A novel mutant variant of the CYP2D6 gene (CYP2D6*17) common in a black African population: association with diminished debrisoquine hydroxylase activity

COLLEN MASI MIREMBWA1, IRENE PERSSON1, LEIF BERTILSSON2, JULIA HASLER3 & MAGNUS INGELMAN-SUND BERG1
1Department of Medical Biochemistry and Biophysics, Karolinska Institutet, S-17177 Stockholm, Sweden and 2Department of Medical Laboratory Sciences and Technology, Huddinge University Hospital, Huddinge, Sweden and 3Department of Biochemistry, University of Zimbabwe, Harare, Zimbabwe

1 The debrisoquine hydroxylase (CYP2D6) is polymorphically distributed. Not only are there differences in the proportions of extensive metabolisers to poor metabolisers in various ethnic groups, but there are also pronounced variations in the metabolic capacity among those classified as extensive metabolisers.

2 The mean debrisoquine metabolic ratio of Caucasian extensive metabolisers is lower than that for a number of African populations. In the present study, we have searched for novel CYP2D6 mutations to explain the diminished enzyme activity in African populations.

3 Three Zimbabwean Shona subjects with EM phenotypes (metabolic ratios for debrisoquine of 0.4, 1.5 and 10.5 respectively) were selected and the open reading frame of the CYP2D6 gene of each was sequenced.

4 The subject with metabolic ratio of 10.5 was found to be homozygous for an allele with a nucleotide exchange in exon 2, 1111C→T causing a 107Thr→Ile amino acid exchange in a conserved region of the enzyme. In addition, he was homozygous for the 2938C→T and 4268G→C mutations causing 296Arg→Ser and 486Ser→Thr amino acid substitution found in the CYP2D6*2 allele.

5 Seventy-six Zimbabwean Shona subjects were subsequently genotyped for the 1111C→T mutation and for the intron 1 gene conversion present in the CYP2D6*2 gene. The 1111C→T mutation was found at an allele frequency of 34% and was only present in alleles carrying the gene conversion in intron 1 indicative for the CYP2D6*2 gene.

6 This allele (CYP2D6*17), containing the 1111C→T, 2938C→T and 4268G→C mutations, was found to be strongly associated with lower capacity for debrisoquine hydroxylation. We therefore postulate that the CYP2D6*17 allele might contribute to the molecular basis of the previously established diminished debrisoquine hydroxylase activity in African Bantu populations.

Keywords African population Bantu Zimbabwe drug metabolism debrisoquine genetic polymorphism interethnic differences

* In this paper we have used the new nomenclature for the CYP2D6 alleles [1]. Accordingly, the following allelic designations is used for those CYP2D6 genes here discussed: CYP2D6*2, CYP2D6*3, CYP2D6*4, CYP2D6*6, CYP2D6*9, CYP2D6*10, CYP2D6*17, CYP2D6Z.
Introduction

The genetic polymorphism of debrisoquine hydroxylase (CYP2D6) has been a subject of great interest, particularly as the enzyme metabolises over 40 clinically important drugs [1, 2]. Clinical implications of the poor metaboliser (PM) vs the extensive metaboliser (EM) phenotypes have been investigated and these studies showed that PM subjects experience side effects at normal doses of drugs that are substrates of CYP2D6 [2]. Using debrisoquine as a probe drug, the prevalence of PMs is 0–1% in Orientals (Chinese, Koreans and Japanese) [3], 5–10% in Caucasians (Europeans) [4] and 0–5% in some black African populations [5, 6]. The molecular genetic basis for the poor metaboliser status in various populations has been shown to be the homozygous occurrence of any of a number of defective alleles, the most common being the CYP2D6*5 (frame shift mutation), CYP2D6*4 (splice mutation) CYP2D6*9 (gene deletion) and CYP2D6*6 (frame shift) [7–11].

Attention has now also been directed to the EM category since within this group there is a variation in the debrisoquine metabolic ratios (MRs) of up to 100-fold [12]. The debrisoquine MRs in EMs can therefore, be divided into ultrarapid EMs (MR < 0.2), intermediate EMs and slow EMs (MR > 1.0). Ultrarapid metabolisers, demanding very high doses of drugs which are substrates of CYP2D6 in order to attain therapeutic levels [13], have been observed in Caucasians to a greater extent than in Orientals or black Africans [6, 10, 14]. The molecular basis of the enhanced metabolic capacity was found to be gene duplication or gene amplification of the CYP2D6*2 allele and subjects with up to 13 functional copies have been described [15–17].

In a previous study on a Zimbabwean Shona population, as well as in retrospective analysis of phenotyping studies done in other black African populations, a general tendency towards higher metabolic ratios for CYP2D6 probe drugs can be noted [6]. The low frequency in the Zimbabwean population of the CYP2D6*10 variant and the lack of the CYP2D6*9 allele, both of which encode an enzyme with low debrisoquine hydroxylase activity in Orientals and Caucasians, respectively [18–20], showed that these alleles could not account for the shift to higher metabolic ratios in the black Bantu population. Thus, in order to establish the genetic basis for the diminished CYP2D6 activity among EMs in the Zimbabwean population, we have investigated the CYP2D6 sequence in several individuals with known phenotypes.

Methods

DNA samples

DNA was isolated from 76 subjects who participated in our previous CYP2D6 phenotyping and genotyping studies on a Zimbabwean Shona population [6]. Analysis of the number of genes in the locus, and the genotype for CYP2D6*3, *4, *9 and *10 alleles in these subjects were performed in the previous study [6] with methods described [19–21].

Materials

Heat stable DNA polymerase was purchased from Advanced Biotechnologies, London, UK and PCR primers were performed on a Perkin Elmer Thermocycler 480. Sequenase version 2.0 T7 DNA polymerase and the Sequenase kit used were from United States Biochemicals. Allele specific primers were obtained from KEBO (Sweden). All chemicals used were of highest available quality.

CYP2D6 gene sequencing

CYP2D6 specific primers were used to amplify segments of the gene and single strands were generated by using biotinylated primers and streptavidin (Dynal) separation, or asymmetric PCR. For amplification of specific exons of the CYP2D6 gene, 35 cycles of PCR on genomic DNA (200 ng) were performed as follows: initial denaturation at 94 °C for 1.5 min, thirty-five cycles of 1 min at 94 °C (denaturation), 1.5 min at either 52 °C or 60 °C (primer annealing), 1.5 min at 72 °C (elongation) and a final extension period of 7 min at 72 °C. Magnesium concentrations were titrated for the different primers for optimal (specific) amplifications. Asymmetric PCR was performed under the same conditions as above with minor modifications, 5 μL of the first PCR and an excess of one of the primers flanking the exon of interest was used. Shorter annealing and elongation times of 30 s were used and only 25 cycles were run. The PCR products were analysed on agarose gel and single stranded DNA (ssDNA) was visible as a band additional to the original one. The ssDNA was precipitated and then dissolved in water, ready for sequencing. Where biotinylated primers were used, streptavidin coated magnetic beads (Dynal) and alkaline denaturation was used to separate the strands from the first PCR reaction. The non-biotinylated strand was also used for sequencing after neutralisation with HCl. Table 1 shows the primers used for the amplification of CYP2D6 exons and for sequencing. Sequencing was performed by the double-stranded dideoxynucleotide chain-termination method on all exons from both directions, covering the intron-exon junctions.

PCR-based allele-specific analysis

Genotyping for the exon 2 1111C→T mutation From sequence analysis, a mutation (1111C→T) was identified in exon 2 of an extensive metaboliser with a high debrisoquine metabolic ratio. A PCR-based genotyping assay for this mutation was developed so that all 76 DNA samples could be assessed for the mutation.
Exon 3 and 4: Pr 1: 5’-ATTTCCAGCTGGAGAATCC-3’ (F)
Pr 2: 5’-GAGACTTCCTGGTCTCTC-3’ (R)

Exon 5 + 6: Pr ex5F: 5’-GGCTGAGACTCTTGCAGG-3’ (F)
Pr 4: 5’-CAGGAGGCCAGGGAGAGGAC-3’ (R)

Exon 7 + 8 + 9: Pr ex7F: 5’-CACATAGGGAGCGAGAAG-3’ (F)
Pr 16R: 5’-ATATAGCTCCCTGAGCC-3’ (R)

CYP2D6*17: Pr ex2F: 5’-CCAGAGTTCAAATAGGACTA-3’ (F)
Pr ex2R: 5’-CTTCTGAGGAGCAGGACG-3’ (R)

CYP2D6*2: Pr ex10F: 5’-CTTCTGAGGAGCAGGACG-3’ (F)
Pr ex10B: 5’-CTTCTGAGGAGCAGGACG-3’ (R)

*Primer direction: (F)-forward, (R)-reverse.
*Biotinylated primer.

Table 1 Primers for PCR and sequencing of CYP2D6 exons 1–9, and allele specific PCR for CYP2D6*17

<table>
<thead>
<tr>
<th>Site</th>
<th>Name</th>
<th>Sequence</th>
<th>Exon</th>
<th>Name</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 1 + 2</td>
<td>Pr 9F</td>
<td>5’-ACCAGGCCCCCTCCACCCCGG-3’ (F)*</td>
<td>1  Pr 1</td>
<td>Pr ex1F</td>
<td>5’-GGTGGGGTGGCAGCGTG-3’ (F)</td>
</tr>
<tr>
<td></td>
<td>Pr ex2R</td>
<td>5’-CTCCTGACCCACCTCGGG-3’ (R)</td>
<td>2  Pr 2</td>
<td>Pr ex1R</td>
<td>5’-GCTCTGAGGAGGAGGC-3’ (R)</td>
</tr>
<tr>
<td>Exon 3 + 4</td>
<td>Pr 1</td>
<td>5’-ATTTCCAGCTGGAGAATCC-3’ (F)</td>
<td>3  Pr 1</td>
<td>5’-ATTTCCAGCTGGAGAATCC-3’ (F)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pr 2</td>
<td>5’-GAGACTTCCTGGTCTCTC-3’ (R)</td>
<td>4  Pr 7</td>
<td>5’-CAGAAGGAGGCGTCC-3’ (R)</td>
<td></td>
</tr>
<tr>
<td>Exon 5 + 6</td>
<td>Pr ex5F</td>
<td>5’-GGCTGAGACTCTTGCAGG-3’ (F)</td>
<td>5  Pr 5</td>
<td>5’-GGCGGCTGACACCTTCTCTT-3’ (R)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pr 4</td>
<td>5’-CAGGAGGCCAGGGAGAGGAC-3’ (R)</td>
<td>6  Pr 5</td>
<td>5’-GGCGGCTGACACCTTCTCTT-3’ (R)</td>
<td></td>
</tr>
<tr>
<td>Exon 7 + 8 + 9</td>
<td>Pr ex7F</td>
<td>5’-CACATAGGGAGCGAGAAG-3’ (F)</td>
<td>7  Pr 5</td>
<td>5’-CACATCGGGGAGGAGGAC-3’ (F)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pr 16R</td>
<td>5’-ATATAGCTCCCTGAGCC-3’ (R)</td>
<td>8  Pr 5</td>
<td>5’-CACATCGGGGAGGAGGAC-3’ (F)</td>
<td></td>
</tr>
<tr>
<td>CYP2D6*17</td>
<td>Pr ex2F</td>
<td>5’-CCAGAGTTCAAATAGGACTA-3’ (F)</td>
<td>9  Pr 5</td>
<td>5’-GGCGGCTGACACCTTCTCTT-3’ (F)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pr ex2R</td>
<td>5’-CCAGAGTTCAAATAGGACTA-3’ (R)</td>
<td>Pr ex9R</td>
<td>5’-CTACCACTGCTTAT-3’ (R)</td>
<td></td>
</tr>
<tr>
<td>CYP2D6*2</td>
<td>Pr ex10F</td>
<td>5’-CTTCTGAGGAGCAGGACG-3’ (F)</td>
<td>Pr ex9R</td>
<td>5’-CTACCACTGCTTAT-3’ (R)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pr ex10B</td>
<td>5’-CTTCTGAGGAGCAGGACG-3’ (R)</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

Genotyping for the CYP2D6*2 variant: From sequence analysis, the exon 2 mutation was also associated with the mutations in exon 3, 6 and 9 found in the CYP2D6*2 allele. Previously, Johansson et al. [15] have shown that the CYP2D6*2 variant always is associated with a mutation in intron 1 (a gene conversion event from pseudogene CYP2D7), and that the genotype of the former can be established by analysis of the latter. To identify the CYP2D6 genotype with respect to the CYP2D6*2 variant we therefore analysed the intron 1 sequence. A primer in the pre-exon 1 sequence (Pr 9B) and a primer in the intron 1 sequence (either one with the wild type sequence, Pr 10, or the one, Pr 10B, specific for the sequence between bp302 and 333 in intron 1, which is identical to the converted sequence from pseudogene CY2D7/P), were used for allele-specific amplification [15].

Results

All nine exons of CYP2D6 in DNA from three subjects, Z69 (metabolic ratio, 0.4), Z5 (metabolic ratio, 1.5), and Z38 (metabolic ratio, 10.5) were sequenced. No nucleotide differences were found in the sequences of exons 1, 4, 5, 7, and 8 compared with the wild type published sequences [22]. Table 2 shows the mutations found in exons 2, 3, 6, and 9. A novel mutation was found in exon 2 of DNA from Z5 and Z38, whereas the other mutations in exons 3, 6, and 9 are those characteristic of the CYP2D6*2 allele. Figure 1 shows sequencing results for Z69 and Z38 illustrating the C→T mutation found at base pair 1111 in exon 2 of Z38 with the wild type sequence exhibited by Z69. This allele, thus containing the 1313C→T, 1726G→C, 2091C→T and
wt/wt, wt/mt assay developed for the exon 2 mutation. All subjects with *10 and one with the variant by the intron 1 analysis method [15]. Figure 2 Three individuals with the *17 allele were also omitted CYP2D6*2 carrying this mutation also had the allele and those with one or two alleles bearing CYP2D6*17 conversion into the CYP2D6 sequence in intron 1 derived from CYP2D7P. We report here on a mutant variant of the CYP2D6 genes of the three DNAs sequenced from Zimbabwean subjects. A significant difference between the MRs in the Hardy–Weinberg principle predicting the frequency of the MR allele co-incides with diminished debrisoquine hydroxylase activity. The allele does not occur without post-test for comparisons between the individual groups, it is evident that the CYP2D6*2 allele does not significantly influence the enzyme activity, whereas the CYP2D6*17 allele, is strongly associated with metabolic ratios that are higher than those of the wild-type and CYP2D6*2 allele combinations (Table 3). Figure 3 shows the frequency distribution of debrisoquine metabolic ratios for 59 subjects classified as extensive metabolisers in a previous study [6]. In this figure, only subjects of XbaI 29/29 kb haplotype (without CYP2D6*3, *4, *5, *9 and *10 mutations or other known XbaI haplotype variants) were included so as to illustrate the relationship of the CYP2D6*17 allele with the MR for debrisoquine. The subjects with the CYP2D6*2 allele were included in the wild type group since it is apparent from Table 3 and the findings of [15] that this variant has no great influence on the debrisoquine MR unless present in multiple copies. It is evident from the Figure 3 that the presence of the CYP2D6*17 allele co-incides with diminished debrisoquine hydroxylase activity.

Table 2 Mutations in the CYP2D6 genes of the three DNAs sequenced from Zimbabwean subjects

<table>
<thead>
<tr>
<th>Location</th>
<th>Mutation</th>
<th>Amino acid change</th>
<th>Z69 (0.4)</th>
<th>Z5 (1.5)</th>
<th>Z38 (1.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exon 2</td>
<td>1111C→T</td>
<td>1111Thr→Ile</td>
<td>wt/wt</td>
<td>wt/mt</td>
<td>mt/mt</td>
</tr>
<tr>
<td>Exon 3</td>
<td>2964G→C</td>
<td>Silent</td>
<td>wt/wt</td>
<td>wt/mt</td>
<td>mt/mt</td>
</tr>
<tr>
<td>Exon 6</td>
<td>2964G→C</td>
<td>2964Arg→Cys</td>
<td>wt/wt</td>
<td>wt/mt</td>
<td>mt/mt</td>
</tr>
<tr>
<td>Exon 9</td>
<td>4603G→C</td>
<td>4603Ser→Thr</td>
<td>wt/wt</td>
<td>wt/mt</td>
<td>mt/mt</td>
</tr>
</tbody>
</table>

All the other exons had sequences identical to those reported for the wildtype allele [22].

Discussion

We report here on a mutant variant of the CYP2D6 gene in a black African population termed CYP2D6*17. This allele contains a 1111C→T mutation in exon 2, together with the previously characterised mutations in exon 3, 6, and 9 and the gene conversion in intron 1 which are characteristics of CYP2D6*2. In the population studied, the CYP2D6*2 allele does occur without the CYP2D6*17 exon 2 mutation, whereas the opposite combination was not found. The CYP2D6*17 allele was present at a very high frequency of 34% and obeys the Hardy–Weinberg principle predicting the frequency distribution of a mutant allele in a population. Since previously described defective alleles, such as...
CYP2D6 mutant variant in an African population

Figure 2  (a) A schematic diagram of the strategy used for allele specific genotyping of the 311C→T mutation of the CYP2D6*17 allele. A gene specific fragment of 1417 bp was first amplified by PCR and the 311C→T mutation was evaluated in a second PCR step using mutation specific and wild type specific primers. (b) Agarose gel separation of the PCR amplification products obtained in the first (top) and the second PCR steps (bottom).

Table 3  The effect of CYP2D6*2 and CYP2D6*17 (CYP2D6*2 plus the exon 2 311C→T mutation) on debrisoquine metabolic ratios

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Debrisoquine MR</th>
<th>mean*</th>
<th>median (25 and 75 percentiles)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(number of subjects)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>wt/wt (n=12)</td>
<td>0.56</td>
<td>0.46</td>
<td>(0.41–0.61)</td>
</tr>
<tr>
<td>wt/*2 (n=13)</td>
<td>0.59</td>
<td>0.54</td>
<td>(0.48–0.74)</td>
</tr>
<tr>
<td>*2/*2 (n=1)</td>
<td>1.34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>wt/*17 (n=23)</td>
<td>1.45</td>
<td>1.25</td>
<td>(1.04–1.56)</td>
</tr>
<tr>
<td>*17/*17 (n=10)</td>
<td>3.94</td>
<td>3.30</td>
<td>(2.87–3.57)</td>
</tr>
</tbody>
</table>

*a Antilog of mean logarithmic MR for debrisoquine.

Differences between the individual groups were assessed by the Mann–Whitney U test:

b wt/*2 not significantly different from wt/wt (P > 0.7).

c wt/*17 significantly different from wt/wt and wt/*2 (P < 0.001 and P < 0.001).

d *17/*17 significantly different from wt/wt, wt/*2 and wt/*17 (P < 0.001, P < 0.001 and P < 0.001).

CYP2D6*2, *3, *4, *5, *9 and *10, occur at a very low frequency in the Zimbabwean Shona population [6], their contribution to the general shift towards higher debrisoquine MRs in the population is minor. Our results indicate a convincing association of the CYP2D6*17 allele with high debrisoquine metabolic ratios (diminished enzyme activity) of extensive metabolisers. The Zimbabwean Shona population belongs to the Bantu people (black Africans populating the region from Cameroon to Central and Eastern Africa, and most of Southern Africa) who are genetically closely related [23]. We have examined the CYP2D6*17 prevalence in some Oriental (Chinese) and Caucasian (Swedish) subjects where it was absent (Oscarsson et al., unpublished data). We have, however, found it among Ethiopians, a non-Bantu African population, at an allele frequency of 10% and associated with diminished enzyme activity [24]. It is conceivable that the molecular basis for diminished CYP2D6 enzyme activity in Bantu populations is the high frequency of occurrence of the CYP2D6*17 allele.

The 311C→T mutation in exon 2 causes an amino acid change from the hydrophilic 107Thr to the hydrophobic Ile. This change takes place in a region (β-helix) which is shown to be conserved in alignment studies between CYP2D6 and CYP101 [25]. This amino acid change may therefore disturb the structure of CYP2D6. The 107Thr is located in a region corresponding to a putative substrate-recognition site (SRS1) in Gotoh's model of six SRSs for the CYP2 family [26]. Amino...
acid residues identified as being critical for substrate specificity of CYP2 forms fall within or near the putative SR6s [27]. In addition, using chimeric constructs of the CYP2D6*4 allele which contains three mutations in exon 2, [28] showed that two of these mutations caused impaired enzyme function. The chimeric constructs causing ^180Leu→Met and ^188His→Arg changes gave rise to enzymes with only 25% and 50% of wild type activity respectively. Further studies in different expression systems are needed to elucidate the molecular basis for the diminished enzyme activity of the CYP2D6*17 enzyme variant in detail.

No studies have been performed in African populations on the effect of various CYP2D6 phenotypes in relation to drug response and so it is difficult to demonstrate the clinical implications of diminished enzyme activity in these populations. However, it is possible to extrapolate the consequences of diminished activity in Orientals to Bantu populations. For example, diminished CYP2D6 activity has been proposed to explain why Orientals experience side effects to psychotropic medications [29] when prescribed in doses established in Caucasians [30]. For drugs that are substrates of CYP2D6 and have a narrow therapeutic window, it might, therefore, be desirable to reduce doses in Oriental and Bantu populations compared with Caucasian populations.

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