

An unequal cross-over event within the *CYP2D* gene cluster generates a chimeric *CYP2D7/CYP2D6* gene which is associated with the poor metabolizer phenotype

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- 1 The study of the *CYP2D* genotype and phenotype of a Caucasian family revealed that a XbaI-9 kb allele was associated with the poor metabolizer phenotype.
- 2 A Polymerase Chain Reaction (PCR)-based assay showed that the previously described mutations *D6A* and *D6B* are not associated with the XbaI-9 kb allele.
- 3 To explore the molecular basis of the poor metabolizer phenotype associated with the XbaI-9 kb allele, complete sequencing of the nine exons and intron-exon boundaries of the *CYP2D6* gene was undertaken after amplification by PCR.
- 4 All the exons were successfully amplified using *CYP2D6* gene-specific primers except exon 1 which required a combination of *CYP2D7* gene-specific 5' primer and a *CYP2D6* gene-specific 3' primer.
- 5 Sequence data derived from this amplified product revealed that the XbaI-9 kb allele corresponds to a novel rearrangement of the locus. This involved a deletion of an approximately 20 kilobase (kb) DNA segment generating a hybrid 5' *CYP2D7/CYP2D6* 3' gene.
- 6 The chimeric gene is non-functional presumably due to an insertion in exon 1 (characteristic of the exon 1 of the *CYP2D7* gene) which causes a shift in the reading frame with premature termination of translation.

Keywords cytochrome P450-debrisoquine 4-hydroxylase (*CYP2D6*) genetic polymorphism poor metabolizer *CYP2D6* allele

Introduction

The polymorphism of debrisoquine/sparteine type oxidation is one of the most extensively studied genetic deficiencies of drug metabolism in humans [1, 2]. Phenotypically, individuals are either poor metabolizers (PM) or extensive metabolizers (EM). About 5–10% of Caucasians exhibits PM phenotype [3, 4] and are characterized by their inability to metabolize marker drugs such as debrisoquine, sparteine and dextromethorphan as well as many clinically important drugs. This PM phenotype, inherited as an autosomal recessive trait, is due to the deficient activity of a cytochrome P450 enzyme, which is encoded by the *CYP2D6* gene in man [5, 6]. This gene is a member of a multigene cluster (*CYP2D* cluster) on chromosome 22q13.1 [7, 8]. The two other members of this cluster, namely *CYP2D7* and *CYP2D8P*, exhibit high sequence homology to the functional

CYP2D6 gene [9]. The *CYP2D8P* gene contains several gene-disrupting insertions, deletions and termination codons within its exons, and is therefore clearly a pseudogene. However the *CYP2D7* gene (located between the *CYP2D8P* and *CYP2D6* genes) has a similar structure to that of the functional *CYP2D6* gene, except for a characteristic single reading frame-disrupting insertion (T₂₂₆) in its first exon.

It has been hypothesised that both gene conversions and unequal crossing-overs within this family of tandemly repeated genes could account for its high degree of polymorphism in man. To date, only five variant *CYP2D6* alleles namely, *D6A*, *D6B*, *D6D*, *D6E* and *D6T* have been characterized at the molecular level and shown to be associated with the PM phenotype [10–15]. The existence of additional mutant alleles has been predicted on the basis of

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phenotype data [16]. In this study, we report the molecular characterization of a novel PM allele. This XbaI-9 kb allele is a *CYP2D7/CYP2D6* fusion gene containing exon 1 and the major portion of intron 1 of the *CYP2D7* gene at its 5' end, while the rest of the sequence is the same as that of the *CYP2D6* gene.

Methods

Subjects, phenotypes and genotypes

The present work involves the study of one Caucasian nuclear family. The designation of *CYP2D* genotypes and dextromethorphan phenotypes has already been reported [17]. A detailed description of the polymorphic profiles for the BamHI and NcoI enzymes and the corresponding genotypes derived from these data are depicted in Figure 1 and Figure 3a. Nucleotide sequencing of the *CYP2D6* exons and exon-intron junctions was carried out as described earlier [18].

Specific amplification by PCR of the new allele

Specific amplification of a region of the XbaI-9 kb allele was performed with a pair of primers, one specific to a region upstream from the *CYP2D7* gene, P1 (5'-CGTGACAGCTTTGAGGCTCA-3') -799/-780 and the other P2 (5'-CCCGGGTCCCACGGAAATCT-3') +1191/+1172 complementary to a region in intron 2 of the *CYP2D6* gene (we have adopted the numbering system of Kimura *et al.* [9]). The amplification reaction was carried out in a final volume of 25 µl in the presence of 10 mM Tris-HCl, 2.5 mM MgCl₂, 50 mM KCl, 125 µM of each dNTP, 10 pmol of each primer, 250 ng of genomic DNA as template and 0.5 U of Taq polymerase. Thirty cycles of denaturation at 94° C (30 s), annealing at 58° C (30 s) and extension at 72° C (2 min) were done.

Direct sequencing of the PCR product

The PCR product derived from this experiment was purified by electrophoresis on 3% Nusieve-agarose gel and the purified product was sequenced by the dideoxynucleotide chain termination method [19] using Sequenase kit with the following primers: P1, (5'-TGTGCTGAGAGTGTCTGCC-3') -2/+17, (5'-TGATAGTGGCCATCTTCCTG-3') +123/+142 (5'-CACCCACCATCCATGTTTGC-3') +344/+325, (5'-AGCCAGGCTGAGAAGGGGAA-3') +365/+384, (5'-AGGTGGAGCTGGACTTGGCA-3') +477/+496, (5'-TTTTGCACTGTGGGTCTCGG-3') +661/+680, and P2.

Results

In the nuclear family studied, both parents were phenotyped as EMs while the son had a PM phenotype (Figure 2a). A PCR-based assay using their

genomic DNA as template failed to reveal any of the previously described mutations (*D6A*, *D6B*) [11–12]. Southern blot analysis of genomic DNA probed with the full-length *CYP2D6* cDNA (a generous gift from Professor U. Meyer, Biocentrum, Basle, Switzerland) revealed that the father was heterozygous for the XbaI-29 kb allele and a known XbaI-11.5 kb PM allele (*D6D*), while the mother was heterozygous for the wild-type XbaI-29 kb allele and a mutant XbaI-9 kb allele (Figure 2b). The son with the PM phenotype was heterozygous for the XbaI-11.5 kb and the XbaI-9 kb allele reflecting a mendelian segregation pattern and demonstrating that the latter is also a PM allele.

The father exhibited two characteristic polymorphic fragments for BamHI (*viz.*: *CYP2D7* specific 6.6 kb (BamHI-) and *CYP2D6* specific 4.1 kb (BamHI-) fragments (for a detailed description see legend to Figure 1)). Since the father had only one *CYP2D6* gene (the other being deleted in the XbaI-11.5 kb related chromosome), the deduced genotype for BamHI polymorphism is -del/- corresponding to genotype XbaI-11.5 kb/XbaI-29 kb (Figure 2b and 2c). This was further confirmed by the son's profile which showed cosegregation of the XbaI-11.5 kb fragment with the BamHI-6.6 kb fragment. The mother had characteristic *CYP2D7* specific 4.9 kb (BamHI+) and *CYP2D6* specific 2.2 kb (BamHI+) fragments (Figure 2b and 2c). However, she exhibited a BamHI-3.9 kb fragment of abnormal size which cosegregated with the XbaI-9 kb fragment as evidenced by the presence of both atypical fragments (XbaI-9 kb and BamHI-3.9 kb) in the son. Absence of any of the two typical (4.1 kb and 2.2 kb) *CYP2D6* specific fragments in the son was intriguing and suggested that both the deletion of the *CYP2D6* gene associated with XbaI-11.5 kb fragment on one chromosome and a rearranged *CYP2D6* gene associated with XbaI-9 kb fragment on the other failed to produce *CYP2D6* specific fragments of appropriate size. Arguments for a rearranged *CYP2D6* gene were further provided by the son's NcoI enzyme profiles which did not contain the 9.2 kb *CYP2D6* specific fragment but a 4.2 kb fragment of abnormal size, the latter cosegregating with XbaI-9 kb allele (Figure 2b and 2c). Although the son appeared to be homozygous for *CYP2D7* specific BamHI 6.6 kb fragment, the maternal contribution of the *CYP2D7* related allele was not retrieved. This raised the possibility that the *CYP2D7* region might also be involved in the proposed rearrangement. Overall, the data suggested that a gross locus rearrangement of the *CYP2D* cluster had occurred. On the basis of a detailed examination of the restriction map of the *CYP2D* cluster (Figure 3a), we postulated that deletion of a substantial segment (20 kb) of the *CYP2D7* gene region and the *CYP2D6-CYP2D7* intergenic region had occurred, generating the XbaI-9 kb, NcoI-4.2 kb and BamHI-3.9 kb fragments as depicted in Figure 3b.

In order to explore the molecular basis of the PM phenotype associated with the XbaI-9 kb mutant allele, systematic sequencing of all nine exons and exon-intron boundaries of the presumptively mutant *CYP2D6* allele was carried out by PCR amplification using *CYP2D6* gene specific primers [18]. For this

purpose, we chose to use the son's DNA because it carried a deletion of the entire *CYP2D6* gene (allele XbaI-11.5 kb or *D6D*) on the chromosome opposite to that of the XbaI-9 kb allele. Therefore we expected that characterisation of the XbaI-9 kb allele would be facilitated without interference from the *CYP2D6* allele on the other chromosome. Experiments targeted

to produce specific amplification products for each exon of the *CYP2D6* gene failed to amplify exon 1, while all other exons were successfully amplified. The sequence data of the amplified exons confirmed that they are authentic exons of the *CYP2D6* gene and no other sequence variation was observed, thus excluding the presence of *D6T*, *D6B*, *D6A* and *D6E*

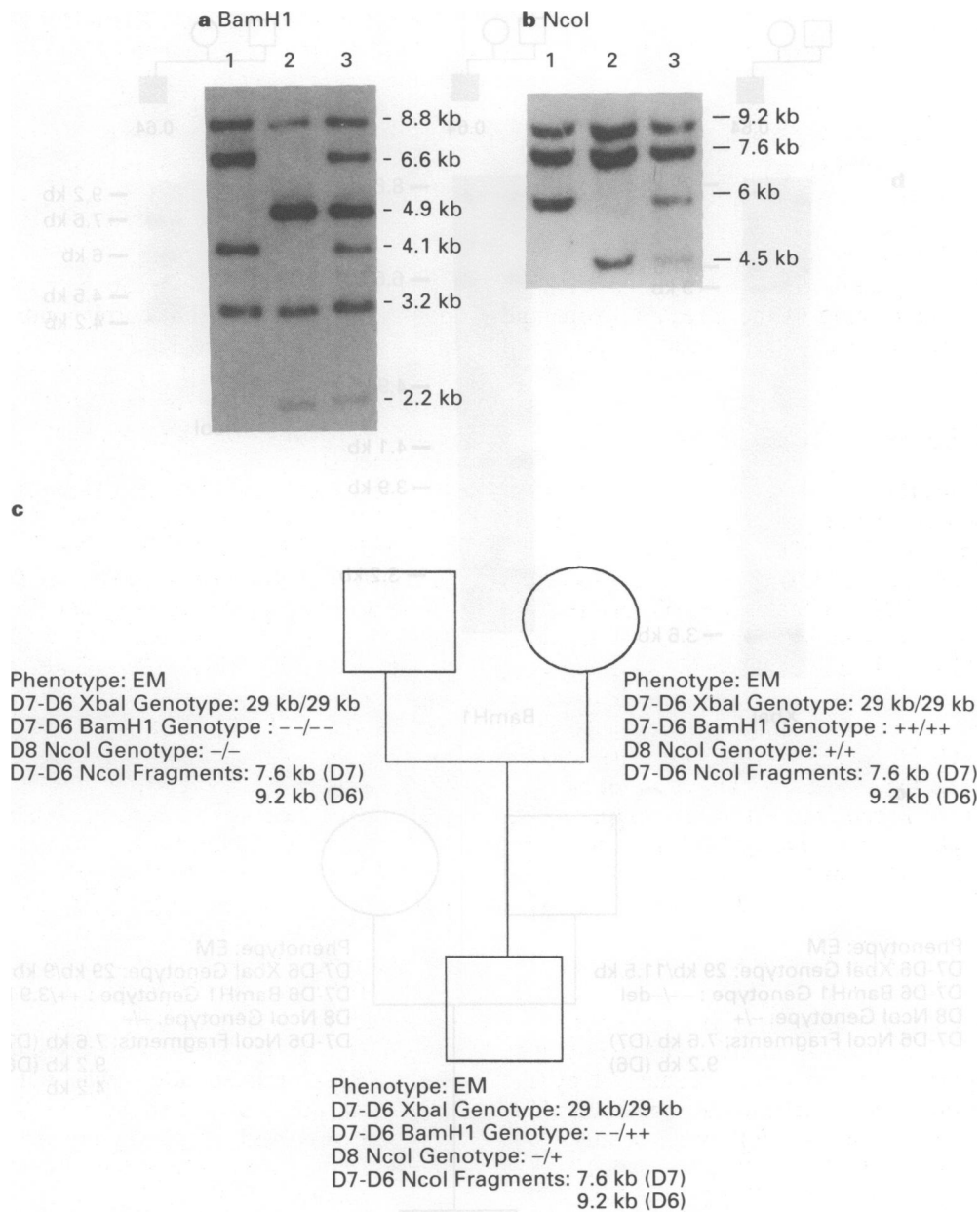


Figure 1 Autoradiographs of the a) BamH1 and b) NcoI restriction profiles. If the polymorphic site is absent (–) in both *CYP2D7* and *CYP2D6* introns 1 (which constitutes the haplotype – –), the BamH1 enzyme would generate characteristic 6.6 kb and 4.1 kb fragments, respectively, together with two constant 8.8 kb and 3.2 fragments, the latter two corresponding to *CYP2D8* and the 3' portion of the *CYP2D6* gene regions. When the polymorphic site is present (constituting the haplotype ++), the *CYP2D7*-specific 6.6 kb fragment will be split into 4.9 kb and 1.7 kb fragments, and the *CYP2D6*-specific 4.1 kb fragment into 2.2 kb and 1.9 kb fragments. Two major haplotypes are observed among Caucasians, namely ++ (*CYP2D7*, *CYP2D6*) and – – (*CYP2D7*, *CYP2D6*) [17]. Two homozygous states for these haplotypes, namely ++/++ and – –/– –, would give rise to 4.9, 2.2, 1.9 and 1.7 kb fragments (subject 2) and 6.6 and 4.1 kb fragments (subject 1), respectively, together with the constant fragments. In the heterozygous state (constituting the genotype +/-) all the fragments described above would appear together on the autoradiographs (subject 3). For the NcoI polymorphism in intron 7 of the *CYP2D8* gene [22], its presence (+) would generate characteristic 4.5 kb and 1.5 kb fragments (subject 2) and its absence (–) would generate a 6 kb fragment (subject 1) together with *CYP2D7*-specific 7.6 kb and *CYP2D6*-specific 9.2 kb constant fragments. As described above, the genotypes deduced from the observed fragment sizes are shown for this EM family (c).

mutations on this XbaI-9 kb. Our failure to amplify exon 1 led us to reexamine our strategy of amplification using the *CYP2D6* sequence-specific primers. If the postulated deletion model was correct, then the 5' sequences of *CYP2D7* gene would be expected to lie upstream from this mutant allele. To test this

hypothesis, we used a primer pair, one specific to the upstream sequence of the *CYP2D7* gene region and the other specific to a sequence in intron 2 of the *CYP2D6* gene. In a normal unrearranged *CYP2D* cluster, these primers are located too far away from each other to generate a PCR product in our experi-

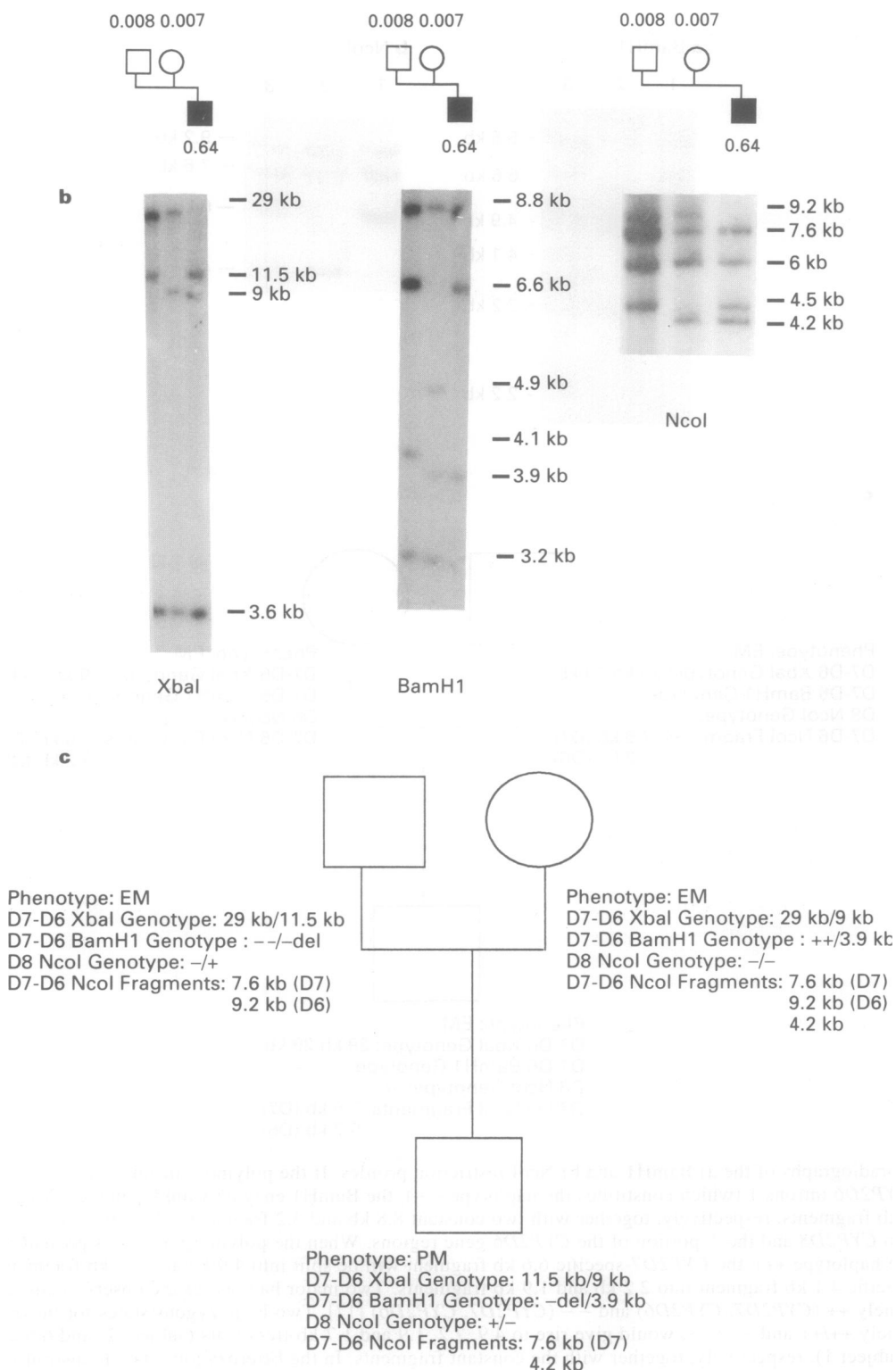


Figure 2 a) The pedigree of the Caucasian family studied. The values shown are urinary metabolic ratios for dextromethorphan/dextrorphan used to assign the phenotype into EM (in white) and PM (in black) b) Autoradiographs of NcoI, BamH1 and XbaI restriction profiles for these subjects and the deduced genotypes (c).

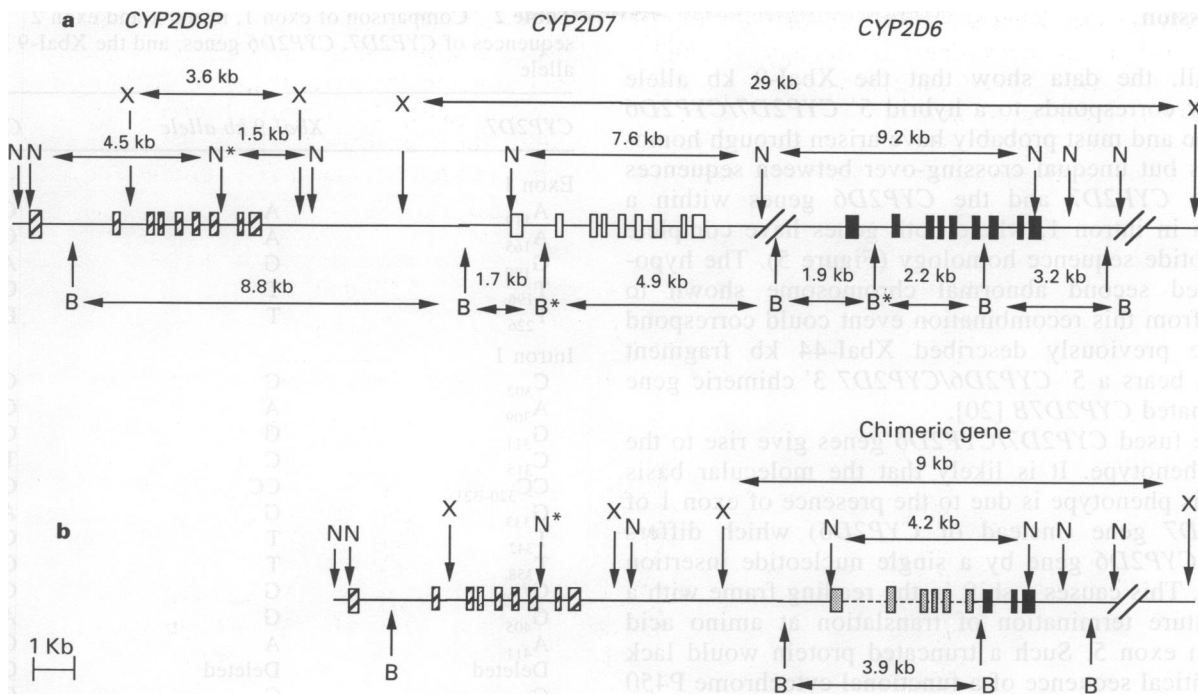


Figure 3 a) A map of the *CYP2D* gene cluster for the *Xba*I (X) restriction enzyme [10], and for the polymorphic (*) *Nco*I (N) and *Bam*HI (B) restriction sites [12, 22]. b) Postulated structure of the *Xba*I-9 kb fragment. The dotted line spanning 6 exons is a region which could correspond either to the *CYP2D7* or *CYP2D6* sequence.

mental conditions. However, in the rearranged cluster the generation of a fragment of 1990 bp would be expected. Out of more than 110 DNA samples of known *Xba*I-defined genotypes examined with this pair of primers (Table 1), the DNA from only two individuals (the mother and the son of the present study) bearing the *Xba*I-9 kb allele was able to produce the fragment of expected size (Figure 4). Subsequently, we have encountered two other *Xba*I-9 kb alleles and two common *Xba*I-16+9 kb alleles in Caucasian families, three of which bear the D6B mutation (Table 1). None of these samples exhibited abnormal profiles for *Bam*HI and *Nco*I enzymes or give amplified product in PCR experiments using the

above-mentioned *CYP2D7/CYP2D6* specific primer combination.

Direct sequencing of the *Xba*I-9 kb specific PCR product obtained with DNA of the son revealed (Table 2) that i) exon 1 carries sequence features of the *CYP2D7* gene, in particular the characteristic T₂₂₆ insertion (as compared with the reference sequence of the *CYP2D6* gene [9]), ii) the sequence of intron 1 is characteristic of the *CYP2D7* gene up to nucleotide C₆₈₈ (deleted in *CYP2D7* but not in *CYP2D6*) after which the sequences of *CYP2D6* and *CYP2D7* were indistinguishable up to nucleotide 833, iii) further downstream, nucleotide C at position 834 is characteristic of the *CYP2D6* gene and iv) exon 2 corresponds to the known sequence of the *CYP2D6* gene.

Table 1 Genotypes of individuals tested with primers specific for the rearranged 5' *CYP2D7-CYP2D6* 3' chimeric gene described in this study. wt : wild type in which no known mutation (D6A, D6B and D6D) was detected

Genotypes	n individuals
29kbwt/29kbwt	64
29kbwt/29kbD6B	20
29kbD6B/29kbD6B	3
29kb/D6D	4
29kbD6B/D6D	1
29kbwt/44kbwt	2
29kbwt/44kbD6B	11
29kbD6B/29kbD6B	1
29kbwt/16+9kbD6B	2
29kbwt/9kbwt	1
29kbwt/9kbD6B	1

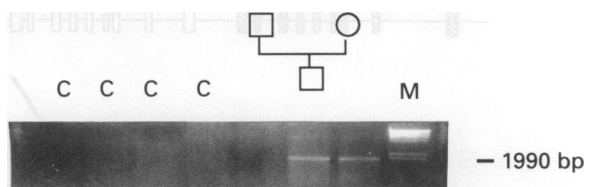


Figure 4 Amplification of a region which includes exon 1-intron 1-exon 2 of the presumably fused *CYP2D7/CYP2D6* gene using a pair of primers, one specific to a region upstream from the *CYP2D7* gene and the other to a region in intron 2 of the *CYP2D6* gene. The results of PCR-amplification as visualized in ethidium bromide stained polyacrylamide gel for DNA from both parents and the son as well as from four controls (C) are shown (M : DNA molecular-weight marker (Boehringer II)).

Discussion

Overall, the data show that the XbaI-9 kb allele clearly corresponds to a hybrid 5' *CYP2D7/CYP2D6* 3' gene and must probably have arisen through homologous but unequal crossing-over between sequences of the *CYP2D7* and the *CYP2D6* genes within a region in intron 1, where both genes have complete nucleotide sequence homology (Figure 5). The hypothesized second abnormal chromosome shown to arise from this recombination event could correspond to the previously described XbaI-44 kb fragment which bears a 5' *CYP2D6/CYP2D7* 3' chimeric gene designated *CYP2D7B* [20].

The fused *CYP2D7/CYP2D6* genes give rise to the PM phenotype. It is likely that the molecular basis for this phenotype is due to the presence of exon 1 of *CYP2D7* gene (instead of *CYP2D6*) which differs from *CYP2D6* gene by a single nucleotide insertion (T_{226}). This causes a shift in the reading frame with a premature termination of translation at amino acid 253 in exon 5. Such a truncated protein would lack the critical sequence of a functional cytochrome P450 enzyme.

This novel PM allele does not appear to be frequent among Caucasians as revealed by our study of 110 individuals with known genotypes. In this study two XbaI-16+9 kb alleles as well as two XbaI-9 kb alleles were identified. These latter alleles failed to produce any PCR product with a *CYP2D7* and *CYP2D6* specific primer combination, which otherwise amplifies the novel XbaI-9 kb allele described here. Furthermore, they did not exhibit abnormal restriction profiles with the various enzymes used to examine the XbaI-9 kb-associated 5' *CYP2D7/CYP2D6* 3' chimeric gene. Therefore, it is obvious that these alleles differ from the one described in the present study and probably arise from different types

Table 2 Comparison of exon 1, intron 1 and exon 2 sequences of *CYP2D7*, *CYP2D6* genes, and the XbaI-9 kb allele

<i>CYP2D7</i>	XbaI-9 kb allele	<i>CYP2D6</i>
Exon 1		
A ₁₁₉	A	G
A ₁₆₅	A	G
G ₁₉₀	G	A
T ₁₉₆	T	C
T ₂₂₆	T	Deleted
Intron 1		
C ₃₀₂	C	G
A ₃₀₉	A	C
G ₃₁₁	G	C
C ₃₁₅	C	T
CC ₃₂₀₋₃₂₁	CC	GA
G ₃₃₃	G	A
T ₃₄₂	T	G
T ₃₄₂	T	C
G* ₃₅₈	G	G
G ₄₀₅	G	A
A ₄₁₁	A	G
Deleted	Deleted	C ₆₈₈
G ₈₃₄	C	C
G ₉₃₁	T	T
Exon 2		
A ₁₀₆₂	C	C
G ₁₀₇₂	A	A
G ₁₀₈₅	C	C
C ₁₁₀₂	T	T
TA ₁₁₁₀₋₁₁₁₁	AC	AC
G ₁₁₁₆	A	A
C ₁₁₂₄	T	T

* : G at position 398 (creating a constant BsaH1 site) was missing in the original sequence reported by Kimura *et al.* [9]. We confirmed its presence by sequencing and restriction enzyme analysis of this region in *CYP2D7*-specific PCR products obtained with several DNA from unrelated EM individuals.

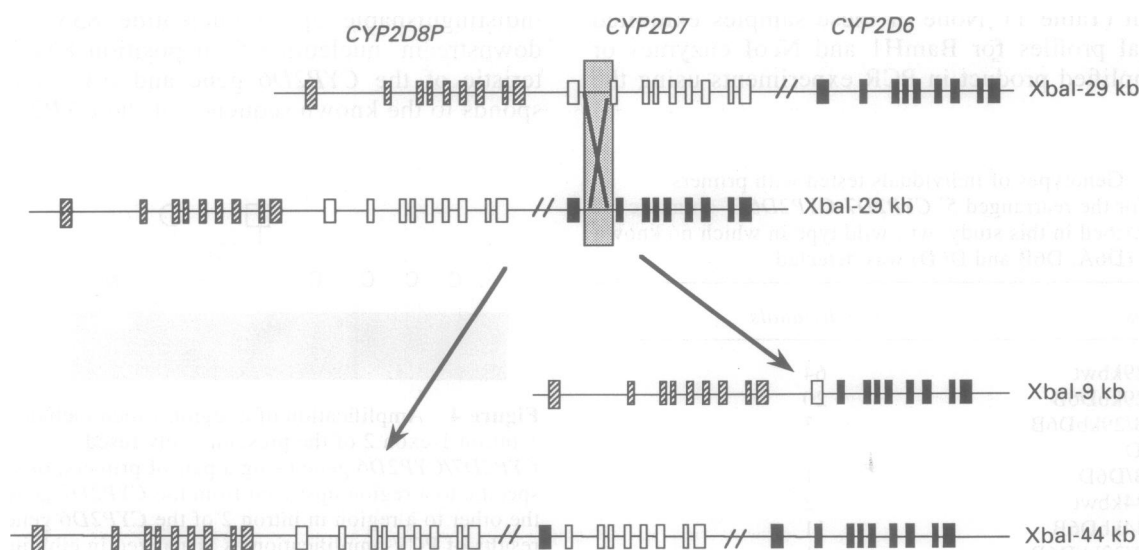


Figure 5 The predicted model for generating the XbaI-9 kb and the related XbaI-44 kb alleles of the *CYP2D* locus through unequal crossing-over between two parent XbaI-29 kb alleles in intron 1 region of the *CYP2D6* and *CYP2D7* genes.

of rearrangement of the *CYP2D* locus or very likely from polymorphisms of the XbaI restriction site. Taken together, these data reveal the molecular heterogeneity of the XbaI defined 9 kb allele in a similar manner to that demonstrated for the XbaI defined 44 kb alleles [21].

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References

- Maghoub A, Idle JR, Dring LG, Lancaster R, Smith RL. Polymorphic hydroxylation of the debrisoquine in man. *Lancet* 1977; **ii**: 584–586.
- Eichelbaum M, Spannbrucker N, Steincke B, Dengler HJ. Defective N-oxidation of sparteine in man: a new pharmacogenetic defect. *Eur J Clin Pharmacol* 1979; **16**: 183–187.
- Evans DAP, Maghoub A, Sloan TP, Idle JR, Smith RL. A family and population study of the genetic polymorphism of debrisoquine oxidation in a white British population. *J Med Genet* 1980; **17**: 102–105.
- Alvan G, Betchel P, Iselius L, Gundert-Remy U. Hydroxylation polymorphisms of debrisoquine and mephenytoin in European populations. *Eur J Clin Pharmacol* 1990; **39**: 533–537.
- Gonzalez FJ, Skoda RC, Kimura S, *et al.* Characterization of the common genetic defect in humans deficient in debrisoquine metabolism. *Nature* 1988; **331**: 442–446.
- Gough AC, Miles JS, Spurr NK, *et al.* Identification of the primary gene defect at the cytochrome P450 CYP2D locus. *Nature* 1990; **347**: 773–776.
- Gonzalez FJ, Vilbois F, Hardwick JP, *et al.* Human debrisoquine 4-hydroxylase (P450IID1): cDNA and deduced amino acid sequence and assignment of the CYP2D locus to chromosome 22. *Genomics* 1988; **2**: 174–179.
- Gough AC, Smith CAD, Howell SM, Wolf CR, Bryant SR, Spurr NK. Localization of the CYP2D gene locus to human chromosome 22q13.1 by polymerase chain reaction, *in situ* hybridization, and linkage analysis. *Genomics* 1992; **15**: 430–432.
- Kimura S, Umeno M, Skoda RC, Meyer UA, Gonzalez FJ. The human debrisoquine 4-hydroxylase locus: sequence and identification of the polymorphic CYP2D6 gene, a related gene and a pseudogene. *Am J Hum Genet* 1989; **45**: 889–904.
- Skoda RC, Gonzalez FJ, Demierre A, Meyer UA. Two mutant alleles of the human cytochrome P450 db1 gene associated with genetically deficient metabolism of debrisoquine and other drugs. *Proc Natl Acad Sci USA* 1988; **85**: 5240–5243.
- Hanioka N, Meyer UA, Gonzalez FJ. The human CYP2D locus associated with a common genetic defect in drug oxidation: a G₁₉₃₄→A base change in intron 3 of a mutant CYP2D6 allele results in an aberrant 3' splice recognition site. *Am J Hum Genet* 1990; **47**: 994–1001.
- Kagimoto M, Heim M, Kagimoto K, Zeugin T, Meyer UA. Multiple mutations of the human cytochrome P450IID6 gene in poor metabolizers of debrisoquine. *J Biol Chem* 1990; **265**: 17209–17214.
- Gaedigk A, Blum M, Gaedigk R, Eichelbaum M, Meyer UA. Deletion of the entire cytochrome P450IID6 gene as a cause of impaired drug metabolism in poor metabolizers of debrisoquine/sparteine polymorphism. *Am J Hum Genet* 1991; **48**: 943–950.
- Saxena R, Shaw GL, Relling MV, *et al.* Identification of a new variant CYP2D6 allele with a single base deletion in exon 3 and its association with the poor metabolizer phenotype. *Hum Mol Genet* 1994; **3**: 923–926.
- Evert B, Griese EU, Eichelbaum M. A missense mutation in exon 6 of the CYP2D6 gene leading to a histidine 324 to proline exchange is associated with the poor metabolizer phenotype of sparteine. *Naunyn-Schmiedeberg's Arch Pharmacol* 1994; **350**: 434–439.
- Daly AK, Armstrong M, Monkman SC, Idle ME, Idle JR. Genetic and metabolic criteria for the assignment of debrisoquine 4-hydroxylation (cytochrome P4502D6) phenotypes. *Pharmacogenetics* 1991; **1**: 33–41.
- Mura C, Panserat S, Vincent-Viry M, Galteau MM, Jacqz-Aigrain E, Krishnamoorthy R. DNA haplotype dependency of debrisoquine 4-hydroxylase (CYP2D6) expression among extensive metabolizers. *Hum Genet* 1993; **92**: 367–372.
- Panserat S, Mura C, Gerard N, *et al.* DNA haplotype dependant amino acid variations in debrisoquine 4-hydroxylase (CYP2D6): evidence for two major allozymes among extensive metabolizers. *Hum Genet* 1994; **94**: 401–406.
- Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* 1977; **74**: 5463–5467.
- Heim MH, Meyer UA. Evolution of a highly polymorphic human cytochrome P450 gene cluster: CYP2D6. *Genomics* 1992; **14**: 49–58.
- Mura C, Gerard N, Vincent-Viry M, Galteau MM, Jacqz-Aigrain E, Krishnamoorthy R. Molecular heterogeneity of the XbaI defined 44 kb allele of the CYP2D locus within the caucasian population. *Br J Clin Pharmacol* 1993; **35**: 161–165.
- Mura C, Broyard JP, Jacqz E, Elion J, Krishnamoorthy R. NcoI RFLP in the pseudogene (CYP2D8P) of the human debrisoquine 4-hydroxylase. *Nucleic Acids Res* 1991; **19**: 1162.

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