

## Response: Letter to the Editor

### RESPONSE TO COMMENT ON "A FRAMESHIFT MUTATION AND ALTERNATE SPLICING IN HUMAN BRAIN GENERATE A FUNCTIONAL FORM OF THE PSEUDOGENE CYTOCHROME P4502D7 THAT DEMETHYLATES CODEINE TO MORPHINE"

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Cytochrome P450 (P450) enzymes play an important role in the metabolism of drugs and thereby are an important determinant of therapeutic outcome. Although liver is a major organ involved in P450-mediated metabolism, drug metabolism occurring in extrahepatic organs at the site of action of drugs is also important, since minor metabolic pathways become significant at the location of action. It is with this objective that our laboratory has been studying drug metabolism in brain with particular reference to human brain cytochromes P450. Our earlier studies (Pai et al., 2002) on metabolism of alprazolam by brain microsomes demonstrated the formation of higher amounts of the pharmacologically active metabolite ( $\alpha$ -hydroxy alprazolam) relative to the inactive metabolite (4-hydroxy alprazolam). This was the first indication that metabolism within the brain could differ from that in the liver. With this

such as the metabolism of codeine exclusively to morphine, unlike CYP2D6, which metabolizes codeine to both norcodeine and morphine. We have more recently reported the presence of an alternative splice variant of CYP1A1 that lacks 87 bp of exon 6 (Chinta et al., 2005). The presence of such distinctive enzymes generated by alternative splicing adds a new dimension to metabolism of drugs at the site of action.

As mentioned by Dr. Hoskins and colleagues, genotyping of CYP2D alleles is fraught with problems due to the high degree of homology between the members of this subfamily. Careful genotyping to separate CYP2D6 and CYP2D7 and CYP2D8 alleles is currently being carried out in our laboratory. Nevertheless, the unique sequence in the 57 bp of intron 6 that is present in the brain variant CYP2D7 (AY220845) clearly shows that it is indeed derived from CYP2D7. Notwithstanding this, the func-

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57 bp      AGGAAGGAGAGT-----GTCCCTGGGTGCTCACCCATTGTGGGGACGCATGCTGTCCAGT
CYP2D6     AAGAAGGAGTGTACAGGGCCGGACCCCTGGGTGCTGACCCATTGTGGGGACGCATGCTGTCCAGT

57 bp      AGGAAGGAGAGTGTTC-----CCCTGGGTGCTCACCCATTGTGGGGACGCATGCTGTCCAGT
CYP2D8     AGGCAGGAGAGTGTACAGGGCTGGTCCCTGGGTGCTGACCCATTGTGGGGACGCATGCTGTCCAGT

57 bp      AGGAAGGAGAGTGTCCCTGGGTGCTCACCCATTGTGGGGACGCATGCTGTCCAGT
CYP2D7     AGGAAGGAGAGTGTCCCTGGGTGCTGACCCATTGTGGGGACGCATGCTGTCCAGT
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FIG. 1. ClustalW alignment of the 57 bp of intron 6 with CYP2D6, CYP2D7, and CYP2D8.

background, we began our search for distinctive P450 enzymes that may be expressed in the human brain. Our focus was on the CYP2D enzyme, which metabolizes several psychoactive drugs. By screening a human cDNA library, we isolated a full-length clone, which translated into a functional P450. Sequencing revealed that the clone isolated by us belonged to the CYP2D subfamily and, importantly, contained 57 bp of intron 6. The 57 bp of intron 6 are unique to CYP2D7 and do not share the similar degree of homology with the intron 6 region of CYP2D6 or CYP2D8, as shown in Fig. 1.

CYP2D7, as stated by Hoskins et al. (2005) is a pseudogene due to a "T" insertion in exon 1 (138T), resulting in a frameshift and premature termination of translation (Kimura et al., 1989). The DNA sequence of the clone isolated by us (AY220845) had a 138delT mutation, which resulted in complete translation of the gene. The most important finding of the above paper (Pai et al., 2004) is that histospecific splicing could result in translation of a unique P450 enzyme in the brain, which is not expressed in other tissues involved in drug metabolism, such as liver or kidney. The alternate spliced P450 enzyme exhibits novel biotransformation pathways,

tional significance of the presence of alternately spliced, distinctive P450 enzymes in the human brain and, maybe, other extrahepatic organs with altered functionality cannot be overlooked.

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ABBREVIATIONS: CYP, cytochrome P450; bp, base pair(s).