FUNCTIONAL ANALYSIS OF CYP2A6*15 AND CYP2A6*16

Kazuma Kiyotani1, Masaki Fujieda1, Tsutomu Shimada2, F. Peter Guengerich2, Andrew Parkinson3, Goro Honda4, Kazuko Nakagawa5, Hiroshi Yamazaki1, and Tetsuya Kamataki1

1Graduate School of Pharmaceutical Sciences, Hokkaido University, Sapporo 060-0812, Japan, 2Vanderbilt University School of Medicine, Nashville, TN 37232-0146, USA, 3XenoTech, Lenexa, KS 66219, USA, 4Kokura Memorial Hospital, Kitakyusyu 802-8555, Japan, and 5Graduate School of Medical and Pharmaceutical Sciences, Kumamoto University, Kumamoto 862-0973, Japan.

Human cytochrome P450 2A6 (CYP2A6) is involved in the metabolism of several pharmaceuticals such as coumarin, nicotine, tegafur, and fadrozole. A wide inter-individual variation has been noted in the expression levels of CYP2A6 and its enzyme activities. To clarify the causes of this variation, we comprehensively analyzed the CYP2A6 gene of 33 Japanese and 28 Caucasian liver samples and discovered novel 23 single nucleotide polymorphisms (SNPs). The purpose of this study is to clarify the function of the novel non-synonymous SNPs, 2134A>G and 2161C>A (termed CYP2A6*15 and CYP2A6*16), located in exon 4. We determined the coumarin 7-hydroxylase and nicotine C-oxidase activities of CYP2A6.15 (Lys194Glu) and CYP2A6.16 (Arg203Ser) using E. coli expression system and human liver microsomes. The Vmax/Km values for coumarin 7-hydroxylation and nicotine C-oxidation catalyzed by CYP2A6.15 and CYP2A6.16 expressed in E. coli membranes were 30-60% of those of wild-type CYP2A6 enzyme. The Km values were increased by Arg203Ser substitution, suggesting that Arg203 was an important residue for substrate binding of CYP2A6. The Km values of liver microsomes from subjects possessing CYP2A6*16 alleles were also higher than those possessing wild-type. These results indicate that these two polymorphisms affect the disposition of drugs which are metabolized by CYP2A6.