal cross-over between two wild-type *CYP2D* alleles (29 kb *Xba*I) involving the 0.6 kb repeats would therefore produce a deletion allele and a *CYP2D6* duplication allele with a 13.7 kb extra fragment when subjected to *Eco*RI RFLP analysis (Fig. 1A). The alternative recombination involves a fragment spanning the 12.1 kb region between the two 2.8 kb repeats. A homologous, unequal cross-over event between two 29 kb *Xba*I alleles affecting these regions would generate the 13 kb (11.5 kb) *CYP2D6*5* deletion allele and a reciprocal *CYP2D6* duplication allele (Fig. 1B). The postulated duplication allele should yield an additional 12.1 kb fragment upon *Eco*RI RFLP analysis. On the basis of currently available information, the scheme shown in Fig. 1B appears the more likely since a 12.1 kb fragment has been observed by *Eco*RI RFLP analysis in individuals with the 42 kb *Xba*I allele [12–14].

To investigate further the mechanism by which the duplication occurred, we decided to amplify and characterize the unique *CYP2D6-CYP2D6* intergenic region of the tandemly arranged genes in the duplication alleles. Two *CYP2D6*-specific primers, one forward primer in exon 9 (cyp-205) and one reverse primer in intron 2 (cyp-42), amplified a 9.3 kb fragment only in subjects with the 42 kb *Xba*I allele (Figs. 2 and 3). Using these primers, a PCR fragment of 10.9 kb, which would be expected for alleles generated by unequal cross-over involving the 0.6 kb repeats (Fig. 1A), was not detected. Moreover, restriction digestion of the 9.3 kb PCR product with *Bam*HI, *Eco*RI, *Hin*dIII, *Kpn*I and *Xba*I yielded fragments with sizes as expected from an allele with break points in the CYP-REP units (Fig. 2 Fig. 3). These results strongly indicated that the *CYP2D6* duplication allele had been generated by homologous unequal cross-over involving the 2.8 kb CYP-REP units, and not the 0.6 kb repeats.

The CYP-REP-7 and CYP-REP-6 units are almost identical except for four basepairs in the 5′-end and four basepairs in the 3′-end [20] (Fig. 3). According to the mechanism described in Fig. 1B, it could be expected that the CYP-REP repeat located in the *CYP2D6-CYP2D6* intergenic region (CYP-REP-DUP) would be a hybrid of CYP-REP-6 in the 5′-end and CYP-REP-7 in the 3′-end. The 9.3 kb cyp-205/cyp-42 PCR product was subjected to sequencing of the 5′- and 3′-ends of the CYP-REP-DUP unit. The DNA sequence was identical for the 42 kb *CYP2D6*1* and *CYP2D6*2* alleles, and demonstrated that CYP-REP-DUP is identical to CYP-REP-6 and CYP-REP-7 in its 5′- and 3′-ends, respectively (Fig. 3). The CYP-REP-DUP unit is therefore a reciprocal of the CYP-REP-DEL repeat of the *CYP2D6*5* gene deletion allele (Fig. 1B), as we previously hypothesized [20]. These data confirm that the duplication of the *CYP2D6* gene has occurred by homologous, unequal cross-over involving the 2.8

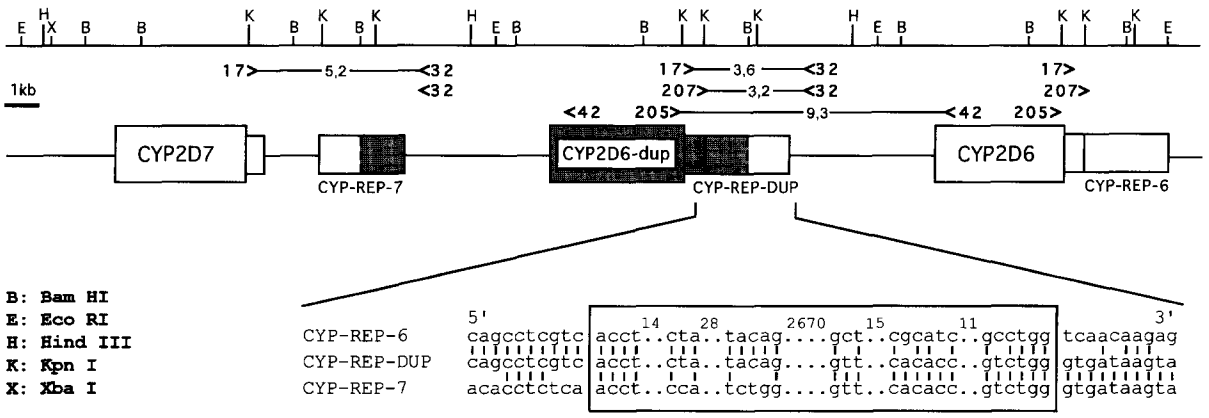

Fig. 3. Genomic organization of the 42 kb *CYP2D Xba*I allele. The 42 kb allele is aligned with respect to restriction maps and sequence similarity according to our present results. The shaded area indicates the 'extra' *CYP2D6* sequence, as compared to a 29 kb *Xba*I allele. Large boxes are *CYP2D* genes and pseudogenes, and smaller boxes represent the 0.6 and 2.8 kb direct repeats. The horizontal arrowheads indicate binding sites for the different upper and lower primers. Arrowheads connected by lines show PCR products which were amplified by the primers. Underneath, nucleotide sequences from the CYP-REP-6 and CYP-REP-7 regions in the 29 kb *CYP2D6*1* allele are aligned with the CYP-REP-DUP region in the 42 kb allele. The alignment shows that CYP-REP-DUP is homologous to CYP-REP-6 in its 5′-end and to CYP-REP-7 in its 3′-end. The 2.8 kb CYP-REP units are boxed with dotted lines representing identical sequence, the extent of which is shown by the numbers of basepairs.

kb CYP-REP-7 and CYP-REP-6 units. Since the central sequences of the CYP-REP units are almost identical [20], the exact break points can probably not be determined.

3.2. PCR assays to identify alleles with duplication of the CYP2D6 gene

The amplification of the 9.3 kb *CYP2D6-CYP2D6* intergenic sequence by the cyp-205/cyp-42 primer pair can be used to identify the 42 kb *Xba*I *CYP2D6* duplication allele (Figs. 2 and 3). However, amplification of such large genomic DNA fragments by PCR may be more prone to failure as compared to shorter fragments, thereby causing false negatives. Also, the cyp-205/cyp-42 PCR assay in some cases produced faint, unspecific PCR products in addition to the main 9.3 kb fragment (results not shown). As a consequence, we decided to develop more reliable PCR assays with shorter and more specific PCR products, based on our findings concerning the structure of the 42 kb *Xba*I allele.

The sequence between the 3′-end of CYP-REP-7 and the 5′-end of *CYP2D6* is normally not present downstream of a *CYP2D6* gene (Fig. 3). However, in the alleles with duplicated *CYP2D6* genes this unique sequence should also appear in the intergenic region between the two *CYP2D6* genes, downstream of the CYP-REP-DUP unit (Fig. 3). Moreover, the 0.6 kb repeat and the 2.8 kb CYP-REP unit upstream of a *CYP2D6* gene are usually separated by a 1.6 kb DNA element, as in the 29 and 44 kb *Xba*I alleles [11]. This is not the case in the postulated *CYP2D6-CYP2D6* intergenic region of the 42 kb *Xba*I allele, where the 0.6 and 2.8 kb CYP-REP-DUP repeats are present together without the 1.6 kb 'extra' sequence (Fig. 3).

First, we synthesized a forward primer (cyp-207) which overlaps the junction between the 0.6 and 2.8 kb repeats. Since this organization of the 0.6 and 2.8 kb units is usually confined to the region downstream of *CYP2D6*, cyp-207 should function as a *CYP2D6*-specific primer (see Fig. 3). The cyp-207 forward primer was used together with the cyp-32 reverse primer and yielded a specific 3.2 kb PCR product in subjects with the 42 kb *Xba*I alleles only (Fig. 4A). As an improvement, we used a forward primer (cyp-17) which binds in the 0.6 kb repeat downstream of both *CYP2D7* and *CYP2D6*. Together with the reverse cyp-32 oligonucleotide, this primer combination should always amplify a 5.2 kb PCR fragment from a *CYP2D7-CYP2D6* intergenic region,

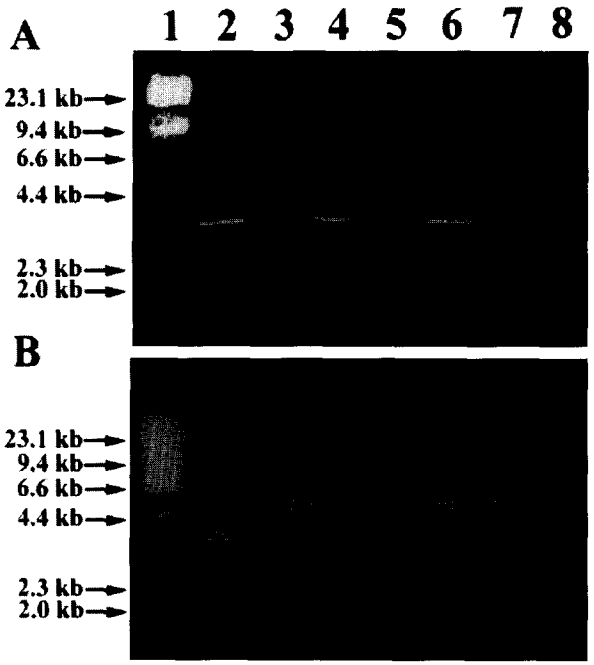

Fig. 4. PCR assays for detection of alleles with duplicated *CYP2D6* genes. The primer pairs cyp-207/cyp-32 (A) and cyp-17/cyp-32 (B) were used to identify the 42 kb *Xba*I allele. DNA samples with the following genotypes are shown: 42 kb/29 kb *CYP2D6*2/CYP2D6*2* (lane 2), 44 kb/29 kb *CYP2D6*4/CYP2D6*4* (lane 3), 42 kb/13 kb *CYP2D6*4/CYP2D6*5* (lane 4), 29 kb/13 kb *CYP2D6*1/CYP2D6*5* (lane 5), 42 kb/29 kb *CYP2D6*1/CYP2D6*1* (lane 6) and 29 kb/29 kb *CYP2D6*3/CYP2D6*4* (lane 7). The DNA marker λ/*Hin*dIII is shown in lane 1.

Table 1
Results of the two different PCR-based gene duplication assays with pretyped positive and negative DNA controls

Classification of pretyped DNA samples			PCR-based CYP2D6 duplication assays	
Genotype		Number of samples	cyp-207/cyp-32 positive	cyp-17/cyp-32 positive
Samples with 42 kb allele; $n=29$				
42/42 kb	CYP2D6*1/CYP2 D6*2	$n=1$	$n=1$	$n=1$
42/29 kb	CYP2D6*1/CYP2 D6*1	$n=3$	$n=3$	$n=3$
42/29 kb	CYP2D6*1/CYP2 D6*2	$n=13$	$n=13$	$n=13$
42/29 kb	CYP2D6*2/CYP2 D6*2	$n=8$	$n=8$	$n=8$
42/13 kb	CYP2D6*2/CYP2 D6*5	$n=1$	$n=1$	$n=1$
42/13 kb	CYP2D6*4/CYP2 D6*5	$n=3$	$n=3$	$n=3$
Samples without 42 kb allele; $n=24$				
44/44 kb	CYP2D6*4/CYP2 D6*4	$n=1$	$n=0$	$n=0$
44/29 kb	CYP2D6*4/CYP2 D6*4	$n=1$	$n=0$	$n=0$
44/16+9 kb	CYP2D6*4/CYP2 D6*4	$n=1$	$n=0$	$n=0$
29/29 kb	CYP2D6*1/CYP2 D6*1	$n=5$	$n=0$	$n=0$
29/29 kb	CYP2D6*1/CYP2 D6*2	$n=6$	$n=0$	$n=0$
29/29 kb	CYP2D6*2/CYP2 D6*2	$n=4$	$n=0$	$n=0$
29/29 kb	CYP2D6*1/CYP2 D6*6	$n=2$	$n=0$	$n=0$
29/29 kb	CYP2D6*3/CYP2 D6*4	$n=1$	$n=0$	$n=0$
29/13 kb	CYP2D6*1/CYP2 D6*5	$n=1$	$n=0$	$n=0$
29/11 kb	CYP2D6*1/CYP2 D6*16	$n=1$	$n=0$	$n=0$
n.d.	CYP2D6*1/CYP2 D6*7	$n=1$	$n=0$	$n=0$

n.d., not determined.

and thus function as an internal control of the PCR reaction (see Fig. 3). In addition, a 3.6 kb PCR fragment amplified from the *CYP2D6-CYP2D6* region was observed in subjects with the 42 kb *Xba*I alleles (Fig. 4B).

In preliminary experiments, the cyp-207/cyp-32 and cyp-17/cyp-32 assays were superior to the cyp-205/cyp-42 reaction. Therefore, the two former assays were chosen for diagnostic purposes (Fig. 4). Both the cyp-207/cyp-32 and cyp-17/cyp-32 assays detected 26 out of 26 subjects with the 42 kb *Xba*I *CYP2D6*1* or *CYP2D6*2* allele (Table 1), including the following *Xba*I genotypes: 42 kb/42 kb ($n=1$, Caucasian), 42 kb/29 kb ($n=24$, 12 Caucasians and 12 black Americans or Africans) and 42 kb/13 kb ($n=1$, black American). Debrisoquine phenotyping of some of these subjects yielded a MR of 0.41 (median value; range 0.11–4.02, $n=6$) for the 42 kb/29 kb genotype and 0.12 for the person with the 42 kb/42 kb pattern. In addition, three black Americans with the 42 kb/13 kb genotype turned out to be positive for the assays despite the presence in these subjects of the *CYP2D6*4/CYP2D6*5* genotype (and absence of *CYP2D6*1* or *CYP2D6*2* allele) (Table 1; see below). No false positive reactions were detected when the assays were run with 24 negative controls containing different combinations of the most common *CYP2D* alleles and *CYP2D6* polymorphisms (Table 1). In addition, no false negative reactions due to technical failure of amplification were encountered, verified by the presence of the internal control product in the cyp-17/cyp-32 reactions. These results confirm that the PCR-based cyp-207/cyp-32 and cyp-17/cyp-32 assays are reliable in detecting subjects having alleles with duplicated *CYP2D6* genes.

3.3. Alleles with duplicated non-functional CYP2D6 genes

In Caucasians, the 42 kb *Xba*I haplotype is usually indicative of a duplication of the functional *CYP2D6* gene. However, in a Zimbabwean population the 42 kb allele has been associated with the *CYP2D6*4* mutation [25] indicating a duplication of non-functional *CYP2D6* genes. In this study we encountered three black Americans with the 42 kb/13 kb *CYP2D6*4/CYP2D6*5* genotype. DNA samples from these three subjects all yielded positive reactions with the cyp-207/cyp-32 and cyp-17/cyp-32 assays, thereby indicating the presence of alleles with duplicated, but non-functional *CYP2D6* genes (Table 1). These results were further verified by MR values obtained from debrisoquine phenotyping of 2 out of the 3 persons. Both subjects had a metabolic ratio above 50, clearly demonstrating the PM phenotype. The PM-associated 42 kb allele with duplication of two non-functional *CYP2D6* genes has most likely been generated through a similar mechanism as described in Fig. 1B, involving two *CYP2D6*4* alleles. In fact, sequencing of the 5′- and the 3′-end of the CYP-REP-DUP unit in this allele revealed identical results, as shown for the 42 kb *CYP2D6*1* and *CYP2D6*2* alleles in Fig. 3. As a consequence, the 42 kb *Xba*I genotype may be heterogeneous, associated with both the PM and UM phenotype. Special caution should therefore be taken to avoid misclassification, especially of black American or African subjects, when interpreting the results from PCR assays designed to detect duplicated *CYP2D6* genes.

Acknowledgements: The work is supported by Dr. Einar Martens' Research Fund and the Norwegian Research Council (NFR, Mental Health Programme), and in part by Lundbeckfonden.

References

[1] Cholerton, S., Daly, A.K. and Idle, J.R. (1992) Trends Pharmacol. Sci. 13, 434–439.
[2] Evans, D.A.P., Maghoub, A., Sloan, T.P., Idle, J.R. and Smith, R.L. (1980) J. Med. Genet. 17, 102–105.
[3] Brøsen, K. and Gram, L.F. (1989) Eur. J. Clin. Pharmacol. 36, 537–547.
[4] Kagimoto, M., Heim, M., Kagimoto, K., Zeugin, T. and Meyer, U.A. (1990) J. Biol. Chem. 265, 17209–17214.
[5] Daly, A.K., Armstrong, M., Monkman, S.C., Idle, M.E. and Idle, J.R. (1991) Pharmacogenetics 1, 33–41.
[6] Gaedigk, A., Blum, M., Gaedigk, R., Eichelbaum, M. and Meyer, U.A. (1991) Am. J. Hum. Genet. 48, 943–950.
[7] Evert, B., Griese, E.-U. and Eichelbaum, M. (1994) Naunyn-Schmiedelberg's Arch. Pharmacol. 350, 434–439.
[8] Evert, B., Griese, E.-U. and Eichelbaum, M. (1994) Pharmacogenetics 4, 271–274.
[9] Saxena, R., Shaw, G.L., Relling, M.V., Frame, J.N., Moir, D.T., Evans, W.E., Caporaso, N. and Weiffenbach, B. (1994) Hum. Mol. Genet. 3, 923–926.
[10] Daly, A.K., Leathart, J.B.S., London, S.J. and Idle, J.R. (1995) Hum. Genet. 95, 337–341.
[11] Steen, V.M., Andreassen, O.A., Daly, A.K., Tefre, T., Børresen, A.-L., Idle, J.R. and Gulbrandsen, A.-K. (1995) Pharmacogenetics 5, 215–223.
[12] Johansson, I., Lundqvist, E., Bertilsson, L., Dahl, M-.L., Sjøqvist, F. and Ingelman-Sundberg, M. (1993) Proc. Natl. Acad. Sci. USA 90, 11825–11829.
[13] Agundez, J.A.G., Ledesma, M.C., Ladero, J.M. and Benitez, J. (1995) Clin. Pharmacol. Ther. 57, 265–269.
[14] Dahl, M.-L., Johansson, I., Bertilsson, L., Ingelman-Sundberg, M. and Sjøqvist, F. (1995) J. Pharmacol. Exp. Ther. 274, 516–520.
[15] Bertilsson, L., Åberg-Wistedt, A., Gustafsson, L.L. and Nordin, C. (1985) Ther. Drug. Monit. 7, 478–480.
[16] Bertilsson, L., Dahl, M.-L., Sjøqvist, F., Åsberg-Wistedt, A., Humble, M., Johansson, I., Lundqvist, E. and Ingelman-Sundberg, M. (1993) Lancet 341, 63.
[17] Skoda, R.C., Gonzalez, F.J., Demierre, A. and Meyer, U.A. (1988) Proc. Natl. Acad. Sci. USA 85, 5240–5243.
[18] Kimura, S., Umeno, M,. Skoda, R.C., Meyer, U.A. and Gonzales, F.J. (1989) Am. J. Hum. Genet. 45, 889–904.
[19] Heim, M. and Meyer, U.A. (1992) Genomics 14, 49–58.
[20] Steen, V.M., Molven, A., Aarskog, N.K. and Gulbrandsen A-.K. (1995) Hum. Mol. Genet. 4, 2251–2257.
[21] Daly, A.K., Brockmöller, J., Broly, F., Eichelbaum, M., Evans, W.E., Gonzales, F.J., Huang, J.-D., Idle, J.R., Ingelman-Sundberg, M., Ishizaki, T., Jacqz-Aigrain, E., Meyer, U.A., Nebert, D.W., Steen, V.M., Wolf, C.R. and Zanger, U.M. (1996) Pharmacogenetics, in press.
[22] London, S.J., Daly, A.K., Cooper, J., Navidi, W.C., Carpenter, C.L. and Idle, J.R. (1995) J. Natl. Cancer Inst. 87, 1246–1253.
[23] Tefre, T., Daly, A.K., Armstrong, M., Leathart, J.B.S., Idle, J.R., Brøgger, A. and Børresen, A.-L. (1994) Pharmacogenetics 4, 47–57.
[24] Armstrong, M., Idle, J.R. and Daly, A.K. (1993) Hum. Genet. 91, 616–617.
[25] Masimirembwa, C.M., Johansson, I., Hasler, J.A. and Ingelman-Sundberg, M. (1993) Pharmocogenetics 3, 275–280.