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CYP2C19 polymorphisms account for inter-individual variability of drug metabolism in cynomolgus macaques

Yasuhiro Uno [a,*], Akinori Matsushita [a,b], Mitsunori Shukuya [c], Yasuka Matsumoto [c], Norie Murayama [c], Hiroshi Yamazaki [c,*]

[a] Pharmacokinetics and Bioanalysis Center, Shin Nippon Biomedical Laboratories, Ltd., Kainan 642-0017, Wakayama, Japan
[b] Drug Safety Research Center, Shin Nippon Biomedical Laboratories, Ltd., Kagoshima 891-1394, Kagoshima, Japan
[c] Laboratory of Drug Metabolism and Pharmacokinetics, Showa Pharmaceutical University, Machida 194-8543, Tokyo, Japan

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ABSTRACT

CYP2C19 (formerly known as CYP2C75), highly homologous to human CYP2C19, has been identified in cynomolgus and rhesus macaques, non-human primate species widely used in drug metabolism studies. *CYP2C19* is predominantly expressed in liver and encodes a functional drug-metabolizing enzyme. Genetic variants in human *CYP2C* genes account for the inter-individual variability in drug metabolism; however, genetic variants have not been investigated in macaque *CYP2C19*. In the present study, re-sequencing of *CYP2C19* in 78 cynomolgus and 36 rhesus macaques identified 34 non-synonymous variants. Among these, 6 were located in substrate recognition sites, the domains important for protein function. Eighteen and 6 variants were unique to cynomolgus and rhesus macaques, respectively. Four variants were characterized by site-directed mutagenesis and metabolic assays, and 3 variants (p.Phe100Asn, p.Ala103Val, and p.Ile112Leu) showed substantially reduced activity as compared with wild type in flurbiprofen 4′-hydroxylation, omeprazole 5-hydroxylation, and *R*-/*S*-warfarin 7-hydroxylation. These variants, co-segregating in the animals analyzed, influenced metabolic activities because the homozygotes and/or heterozygotes showed significantly reduced catalytic activities in liver toward flurbiprofen 4′-hydroxylation and omeprazole 5-hydroxylation as compared with wild type. Kinetic analysis for *R*-warfarin 7-hydroxylation and docking simulation indicated that CYP2C19 Ala103Val would change the function and conformation of this enzyme. Ala103Val variation diminished homotropic cooperativity of CYP2C19 with *R*-warfarin yielding low metabolic capacity. These results indicated that the interindividual variability of CYP2C-dependent drug metabolism is at least partly accounted for by *CYP2C19* variants in cynomolgus macaques.

1. Introduction

Human CYP2Cs, including CYP2C8, CYP2C9, and CYP2C19, are essential drug-metabolizing enzymes, involving the metabolism of approximately 20% of the prescribed drugs such as flurbiprofen and *S*-mephenytoin [1]. In the human *CYP2C* genes, a number of genetic variants have been identified (see http://www.cypalleles.ki.se/), including *CYP2C9*2* and *CYP2C9*3*. These alleles, resulting in the reduced activity of the enzyme, are more prevalent in Caucasians than in Africans while Asians do not appear to possess *CYP2C9*2* [2]. In human *CYP2C19*, *CYP2C19*2* and *CYP2C19*3*, accounting for the majority of poor metabolizer phenotypes, are more prevalent in Asians than in Caucasians or Africans, among which *CYP2C19*3* is largely found in Asians [2]. These defective alleles of human *CYP2C9* and *CYP2C19* genes need to be considered in drug metabolism studies during drug development.

Cynomolgus macaques (*Macaca fascicularis*), and rhesus macaques (*Macaca mulatta*), are non-human primate species widely used in drug metabolism studies. Cynomolgus CYP2C19, formerly known as CYP2C75, is highly homologous to human CYP2C9 and CYP2C19, and the gene is abundantly expressed in liver [3]. Cynomolgus CYP2C19 metabolizes human CYP2C substrates; *S*-mephenytoin, flurbiprofen, and tolbutamide [3,4]. The previous study reported that pharmacokinetics of *S*-mephenytoin was highly variable among 64 cynomolgus macaques [5], and such inter-animal variations might be accounted for by genetic variants.

* Corresponding authors.
E-mail addresses: uno-yasuhiro@snbl.co.jp (Y. Uno), hyamazak@ac.shoyaku.ac.jp (H. Yamazaki).

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Genetic polymorphisms have been found in macaque P450 genes [6–10]: however, the genetic variants have not been investigated in macaque *CYP2C19*.

In the present study, the genomes of 78 cynomolgus macaques (38 from Indochina and 40 from Indonesia) and 36 rhesus macaques were analyzed to identify *CYP2C19* genetic variants. Several identified alleles were analyzed by metabolic assays using flurbiprofen, omeprazole, and *S*-/*R*-warfarin as substrate with monkey liver microsomes and the proteins heterologously expressed in *Escherichia coli* (*E. coli*).

2. Materials and methods

2.1. Preparation of DNA and microsome samples

Whole blood samples were collected from 78 cynomolgus macaques (38 from Indochina and 40 from Indonesia, 4–5 years of age, weighing 3–5 kg) and 36 rhesus macaques (from China, 7 years of age, weighing 3–5 kg). From these samples, genomic DNA was prepared using the Puregene DNA isolation kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Microsome samples were prepared from the livers of 7 Indochinese cynomolgus macaques as described previously [11]. The study was reviewed and approved by the Institutional Animal Care and Use Committee of Shin Nippon Biomedical Laboratories, Ltd.

2.2. PCR amplification and DNA sequencing

Direct sequencing of *CYP2C19* exons was carried out using genome samples of 78 cynomolgus macaques and 36 rhesus macaques. PCR reactions were carried out in a mixture (20 μl) containing 1 ng of genomic DNA, 5 pmol of each primer, and 1 unit of ExTaq HS polymerase (Takara, Otsu, Japan). The *CYP2C19* gene fragments were amplified in the following condition: 95 °C for 2 min (1 cycle); 20 s at 95 °C, 30 s at 60 °C, and 1.5 min at 72 °C (35 cycles); and 10 min at 72 °C (1 cycle). Sequencing was performed using ABI PRISM BigDye Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems, Foster City, CA), followed by electrophoresis with the ABI PRISM 3730 DNA Analyzer (Applied Biosystems). The primers used for PCR and sequencing are listed in Table 1. Sequence analyses were conducted using DNASIS Pro (Hitachi Software, Tokyo, Japan) and Sequencher (Gene Codes, Ann Arbor, MI). Genetic variants were determined by comparison of the sequence with cynomolgus CYP2C19 cDNA sequence (GenBank accession no. DQ074805).

2.3. Site-directed mutagenesis and expression of CYP2C75 proteins

For functional analysis of CYP2C19 variants, protein variants were heterologously expressed in *E. coli* using coexpression plasmids with NADPH-cytochrome P450 reductase, and membrane preparation was performed, as previously described [3]. Each mutation was introduced into the expression plasmid of cynomolgus CYP2C19 using the QuikChange XL II kit (Agilent, Santa Clara, CA) according to the manufacturer's instructions. The primers used are listed in Table 1. For the CYP2C19 variant p.[Phe100Asn; Ala103Val; Ile112Leu], the expression plasmid was prepared using the plasmid for the variant p.Ile112Leu as a template, with the primers mfCYP2C75_c298/308 (5qc1) and mfCYP2C75_c298/308 (3qc1). The entire sequence of the insert was confirmed by sequencing.

Table 1
The primers used for amplification and site-directed mutagenesis in *CYP2C19*.

Exons			Sequence (5′ → 3′)
PCR			
1	F	mmCYP2C75 (5flk1a)	GTGCATTGGAACCACTTGG
	R	mmCYP2C75 (3int1a)	GAACACAACATTTCAAACTATCGACAC
	S	mmCYP2C75 (5flk1b)	CTCCAAAGTGGGCACTGG
2–3	F	mmCYP2C75 (5int1a)	CTCAAGCATGAGTGTTTGGGTAAG
	R	mmCYP2C75 (3int3a)	CCCACCATGTCCACTTGC
	S	mmCYP2C75 (5int1b)	TAGGCCATCTGAGTGGCAAG
	S	mmCYP2C75 (5int2_seq1)	CCTATTTCAGCATAGGTCATGG
4	F	mmCYP2C75 (5int3a)	CAGGATTAATGTAAAAGTGGTGCG
	R	mmCYP2C75 (3int4a)	GATCTATATTGGGACATTCATTTCTTG
	S	mmCYP2C75 (3int4_seq1)	GGCTGTATGGGCAAGACTGTAG
5	F	mmCYP2C75 (5int4a)	GAAATGATTATCATCTTTGATTCTCTGG
	R	mmCYP2C75 (3int5b)	CTTAAAATACTTCTCAAGCATTACTCC
	S	mmCYP2C75 (5int4_seq1)	ACAACCAGAGCTTGGTATATGGTATG
6	F	mmCYP2C75 (5int5a)	ATGAAAGAGGAAATGAAAGTGGAC
	R	mmCYP2C75 (3int6a)	AGAGATGCCCTACCCACCTTG
	S	mmCYP2C75 (5int5b)	CTACAGCCTCCGCTACACCAC
7	F	mmCYP2C75 (5int6a)	CCTATGTGTGTCTTTATAAGTGAAGTGTG
	R	mmCYP2C75 (3int7a)	CTATGACAGCAAACTTCTCTAATCTGATC
	S	mmCYP2C75 (3int7_seq1)	TCTCTCACCCAGTGATGGTAGA
8	F	mmCYP2C75 (5int7a)	CCAAAGTGATGGAATAGAGCAG
	R	mmCYP2C75 (3int8a)	CCTGCTTCTGTGGCTCATTATC
	S	mmCYP2C75 (5int7b)	GCACCCAAGTATCCAAGTACCGA
9	FS	mmCYP2C75 (5int8a)	CATCTATCTCCTCATCCCTCTTACA
	R	mmCYP2C75 (3flk1b)	CTCTATTTTTCTTTAACTTCTGACCTGCA
Site-directed mutagenesis			
c.298TT>AA	F	mfCYP2C75 (QCex2-F)	CTGGAAGAGGACATaaTCCATTGGCTGA
	R	mfCYP2C75 (QCex2-R)	TCAGCCAATGGAttATGTCCTCTTCCAG
c.308C>T	F	mfCYP2C75 (QCex2_308-F)	ATTTTCCATTGGtTGACAGAGCTAACAGAGGAT
	R	mfCYP2C75 (QCex2_308-R)	ATCCTCTGTTAGCTCTGTCAaCCAATGGAAAAT
c.298TT>AA/c.308C>T	F	mfCYP2C75_c298/308 (5qc1)	AGTTTTCTGGAAGAGGACATaaTCCATTGGtTGACAGAGCTAACAGAGG
	R	mfCYP2C75_c298/308 (3qc1)	CCTCTGTTAGCTCTGTCAaCCAATGGAttATGTCCTCTTCCAGAAAACT
c.334ATC>CTT	F	mfCYP2C75 (QCex3-F)	ACAGAGGATTTGGAcTtGTTTTCAGCAATG
	R	mfCYP2C75 (QCex3-R)	CATTGCTGAAAACaAgTCCAAATCCTCTGT
c.709A>G	F	mfCYP2C75 (QCex5_709-F)	AAATTACTTAAAAACgTTGCTTTTTTGAA
	R	mfCYP2C75 (QCex5_709-R)	TTCAAAAAAGCAAcGTTTTTAAGTAATTT

F, forward primer, R, reverse primer, S, sequence primer. Nucleotides to be changed are indicated in small letters.