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Marmoset pulmonary cytochrome P450 2F1 oxidizes biphenyl and 7-ethoxycoumarin and hepatic human P450 substrates

Shotaro Uehara [1], Yasuhiro Uno [2], Toru Oshio [1], Takashi Inoue [3], Erika Sasaki [3,4], and Hiroshi Yamazaki [1]

[1] Laboratory of Drug Metabolism and Pharmacokinetics, Showa Pharmaceutical University, Machida, Tokyo 194-8543, Japan, [2] Pharmacokinetics and Bioanalysis Center, Shin Nippon Biomedical Laboratories, Ltd., Kainan, Wakayama, Japan, [3] Department of Applied Developmental Biology, Central Institute for Experimental Animals, Kawasaki, Japan, and [4] Keio Advanced Research Center, Keio University, Minato-ku, Tokyo, Japan.

Running title: Cloning of marmoset cytochrome P450 2F1 cDNA

Shotaro Uehara and Yasuhiro Uno equally contributed.

Correspondence author: Hiroshi Yamazaki, PhD, Professor

Showa Pharmaceutical University, 3-3165 Higashi-tamagawa Gakuen, Machida, Tokyo 194-8543, Japan. Phone: +81-42-721-1406; Fax: +81-42-721-1406. E-mail: hyamazak@ac.shoyaku.ac.jp

Abstract

1. A potentially useful animal model for preclinical studies is the common marmoset (*Callithrix jacchus*). In this study, using reverse-transcription polymerase chain reaction from marmoset livers, we identified a novel cytochrome P450 (P450) 2F1 cDNA with an open reading frame of 1473 bp.
2. High sequence identities of 92-94% with primate P450 2F amino acid sequences were indicated by deduced amino acid sequences of P450 2F1 cDNA. Phylogenetic analysis indicates that marmoset P450 2F1 is more congruent with primate P450 2F forms than those of other species such as rodents.
3. Among five tissue types examined, abundant expression of marmoset P450 2F1 mRNA and P450 2F1 protein in lungs was shown. Cynomolgus monkey P450 2F1 mRNA was abundantly expressed in lungs as well as testes and ovaries in ten tissue types.
4. Similar to those of humans and cynomolgus monkeys, marmoset P450 2F1 heterologously expressed in *Escherichia coli* membranes efficiently catalyzed 7-ethoxycoumarin *O*-deethylation and biphenyl hydroxylation, however unlike human P450 2F1, marmoset P450 2F1 exhibited hydroxylation activity toward coumarin and chlorzoxazone.
5. These findings indicated that P450 2F1 enzyme expressed in marmoset lungs and also catalyzed metabolism of xenobiotics, suggesting the importance of P450 2F-dependent drug metabolism in marmoset lungs.

Keywords: Common marmoset; CYP2F1; drug oxidation.

Introduction

Cytochrome P450s (P450s, EC 1.14.14.1) are the major enzymes that influence the metabolism of many xenobiotic compounds, including environmental carcinogens and drugs. Human P450 2F1 is one such catalyst, and involved in the bioactivation of pulmonary toxins, including 3-methylindole, naphthalene, and styrene. The P450 2F subfamily contains a single member in each species, which is primarily expressed in lungs; P450 2F1 in humans (Nhamburo et al., 1990), P450 2F5 in gorillas (Chen et al., 2002), P450 2F3 in goats (Wang et al., 1998), P450 2F4 in rats (Baldwin et al., 2005), and P450 2F2 in mice (Ritter et al., 1991).

Although the Old World primate cynomolgus monkey (*Macaca fascicularis*) has been used for drug development and safety assessment, the common marmoset (*Callithrix jacchus*), generally referred to as The New World primate, is an ideal animal model for preclinical pharmacokinetics, toxicity, and assessment of metabolism of drugs because of their genetic closeness and phenotypic similarities to humans, small body size, ease of handling, high fertility, and early sexual maturity ('t Hart et al., 2012; Sasaki, 2015). Drug- and xenobiotic-metabolizing enzymes have not been sufficiently analyzed in marmosets, nor adequately compared with humans and cynomolgus monkeys.

Our group has identified more than twenty P450 forms belonging the P450 1-4 families in marmosets. Their amino acid sequences and substrate selectivity have been found to be similar to those of humans and cynomolgus monkeys (Uno et al., 2016). In this study, we isolated novel P450 2F1 cDNA from marmoset lungs, analyzed sequence identity, gene expression patterns, drug-metabolizing activity, and also compared its characteristics to the corresponding human and cynomolgus monkey P450 2F forms.

Materials and Methods

Chemicals and tissue samples

Coumarin, 7-hydroxycoumarin, and biphenyl were purchased from Wako Pure Chemicals (Osaka, Japan). 2- and 4-Hydroxybiphenyl were purchased from Tokyo Kasei (Tokyo, Japan). 7-Ethoxycoumarin and chlorzoxazone were purchased from Sigma-Aldrich (St. Louis, MO, USA). 6-Hydroxychlorzoxazone was purchased from Toronto Research Chemicals (Toronto, Canada). DNA oligonucleotides were synthesized by Sigma Chemical Co. (Tokyo, Japan). Human P450 2F1 cDNA was purchased from NovoPro Bioscience (Shanghai, China). Pooled liver microsomes from marmosets and humans were purchased from Corning Life Sciences (Woburn, MA, USA). Pooled liver microsomes from cynomolgus monkeys and pooled lung microsomes from humans were purchased from Xenotech (Lenexa, KS, USA). Lung microsomes from marmosets and cynomolgus monkeys were prepared from lung tissues of 18 (10 males and 8 females, 2-6 years of age) and 6 (3 males and 3 females, 4-5 years of age) animals, respectively. Marmoset tissues from brains, lungs, livers, kidneys, and jejunums were collected from 20 marmosets (10 males and 10 females, 2-6 years of age) raised at the Central Institution for Experimental Animals (Kawasaki, Japan). Cynomolgus monkey brains, lungs, hearts, livers, kidneys, jejunums, adrenal glands, testes, ovaries, and uteri were collected from 6 cynomolgus monkeys (3 males and 3 females from Indochina, 4-5 years of age) at Shin Nippon Biomedical Laboratories, Ltd. (Kainan, Japan). The study was approved by the Institutional Animal Care and Use Committee. Pooled marmoset tissue microsomes were prepared as described previously (Uehara et al., 2015b). All other chemicals were of the highest grade commercially available.

P450 2F1 cDNA cloning

Marmoset and cynomolgus monkey P450 2F1 cDNAs were isolated by reverse transcription (RT)-polymerase chain reaction (PCR) using total RNA from lung tissues. Briefly, total RNAs were extracted from lungs of marmosets and cynomolgus monkeys with RNeasy Mini Kit (Qiagen, Valencia, CA). The first-strand cDNA was synthesized with 1.0 μg of total RNA using the Superscript III First Strand Synthesis kit (Invitrogen, Carlsbad, CA, USA). PCR was performed using the high-fidelity DNA polymerase KOD-Plus-Neo (Toyobo, Osaka, Japan) with an ABI GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Foster City, CA, USA). The primers used were cjCYP2F1(5rt2) 5′-GATCCTACTCTCTCCTCAGCG-3′ and cjCYP2F1(3rt2) 5′-CTCATCGCTCACAGGGGAATC-3′ for marmoset P450 2F1, and mmCYP2F1 (5rt1) 5′-GACGCCAGCAGCTGCCTTCACC-3′ and mmCYP2F1 (3rt1) 5′-GCCTCATTGCTCACAGGCGAATC-3′ for cynomolgus monkey P450 2F1. Thermal cycler conditions included an initial 2-minute denaturation at 94°C, followed by 30 cycles of 98°C for 15 seconds, 60°C for 30 seconds, and 68°C for 2 minutes, with a final 7-minute extension at 68°C. For cynomolgus monkey P450 2F1, annealing was performed at 65°C for 30 seconds. The amplified fragments were cloned into pGEM-T easy vectors (Promega, Madison, WI, USA) or pCR4 Blunt TOPO vectors (Invitrogen), and an inserted DNA fragment was sequenced using ABI PRISM BigDye Terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems) with an ABI PRISM 3730 DNA Analyzer (Applied Biosystems).

Bioinformatics

The homology of amino acid sequences was determined using BLAST (National Center for

Biotechnology Information). The multiple alignment of sequences was performed using a Genetyx system (Software Development, Tokyo, Japan). The phylogenetic tree was created using DNASIS Pro (Hitachi Software, Tokyo, Japan). The P450 amino acid sequences from GenBank used for the analysis included; human P450 2F1 (NP_000765), gorilla P450 2F5 (AAL72278), rhesus monkey P450 2F1 (NP_001272197), goat P450 2F3 (NP_001274499), rat P450 2F4 (NP_001274499), and mouse P450 2F2 (NP_031843). The marmoset P450 2F1 amino acid sequence was deduced from the cDNA identified in this study.

Quantitative real-time RT-PCR

The expression levels of P450 2F1 mRNA in marmoset and cynomolgus monkey tissues were measured by real-time RT-PCR. Total RNA was extracted from marmoset and cynomolgus monkey tissues using an RNeasy Mini Kit as described earlier. An equal amount of total RNA was pooled from 3 male marmosets or 6 cynomolgus monkeys (3 males and 3 females) for each tissue type, and pooled total RNA (1 μg) was reverse transcribed according to the procedure described above. Real-time PCR was performed using Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA) with an ABI PRISM 7300 sequence detection system (Applied Biosystems). The PCR reaction mixture contained 400 nM of each primer, 12.5 μl of Power SYBR Green PCR Master Mix, and 2 μl of RT products in a total volume of 25 μl. The PCR primers were cjCYP2F1 (5qrt1) 5'-GGAGAGGAGTTTAGTGGCCG-3' and cjCYP2F1 (3qrt1) 5'-CTTCAGTTTTCCGCAGCTCC-3' for marmoset P450 2F1, mfCYP2F1 (5qrt1) 5'-ACCAACGCATCTTCCAGAACT-3' and mfCYP2F1 (3qrt1) 5'-CTCCTTCTCCTCTGCCATCT-3' for cynomolgus monkey P450 2F1, and mf&h_b-actin (5qrt1) 5′-GGAAATCGTGCGTGACATT-3′ and mf&h_b-actin (3qrt1) 5′-TTTCGTGGATGCCACAGG-3′ for cynomolgus monkey β-actin. Levels of 18S ribosomal

RNA were measured using Eukaryotic 18S ribosomal RNA Endogenous Control (Applied Biosystems) according to the manufacturer's instructions. Thermal cycler conditions were 10 minutes at 95°C, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. All samples were analyzed in triplicate. The abundance of P450 2F1 mRNA in various tissues was expressed as relative to 18S ribosomal RNA levels for marmosets and β-actin mRNA levels for cynomolgus monkeys.

Immunoblotting

The expression of P450 2F1 protein in marmoset liver microsomes was analyzed by immunoblotting with human P450 2F1 polyclonal antibodies [CYP2F1 (H-41), Santa Cruz Biotechnology, Santa Cruz, CA]. In brief, recombinant P450 protein (0.1 pmol of P450/lane) or tissue microsomes (40 μg/lane) were run on 10% sodium dodecyl sulphate-polyacrylamide gel and the resolved proteins were transferred onto polyvinylidene difluoride membrane (Merck Millipore, Billerica, MA). The membranes were blocked with 0.5% skim milk at room temperature for 30 minutes, incubated with anti-human P450 2F1 antibodies (1:1000) at room temperature for 1 hour, and then with goat anti-rabbit secondary antibodies (1:5000; Santa Cruz Biotechnology) at room temperature for 20 minutes. P450 2F1 protein bands were visualized by an ECL Prime Western Blotting Detection System (GE Healthcare, Buckinghamshire, UK) according to the manufacturer's instructions.

Heterologous expression of P450 2F protein in *Escherichia coli*

For enzyme assays, recombinant marmoset P450 2F1 was prepared by heterologous expression in *Escherichia coli* using expression plasmids. The *N*-terminus modification was carried out by PCR using the primers, cjCYP2F1 (5exp3) 5'-CATATGGCTAAGAAAACGAGCTCTAAAGGTAAGCTGCCTCCAGGACCCAGA-3'

and cjCYP2F1(3exp1) 5'-TCTAGATTAGCGCGCGCGCAGGCACAGC-3' for marmoset P450 2F1, cjCYP2F1 (5exp3) and mfCYP2F1(3exp1) 5'-TCTAGATTAGTGCGGGCACAGGCACAGC-3' for cynomolgus monkey P450 2F1, and cjCYP2F1 (5exp3) and hCYP2F1(3exp1) 5'-TCTAGATTAGCGCGGGCGCAGGCACAGC-3' for human P450 2F1. PCR product was subcloned into the pCW vector containing human NADPH-P450 reductase cDNA via the cloning sites NdeI and XbaI (underlined). The sequences of the inserts were verified by DNA sequencing as described earlier. For cynomolgus monkey P450 2F1, the internal XbaI site was mutated without changing the amino acid residue using QuikChange XL II kit (Stratagene, La Jolla, CA) according to manufacturer's instructions. Primers used were mfCYP2F1_c780 (5qc1) 5'- ATTGTTTGTTTCTCACATGATTGACAAACAC-3' and mfCYP2F1_c780 (3qc1) 5'-GTGTTTGTCAATCATGTGAGAAACAAACAAT-3'. The heterologous expression in *Escherichia coli*, preparation of membrane fractions, and the measurement of concentrations of P450 and NADPH-P450 reductase were performed as described previously (Yamazaki et al., 2002).

Enzyme assays

As described previously (Uehara et al., 2015a), 7-ethoxycoumarin *O*-deethylation, coumarin 7-hydroxylation, and chlorzoxazone 6-hydroxylation activities by recombinant P450s and liver microsomes from marmosets and humans were measured by high-performance liquid chromatography. Biphenyl hydroxylation activities were measured as described previously (Shimada et al., 2016) with some minor changes as follows. In brief, a typical incubation mixture (0.25 mL) contained recombinant protein (10 pmol) or liver microsomes (0.125 mg/mL), an NADPH-generating system (0.25 mM $NADP^+$, 2.5 mM glucose 6-phosphate, 0.25 units/mL glucose 6-phosphate dehydrogenase), and 100 μM biphenyl in 100 mM

potassium phosphate buffer (pH 7.4). The reaction was initiated by the addition of NADPH-generating system. The reaction mixtures were incubated at 37°C for 20 min, and terminated by adding 250 μL of methanol, and then 1.0 mL of a mixture of chloroform and ethyl acetate (1:1, v/v). After centrifugation at 20,000g for 10 min, supernatants (50 μL) were injected into a reversed-phase C_{18} column (Mightysil, 5 mm, 150×4.6 mm; Kanto Chemical, Tokyo, Japan) with elution by a mobile phase (19% acetonitrile, 29% methanol, and 52% 12mM ammonium acetate buffer, pH 4.0) at 1.0 mL/min, monitoring at UV 254 nm and fluorescence with an excitation wavelength of 242 nm and emission wavelength of 380 nm.

Results and Discussion

The potential to use marmosets in pharmacokinetic and pharmacological studies, although high, is diminished by limited information being available regarding their drug-metabolizing enzymes. We have identified and characterized more than 20 hepatic P450 in marmosets, and indicated that the P450s have similar tissue expression patterns and enzymatic properties to those of humans (Shimizu et al., 2014; Uno et al., 2016). However, extrahepatic P450s still need to be fully analyzed in marmosets. Analysis of the marmoset genome sequences using BLAT (UCSC Genome Bioinformatics, University of California, Santa Cruz, CA) revealed the novel marmoset P450 2F (P450 2F1) gene which is highly homologous to human P450 2F1 cDNA. Implementing RT-PCR with the gene-specific primers enabled us to isolate P450 2F1 cDNA containing the entire coding region (1668 bp) from marmoset liver. Deduced amino acids (491 residues) contained the primary sequence structure characteristic of P450 proteins, such as substrate recognition sites (SRSs) and the heme-binding region (Fig. 1). When comparing P450 2F cDNA sequences, marmoset P450 2F1 showed high amino acid sequence identity to primate P450 2F forms at 92-94%, whereas those of rodent P450 2Fs

were significantly less similar at 81-82% (Table 1). Phylogenetic analysis of P450 2F amino acid sequences conferred that marmoset and human, together with cynomolgus monkey and rhesus monkey P450 2F1 forms were closely clustered, unlike goat, rat, and mouse P450 2F forms (Fig. 2).

To determine the expression pattern of P450 2F1 mRNA in marmoset tissues, real-time RT-PCR was performed with the gene-specific primers in brain, kidney, liver, lung, and small intestine. Marmoset P450 2F1 mRNA level was notably abundant in lungs (Fig. 3A), also clearly apparent by the single band (approximately 50 kDa) detected in lung microsomes with anti-human P450 2F1 antibodies which cross-reacted recombinant marmoset P450 2F1 proteins (Fig. 4). Species differences of tissue distribution of P450 2F forms are considered in various experimental animals. Marmoset P450 2F1 mRNAs were expressed at a high level in lungs compared to other tissues (Fig 3A). Human P450 2F1 mRNA was mainly expressed in the respiratory tract (lung and trachea), followed by testis, and very low or none in the liver. (Nishimura et al., 2003; Bieche et al., 2007). Interestingly cynomolgus monkey P450 2F1 mRNA, although abundantly expressed in lungs was significantly more so in testes and ovaries (reproductive organs), unlike those of humans where it was 100-fold higher in lungs than testis (Fig 3B) (Nishimura et al., 2003). In mice and rats, P450 2F forms were abundantly expressed in livers, lungs, and nasal olfactory mucosa (Baldwin et al., 2004; Renaud et al., 2011). These results indicated that the abundant expression of P450 2F1 was conserved among marmosets, cynomolgus monkeys and humans.

Using some typical human P450 substrates, including 7-ethoxycoumarin, coumarin, biphenyl, and chlorzoxazone, enzyme assays were carried out to investigate the drug-metabolizing activity of marmoset P450 2F1. Marmoset lung microsomes catalyzed 7-ethoxycoumarin *O*-deethylation and biphenyl hydroxylation at low rates, compared to

marmoset liver microsomes. Recombinant marmoset P450 2F1 catalyzed 7-ethoxycoumarin *O*-deethylation, coumarin 7-hydroxylation, biphenyl hydroxylation, and chlorzoxazone 6-hydroxylation (Table 2), indicating that 7-ethoxycoumarin *O*-deethylation and biphenyl hydroxylation activities by P450 2F1 enzymes were conserved among marmosets, cynomolgus monkeys, and humans. The kinetics analysis indicated that the K_m and V_{max} for 7-ethoxycoumarin *O*-deethylation by P450 2F enzymes were largely different among marmosets, cynomolgus monkeys, and humans (Table 3 and Fig. 5) which further points to differences existing among species in the substrate selectivity of P450 2F enzymes. Goat P450 2F3, as another example of substrate selectivity, metabolizes human P450 2F1 substrates, naphthalene and 3-methylindole, but not 7-ethoxycoumarin, unlike human P450 2F1 (Wang et al., 1998). Studies comparing of the dehydrogenation of 3-methylindole by P450 2F orthologs from humans (P450 2F1), mice (P450 2F2), and goats (P450 2F3) demonstrated relatively large differences in kinetic parameters (Lanza et al., 1999). To understand the congruencies and inconsistencies of the P450 2F-dependent metabolism in marmosets, humans, and other experimental animals, further analysis of enzymatic characteristics and substrate specificity is needed. Previous reports indicated that human P450 2F1, beyond metabolizing classical P450 substrates such as 7-ethoxycoumarin, propoxycoumarin, and pentoxyresorufin, also metabolize pulmonary toxicants or procarcinogens including naphthalene, styrene, 3-methylindole, and benzene (Nhamburo et al., 1990; Nakajima et al., 1994; Lanza et al., 1999; Sheets et al., 2004). Marmoset P450 2F1 also metabolized the air pollutant biphenyl, suggesting that their lung P450 2F1 may be similar to humans in the detoxification of environmental toxicants. There is a very likely significant benefit to investigating the metabolism of various pulmonary toxicants and procarcinogens by marmoset P450 2F1 in the future.

In summary, we have identified in marmosets a novel P450 2F1 highly homologous to

that of humans. This study of P450 2F1 showed: amino acid sequence identities of 93% with similar tissue expression patterns to human, catalysis of 7-ethoxycoumarin *O*-deethylation and biphenyl hydroxylation, also congruently catalyzed by human P450 2F1, and suggested that the metabolism of drugs and environmental chemicals in marmoset lungs are similar among human and cynomolgus monkey P450 2F1, thus making marmosets a contending animal model for preclinical studies.

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Declaration of interest

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Table 1

Sequence identity of P450 2F1 cDNA and amino acids as compared to P450 2Fs of other species.

Species	P450	Identity (%)	
		cDNA	Amino acids
Human	2F1	94	93
Gorilla	2F5	N.A.	93
Cynomolgus monkey	2F1	94	94
Rhesus monkey	2F1	94	92
Goat	2F3	85	83
Rat	2F4	83	82
Mouse	2F2	83	81

N.A., not applicable

Table 2

Xenobiotic-metabolizing activities by tissue microsomes from marmosets, cynomolgus monkeys, and humans, and recombinant P450 2F1 enzymes.

Enzyme source	7-Ethoxycoumarin *O*-deethylation	Coumarin 7-hydroxylation	Biphenyl 2- and 4-hydroxylation	Chlorzoxazone 6-hydroxylation
Microsomes	*pmol/min/mg protein*	*pmol/min/mg protein*	*pmol/min/mg protein*	*pmol/min/mg protein*
Human liver	122 ± 4	550 ± 11	210 ± 14	142 ± 41
Cynomolgus monkey liver	2800 ± 24	1400 ± 19	295 ± 23	352 ± 68
Marmoset liver	144 ± 8	<1.3	210 ± 17	157 ± 51
Human lung	3.8 ± 0.4	<0.32	2.6 ± 0.4	<12
Cynomolgus monkey lung	1.8 ± 0.1	0.38 ± 0.02	2.2 ± 0.7	<12
Marmoset lung	3.7 ± 1.6	<0.32	3.5 ± 0.4	<12
Recombinant P450	*pmol/min/nmol P450*	*pmol/min/nmol P450*	*pmol/min/nmol P450*	*pmol/min/nmol P450*
Human P450 2F1	49 ± 1	<1.3	65 ± 28	<72
Cynomolgus monkey P450 2F1	1.6 ± 0.2	2.9 ± 0.6	29 ± 7	<72
Marmoset P450 2F1	57 ± 1	8.1 ± 0.4	74 ± 20	752 ± 41

Each catalytic activity was measured at a substrate concentration of 100 μM 7-ethoxycoumarin, 100 μM coumarin, 100 μM biphenyl, and 100 μM chlorzoxazone. Data represent the mean ± standard deviation of triplicate determinations.

Table 3

Kinetic analyses for 7-ethoxycoumarin *O*-deethylation by liver microsomes from marmosets and cynomolgus monkeys, and recombinant P450 2F1 enzymes.

Enzyme source	K_m	V_{max}	V_{max}/K_m
Microsomes	*μM*	*pmol/min/mg protein*	*μL/min/mg protein*
Human lung	950 ± 280	64 ± 11	0.067
Cynomolgus monkey lung	760 ± 250	11 ± 2	0.015
Marmoset lung	460 ± 140	5.4 ± 0.8	0.012
Recombinant P450	*μM*	*pmol/min/nmol P450*	*μL/min/nmol P450*
Human P450 2F1	NA	180 [a]	NA
Cynomolgus monkey P450 2F1	NA	30 [a]	NA
Marmoset P450 2F1	209 ± 72	145 ± 18	0.69

Kinetic parameters were determined by non-linear regression analysis (mean ± standard error, n = 14 points of substrate concentrations of 1.7-1250 μM, duplicate determinations) employing the equation, $v = V_{max} \times [S]/(K_m + [S])$ for Michaelis-Menten equation using Kaleidagraph software (Synergy Software, Reading, PA). [a] Activities at 1250 μM, NA, Not available.

Legends for figures

Fig. 1. Comparison of P450 2F1 amino acid sequences from primates. P450 2F amino acid sequences from marmosets (cj), humans (h), gorillas (g), cynomolgus monkeys (mf), and rhesus monkey (mm) were aligned using Genetyx. Six substrate recognition sites (SRS) and a heme-binding site are shown by solid and broken lines, respectively. Amino acid residues conserved and roughly conserved among the five species are shown by asterisks and dots, respectively, under amino acid alignment.

Fig. 2. A phylogenetic tree of P450 2F amino acid sequences from mammalians. A phylogenetic tree was created using the neighbor-joining method using P450 2F amino acid sequences of marmoset (cj), human (h), gorilla (g), cynomolgus monkey (mf), rhesus monkey (mm), goat (c), rat (r), and mouse (m). Human P450 2A13 was used as an outgroup. The scale bar represents a phylogenetic distance of 0.1 amino acid substitutions per site.

Fig. 3. Tissue distribution of P450 2F1 mRNA in marmosets and cynomolgus monkeys. Expression levels of marmoset P450 2F1 mRNA in each pooled tissue from marmosets and cynomolgus monkeys were measured by quantitative reverse-transcription real-time PCR, and normalized with 18S rRNA or β-actin mRNA levels, respectively. P450 2F1 mRNA levels in marmoset and cynomolgus monkey lungs were adjusted to 1, and the relative P450 2F1 mRNA expression levels are shown for other tissues. Each data was mean ± standard deviations of triplicate determinations.

Fig. 4. Detection of marmoset P450 2F1 proteins by immunoblotting. Recombinant P450 2F1 protein (0.1 pmol of P450/lane) was detected by immunoblotting using anti-human P450 2F1 antibodies cross-reacted with recombinant marmoset P450 2F1 protein. Immunoreactive bands of approximately 50 kDa were detected in marmoset lung microsomes (40 μg/lane). Protein disulfide isomerase (PDI) is used in membrane fraction loading control.

Fig. 5. Kinetic parameters for 7-ethoxycoumarin *O*-deethylation by lung microsomes from marmosets and cynomolgus monkeys, and recombinant P450 2F1 enzymes. Kinetic analyses were performed for 7-ethoxycoumarin *O*-deethylation by lung microsomes (A) and recombinant P450 2F1 enzymes (B) . Circles, marmosets; triangles, cynomolgus monkeys; squares, humans.

Fig. 1

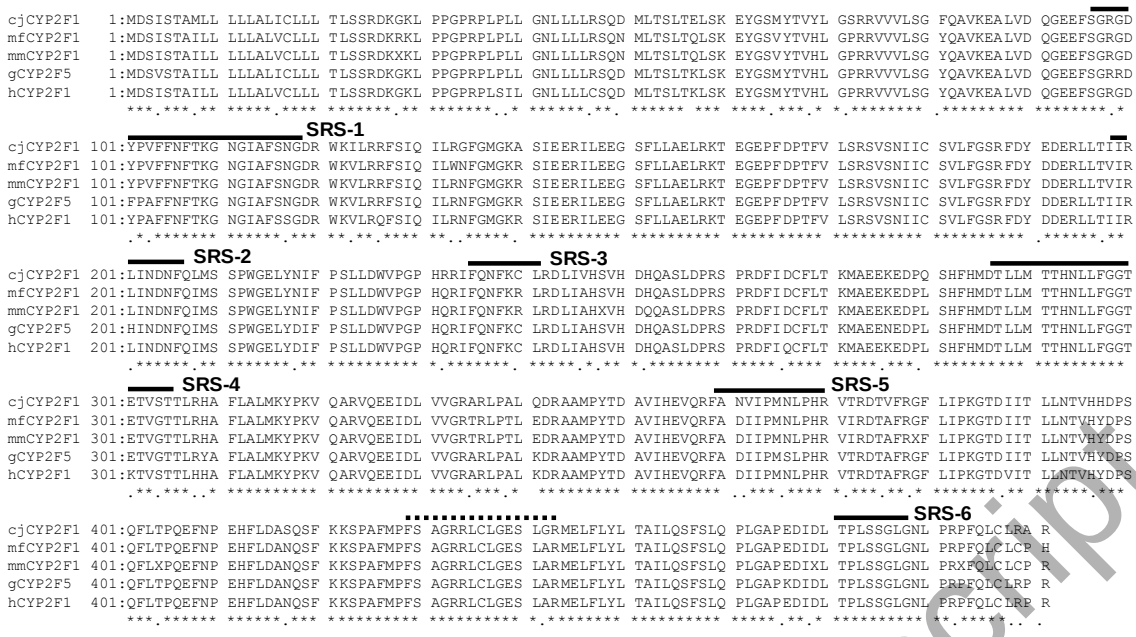

Fig. 2

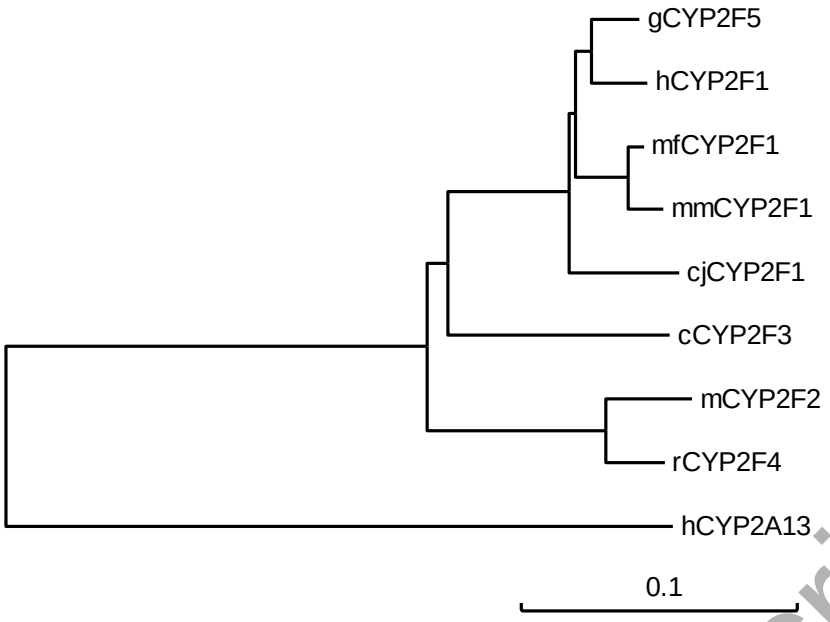

Fig. 3

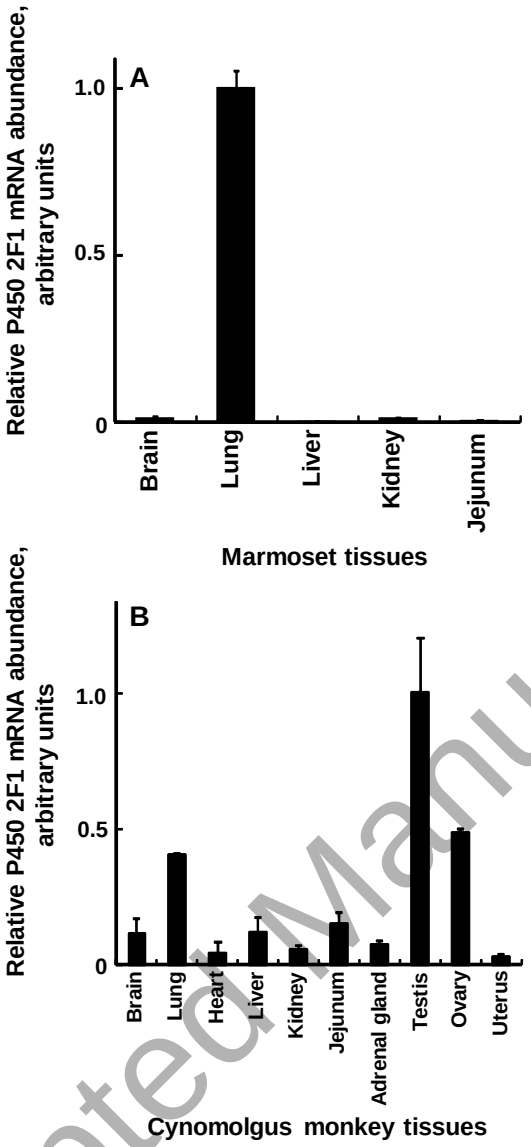

Fig. 4

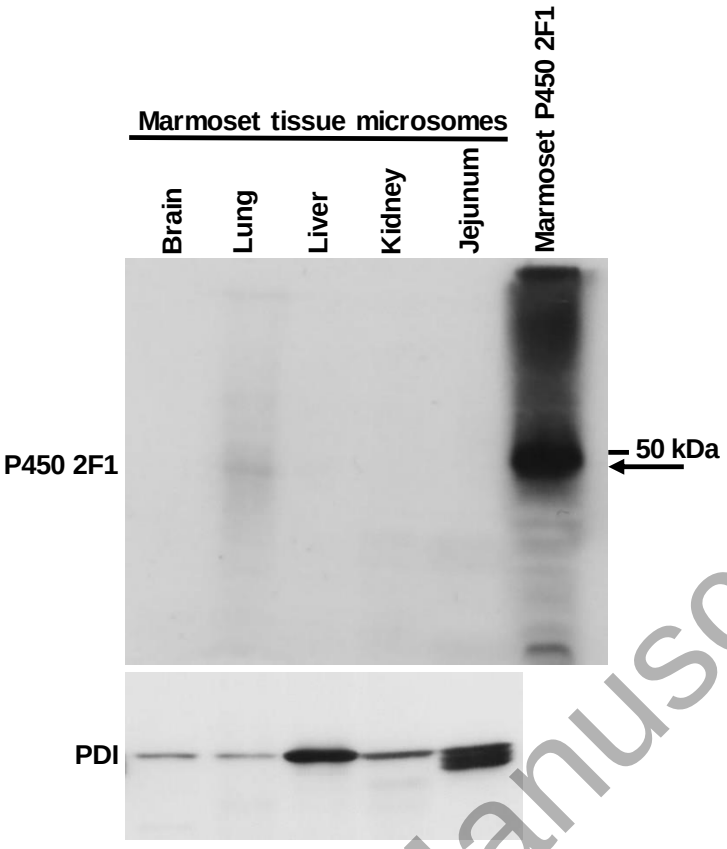

Fig. 5