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ORIGINAL INVESTIGATION

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An additional allelic variant of the CYP2D6 gene causing impaired metabolism of sparteine

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Abstract The identification of a novel *CYP2D6* allele from a healthy Caucasian poor metabolizer was achieved by using a previously described polymerase chain reaction/single-strand conformation polymorphism strategy. Among the four point mutations that this allele carries, a missense mutation in exon 1 (212 G → A or D6–H) seems to be responsible for the loss of CYP2D6 function. Although the mutation D6-H has a low prevalence in a randomly selected population of healthy Caucasians, its identification should further increase the phenotype prediction rate by genotyping.

Introduction

The molecular mechanisms of the CYP2D6 polymorphism that results in a large interindividual variability in drug response (Tucker 1994) and in susceptibility to certain environmentally based diseases (Daly et al. 1994) are now well characterized. About ten allelic variants of the CYP2D6 gene have been described as being associated with the poor metabolizer (PM) phenotype and assays based on the polymerase chain reaction (PCR) are available to identify inactivating mutations in genomic DNA samples (Kagimoto et al. 1990; Saxena et al. 1994; Evert et al. 1994 a, b; Daly et al. 1995; Broly et al. 1996; Marez et al. 1995). However, these genotyping tests fail to detect unknown rare mutations and thus predict fewer than 100% of the PM individuals (Broly et al. 1991; Dahl et al. 1992; Brøsen et al. 1994).

We have recently developed a novel genotyping procedure that consists of single-strand conformation polymorphism analysis of DNA fragments amplified by the polymerase chain reaction (PCR-SSCP; Broly et al. 1995) and have demonstrated the use of this approach in identifying known mutations of the CYP2D6 gene and in screening simultaneously for new ones (Broly et al. 1996; Marez et al. 1995). Here, we report a new inactivating mutation and the characterization of a novel *CYP2D6* defective allele using the above strategy.

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Materials and methods

A DNA sample was taken from a healthy Caucasian volunteer classified as a pM of sparteine (metabolic ratio: 85.5) but genotyped as a heterozygote *CYP2D6*WT/CYP2D6*B* by conventional genotyping assays based on the mutation-specific PCR method (Heim and Meyer 1990). To characterize the subjects' *CYP2D6* alleles, the DNA sample was subjected to PCR-SSCP analysis, sequencing, and allele-specific PCR (AS-PCR) procedure, as previously reported (Marez et al. 1995). The sequence of each exon of the CYP2D6 gene and of its proximal flanking regions was verified using a method and sequencing primers previously described (Broly et al. 1995). The polyadenylation site of the gene was sequenced using previously described primers, viz., 5′-9 and P2 (Broly et al. 1995). In order to sequence the TATA-box region, a 1.6-kb fragment was amplified according to a PCR strategy previously reported (Marez et al. 1995), by using a pair of *CYP2D6*-specific primers (F1: ACCAGGCCCCTCCACCGG: –196 to –179; F2: CCGGATTCCAGCTGGGAAAT: 1404 to 1385). Both strands of the PCR product were sequenced using the forward primer described above and a reverse primer (F3: ACCTGCCTCACTACCAAATG: 88 to 69). A restriction analysis was developed to identify rapidly the mutation 212 G→A in 100 randomly selected healthy Caucasians (the numbering of the nucleotides corresponds to that used by Kimura et al. 1989). Briefly, a PCR fragment amplified as previously described (Marez et al. 1995) was digested at 25° C overnight by the *Sma*I endonuclease and then analyzed by ethidium bromide staining after migration on a 1.6% agarose gel.

Results and discussion

An exon-by-exon PCR-SSCP analysis (Broly et al. 1995) has been used to analyze a DNA sample from a PM subject suspected of carrying an unknown non-functional allele of the CYP2D6 gene. Only the PCR product en-

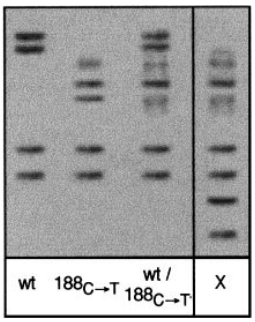

Fig. 1 SSCP analysis of the exon 1 of *CYP2D6* (*wt, 188* $_{C\rightarrow T}$ and *wt/188* $_{C\rightarrow T}$ correspond to the SSCP profiles observed for the PCR product of the exon 1 with a wild-type sequence, a known mutated sequence and a heterozygous sequence, respectively; *X* corresponds to a new SSCP pattern, resulting from unknown sequence alteration(s) in the exon 1 from a PM subject)

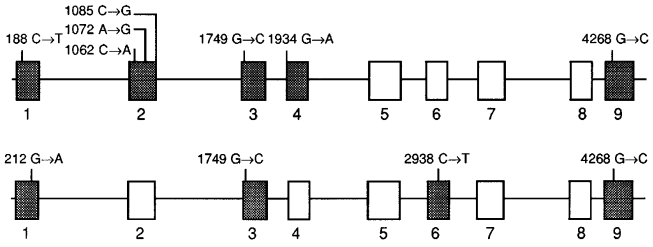

Fig. 2 Nature and position of mutations identified on both *CYP2D6* alleles of the studied PM subject (*filled boxes* exons carrying mutation(s), *open boxes* exons with a wild-type sequence; *upper allele* allele 1, *lower allele* allele 2)

compassing exon 1 shows an abnormal SSCP pattern when compared with control sequences (Fig. 1). Sequencing of the corresponding DNA region has revealed two heterozygous mutations of the *CYP2D6* gene, a known mutation 188 C→T and a new one 212 G→A. The two *CYP2D6* alleles can be distinguished by using an AS-PCR amplification followed by sequencing of each allele-specific fragment (Marez et al. 1995). The nature and the respective positions of all identified mutations on both alleles are depicted in Fig. 2. Our analysis has confirmed that the studied PM subject is a heterozygous carrier of the *CYP2D6*B* defective allele (Fig. 2, allele 1; see also Kagimoto et al. 1990). The other allele (Fig. 2, allele 2) corresponds to a variant of the active *CYP2D6*L* (1749 G→C, 2938 C→T, 4268 G→C; Johansson et al. 1993) and carries a missense mutation (GGG→AGG) at position 212.

Since it is generally accepted that the SSCP technique is not able to detect all sequence deviations (Sheffield et al. 1993), each exon of the *CYP2D6* and its proximal flanking regions have been sequenced to confirm SSCP results. In addition, to verify that the CYP2D6 activity deficiency is not related to sequence alterations in important regulatory regions of the gene, we have analyzed the TATA-box and the polyadenylation site by sequencing. No additional mutation has been identified. Thus, the unique sequence alteration identified as a possible inactivating mutation of *CYP2D6* is the 212 G→A, which results in a $Gly^{42}\rightarrow Arg^{42}$ substitution in the protein sequence.

Although this amino-acid exchange occurs in a region not suggested to participate in the active site of the enzyme (Koymans et al. 1993), its implication in the loss of CYP2D6 function or expression cannot be ruled out. Recently, a point mutation in the *CYP2D6* coding sequence (mutation D6-E) causing an amino-acid change has been described as being associated with the PM phenotype (Evert et al. 1994 a). In addition, several missence mutations in other polymorphic drug-metabolizing enzymes, such as N-acetyltransferase 2, have been shown to result in the loss of enzyme activity (Gonzalez and Idle 1994). The *in vitro* analysis, involving a heterologous expression system, of this novel allelic variant that we have named *CYP2D6*H* should permit us to confirm data obtained *in vivo*.

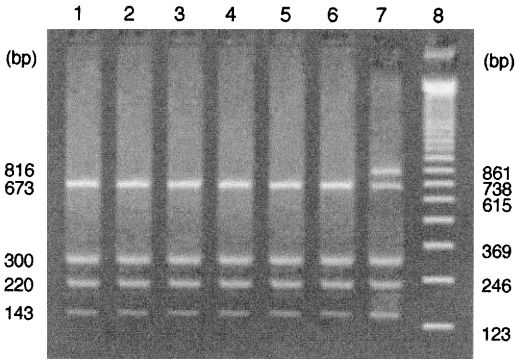

Fig. 3 Identification of the mutation 212 G → A by PCR-*Sma*I restriction analysis. The mutation abolishes a *Sma*I restriction site and generates an 816-bp fragment instead of the 673- and 143-bp fragments for the wild-type sequence. *Lanes 1–6* homozygote wt, *lane 7* heterozygote wt/212 G → A, *lane 8* DNA molecular weight marker, 123-bp ladder (Gibco BRL, Gaithersburg, USA)

The frequency of the mutation 212 G→A, termed D6-H and evaluated using a restriction analysis assay (Fig. 3), appears to be low in 100 unrelated healthy volunteers, since only one other heterozygous carrier of this mutation has been identified. This frequency of 0.01 is comparable to that of other rare *CYP2D6* inactivating mutations (Saxena et al. 1994; Evert et al. 1994 a, b; Daly et al. 1995; Broly et al. 1996; Marez et al. 1995). The identification of this additional mutation D6-H and of the novel defective allele *CYP2D6*H* further extends our knowledge of the molecular mechanism of CYP2D6 polymorphism and confirms the efficiency of our PCR-SSCP strategy as a genotyping method for accurate phenotype prediction.

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