


# Cytochrome P450 2J Genes Are Expressed in Dogs, Cats, and Pigs, and Encode Functional Drug-Metabolizing Enzymes

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## ABSTRACT

Cytochrome P450s (P450s) have been identified and analyzed in dogs and pigs, species that are often used in preclinical drug studies. Moreover, P450s are clinically important for drug therapy not only in humans, but also in species under veterinary care, including dogs and cats. In the present study, seven P450s homologous to human CYP2J2, namely, dog CYP2J2; cat CYP2J2; and pig CYP2J33, CYP2J35, CYP2J91, and CYP2J93, were newly identified and characterized, along with pig CYP2J34 previously identified. The cDNAs of these CYP2Js contain open reading frames of 502 amino acids, except for CYP2J35 (498 amino acids), and share high sequence identity (77%–80%) with human CYP2J2. Phylogenetic analysis revealed that dog and cat CYP2J2 were closely related, whereas pig CYP2Js formed a cluster. All seven CYP2J genes contain nine coding exons and are located in corresponding genomic regions, with the pig CYP2J genes forming a gene cluster. These CYP2J2 mRNAs were predominantly expressed in the small intestine with additional expression in the kidney and brain for dog CYP2J2 and pig CYP2J91 mRNAs, respectively. All seven CYP2Js metabolized human

CYP2J2 substrates terfenadine, ebastine, and astemizole, indicating that they are functional enzymes. Dog CYP2J2 and pig CYP2J34 and CYP2J35 efficiently catalyzed ebastine primary hydroxylation and secondary carebastine formation at low substrate concentrations, just as human CYP2J2 does. Velocity-versus-substrate plots exhibited sigmoidal relationships for dog CYP2J2, cat CYP2J2, and pig CYP2J33, indicating allosteric interactions. These results suggest that dog, cat, and pig CYP2Js have similar functional characteristics to human CYP2J2, with slight differences in ebastine and astemizole oxidations.

## SIGNIFICANCE STATEMENT

Dog CYP2J2; cat CYP2J2; and pig CYP2J33, CYP2J34, CYP2J35, CYP2J91, and CYP2J93, homologous to human CYP2J2, were identified and characterized by sequence, phylogenetic, and genomic structure analyses. Intestinal expression patterns of CYP2J mRNAs were characteristic in dogs, cats, and pigs. Dog, cat, and pig CYP2Js likely play roles as drug-metabolizing enzymes in the small intestine, similar to human CYP2J2.

## Introduction

Cytochrome P450s (P450s) are important drug-metabolizing enzymes that have been identified and analyzed in dogs and pigs, species that are often used in preclinical drug metabolism studies during drug development. P450s are clinically important for drug therapy not only in humans, but also in species under veterinary care, including dogs and cats. Some P450s have been identified in dogs, cats, and pigs and show characteristics similar to those in humans, though some differences from humans have been noted. For example, dog CYP2B11, which is orthologous to human CYP2B6, is abundant in both the liver and small intestine, unlike its prevalent expression in liver in humans, and metabolizes substrates that human CYP2B6 does not metabolize, such as midazolam (Court, 2013). Pig CYP2B22 is homologous to human CYP2B6 but is not

detected in liver at the protein level, unlike CYP2B6 in humans (Elmorsi et al., 2020). In cats, two CYP2C genes are present in the genome, of which one is a pseudogene and the other is expressed at low levels in liver, indicating a limited role of cat CYP2Cs in liver, unlike in humans, where CYP2Cs are the second most important group of P450s in humans after CYP3As (Ono et al., 2019). Therefore, the identification and characterization of individual P450 forms are important to understand P450-mediated drug metabolism in different species.

CYP2J2 is an important P450 in humans because it is a drug-metabolizing enzyme involved in the first-pass metabolism of ebastine and astemizole (Hashizume et al., 2002; Matsumoto et al., 2002). Human CYP2J2 also metabolizes numerous substrates of human CYP3A4 because of the large volume of its active site (Lee et al., 2010); this fact increases the importance of CYP2J2 for drug metabolism. In humans, CYP2J2 is expressed in various tissues, including liver and small intestine, and is abundant in cardiac tissues, such as heart muscle (Wu et al., 1996; Zeldin et al., 1997; Delozier et al., 2007). CYP2J2 plays roles in the metabolism of not only drugs but also endogenous substrates, such as arachidonic acid, thereby generating epoxyeicosatrienoic acids that

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**ABBREVIATIONS:** P450, cytochrome P450; PCR, polymerase chain reaction; RT, reverse transcription; SRS, substrate recognition site.

are important for their protective roles in inflammation and vasodilation (Xu et al., 2013; Murray, 2016; Solanki et al., 2018). Despite the importance of CYP2J enzymes, to date, CYP2Js have not been fully identified and characterized in pigs, dogs, or cats.

In the present study, the following cDNAs homologous to human CYP2J2 were identified in dogs, cats, and pigs: dog CYP2J2; cat CYP2J2; and pig CYP2J33, CYP2J34, CYP2J35, CYP2J91, and CYP2J93. These CYP2Js were characterized by sequence and phylogenetic analyses, genomic structures, tissue expression patterns, and metabolic assays.

## Materials and Methods

**Materials.** Astemizole, terfenadine, and terfenadine alcohol were purchased from Sigma-Aldrich (St. Louis, MO). Desmethyldastemizole was purchased from Toronto Research Chemicals (Toronto, Canada). Ebastine, hydroxyebastine, and carebastine were purchased from Almirall-Prodofarma (Barcelona, Spain). Pooled liver microsomes from dogs and humans were purchased from Corning Life Sciences (Woburn, MA). Oligonucleotides were synthesized by Integrated DNA Technologies (Coralville, IA). All other reagents were purchased from Sigma-Aldrich or Fujifilm Wako Pure Chemicals (Osaka, Japan), unless otherwise specified.

**Tissues and Nucleic Acid Preparation.** Samples of adrenal gland, heart, ileum, jejunum, kidney, liver, and lung were collected from a beagle dog (male, 2 years of age, weighing 10 kg) at Shin Nippon Biomedical Laboratories, Ltd. (Kainan, Japan), a cat (male, 23 months of age, weighing 4.73 kg) at Hokkaido University, and a pig (female, 10 years of age, weighing 13 kg) at Kagoshima University. Additional tissues were collected from the same animals, i.e., brain and testis from the dog; duodenum, spleen, and testis from the cat; and brain, ovary, and uterus from the pig. Moreover, a liver sample was also collected from a cat (female, 6 months of age, unknown weight) at Kagoshima University and used for molecular cloning. Total RNAs were extracted from dog tissues using a mirVana miRNA isolation kit (Ambion; Austin, TX) and from cat and pig tissues using an RNeasy Mini Kit (Qiagen; Valencia, CA), according to the manufacturers' protocols. Cat and pig liver microsomes were prepared as described previously (Uehara et al., 2014, 2015). This study was reviewed and approved by the Institutional Animal Care and Use Committee at Kagoshima University.

**Molecular Cloning.** Reverse transcription (RT)-polymerase chain reaction (PCR) was performed using total RNA extracted from cat, dog, and pig liver and from pig small intestine as described previously (Uno et al., 2006). The primers were designed using Primer3 v.0.4.0 (<https://primer3.ut.ee/>). Briefly, first-strand cDNA synthesis was carried out in a reaction mixture containing 1 µg of total RNA, oligo (dT), and ReverTra Ace (TOYOBO; Osaka, Japan) at 42°C for 1 hour according to the manufacturer's protocols. PCR reactions were performed using the RT product as the template with Q5 Hot Start High-Fidelity DNA Polymerase (New England BioLabs; Ipswich, MA) and a T100 thermal cycler (Bio-Rad Laboratories; Hercules, CA) according to the manufacturers' protocols. The PCR conditions were initial denaturation at 98°C for 30 seconds, followed by 35 cycles of 98°C for 10 seconds, 60°C for 20 seconds, and 72°C for 50 seconds, followed by a final extension at 72°C for 2 minutes. The primers used were dCYP2J2 (5rt1) 5'-CAGCAGAGCGAGAGGACGCGAGA-3' and dCYP2J2 (3rt1) 5'-CCGTAT-TCTCAGAGGACACACCAATTCTTC-3' for dog CYP2J2, fCYP2J2 (5rt1) 5'-CTCAGCCGAGCGAGAGGACTTTG-3' and fCYP2J2 (3rt1) 5'-CTTCAAGA CCGAGGAGTCGACAGA-3' for cat CYP2J2, pCYP2J33 (5rt1) 5'-GAACAC GGAAGAGCGGACACT-3' and pCYP2J33 (3rt1) 5'-GAACACGGAAGAGC GGGACACT-3' for pig CYP2J33, pCYP2J34 (5rt1) 5'-GTCTTTGGCTCAAAC CGCAGAACT-3' and pCYP2J34 (3rt1) 5'-GGACATACTAATCTTCTCTGTA TCGTTCCAAT-3' for pig CYP2J34, pCYP2J35 (5rt1) 5'-GGAAGAGCAGGCG GATGTCTCA-3' and pCYP2J35 (3rt1) 5'-CCAAACCAGTTAAAGTCTTTTAT TTCTCCTGAT-3' for pig CYP2J35, pCYP2J2L (5rt1) 5'-CCGCGGAAGAGCA AGCGTA-3' and pCYP2J2L (3rt1) 5'-GGTTTCCAAACCACTTCAAGTCCA-3' for pig CYP2J91, and pCYP2J93 (5rt1) 5'-AGATCTCCGAAGAGCTGGAGGCT A-3' and pCYP2J93 (3rt1) 5'-CCAAACCAGTTAAAGTCTTTTATTCTCCCA AT-3' for pig CYP2J93. The amplified products were cloned into pMiniT2.0 vectors using a PCR Cloning Kit (New England BioLabs) according to the manufacturer's protocol. The inserts were sequenced using an ABI PRISM BigDye Terminator v3.0

Ready Reaction Cycle Sequencing Kit (Applied Biosystems; Foster City, CA) with an ABI PRISM 3500xl Genetic Analyzer (Applied Biosystems).

**Bioinformatics.** Sequence data were analyzed using the Genetyx system (Software Development; Tokyo, Japan), including the ClustalW program, which was used for alignment of multiple amino acid sequences. A phylogenetic tree was created by the neighbor-joining method. BLAST (National Center for Biotechnology Information) was used for the homology search, and BLAT (UCSC Genome Bioinformatics) and Sequence Viewer (National Center for Biotechnology Information) were used for analysis of the dog, cat, pig, and human genome data. Amino acid and cDNA sequences used for the analyses were from GenBank or the present study.

**Quantification of mRNA Expression.** Expression levels of CYP2J mRNAs were measured using real-time RT-PCR, as reported previously (Uno et al., 2006), with gene-specific primers in pig adrenal gland, brain, heart, ileum, jejunum, kidney, liver, lung, ovary, and uterus; in dog adrenal gland, brain, heart, ileum, jejunum, kidney, liver, lung, and testis; and in cat adrenal gland, duodenum, heart, ileum, jejunum, kidney, liver, lung, spleen, and testis. Briefly, RT reactions were carried out using a ReverTra Ace qPCR RT kit (TOYOBO) according to the manufacturer's protocols, and one-twentieth of the reaction mixture was subsequently used for PCR. The PCR amplification was conducted in a total volume of 20 µl using a THUNDERBIRD SYBR qPCR Mix Kit (TOYOBO) and a StepOnePlus Real-Time PCR System (Applied Biosystems), following the manufacturers' protocols. The following primers were used at final concentrations of 300 nM: d&fCYP2J2 (5qrt1) 5'-CAACTCTTCCATCTGGACTTTGA-3' and d&fCYP2J2 (3qrt1) 5'-TTG ATCAAGGGCAATCCAGTTA-3' for dog and cat CYP2J2, pCYP2J33 (5qrt1) 5'-GCTGGAAGTACACAACTTTGATAAC-3' and pCYP2J33 (3qrt1) 5'-CTTGGACTTTTTCTGGATTTCG-3' for pig CYP2J33, pCY P2J34 (5qrt1) 5'-GTGCAGGTCCAGCTCTACAATATG-3' and pCYP2J34 (3qrt1) 5'-TTTCAATCACACAGGCAACAAAT-3' for pig CYP2J34, pCYP2J35 (5qrt1) 5'-CCCCACCAATCATCTTTAGTAACA-3' and pCY P2J35 (3qrt1) 5'-TGAAGGTGAAGTCCCTTTCTA-3' for pig CYP2J35, pCYP2J2L (5qrt1) 5'-ATCAACGCAATGCCAGTTCTA-3' and pCYP2J2L (3qrt1) 5'-TCTGTCTGTGATTTTCAATCATTC-3' for pig CYP2J91, and pCYP2J93 (5qrt1) 5'-GCTCCATCACATTTGGAGAAC-3' and pCYP2J93 (3qrt1) 5'-GATATTGTAAATCTGGCACCACAC-3' for pig CYP2J93. Relative expression levels were determined, based on three independent amplifications, by normalization of the raw data with the 18S ribosomal RNA levels, which were measured using TaqMan Gene Expression Assays (Assay ID: Hs99999901\_s1, Applied Biosystems) with THUNDERBIRD Probe qPCR Mix (TOYOBO).

**Heterologous Protein Expression.** Expression plasmids were generated with the CYP2J cDNAs isolated in the present study, followed by protein expression in *Escherichia coli*, as reported previously (Iwata et al., 1998; Uno et al., 2006). To enhance protein expression, the N-terminus was modified by PCR amplification of the coding region using Q5 Hot Start High-Fidelity DNA Polymerase (New England BioLabs) as described earlier, with CYP2J cDNA as the template, except that the annealing step was carried out at 55°C. The forward primers used were dCYP2J2 (5exp1) 5'-CGCATTAATGGCTCTGT-TATTAGCAGTTTTTGCGGCCACCCTCTGGGCAGT-3' for dog CYP2J2, fCYP2J2 (5exp1a) 5'-GGAATTCATATGGCTCTGTTATTAGCAGTTTTT GCGGCCGCCGTCTGG-3' for cat CYP2J2, pCYP2J33 (5exp1) 5'-CGCAT-TAATGGCTCTGTTATTAGCAGTTTTTGCAGAGGCTCTCTGGAC-3' for pig CYP2J33, pCYP2J34/93 (5exp1) 5'-GGAATTCATATGGCTCTGTTAT-TAGCAGTTTTTGTCTGAGGCTCTCTGGAC-3' for pig CYP2J34 and CYP2J93, pCYP2J35 (5exp1) 5'-GGAATTCATATGGCTCTGTTATTAGCAGT TTTTGCAGAGTTTTTCGGGAC-3' for pig CYP2J35, and pCYP2J2L (5exp1) 5'-GGAATTCATATGGCTCTGTTATTAGCAGTTTTTGTCTGCGGAG GTTCTCTGCAC-3' for pig CYP2J91. The reverse primers were dCYP2J2 (3exp1a) 5'-GCTCTAGACTCTCCTTCAGGACCGAGGGATTGC-3' for dog CYP2J2, fCYP2J2 (3exp1a) 5'-GCTCTAGACTTCAAGACCGAGGGACT GC-3' for cat CYP2J2, and pCYP2Js (3exp1a) 5'-GCTCTAGA-CCACAACC TCATGCCCT-3' for all pig CYP2Js. The forward and reverse primers contained the NdeI and XbaI sites (underlined), respectively, which were used for subcloning into the pCW vector containing human NADPH-P450 reductase cDNA. For dog CYP2J2 and pig CYP2J33, the forward primers contained the AseI site, which generates compatible overhangs for the NdeI site. Membrane preparation was performed as described previously (Iwata et al., 1998; Uno et al., 2006). The

hCYP2J2	1:	MLAAMGSLAA	ALWAVVHPT	LLLGTVAFL	AADFLKRRR	KNYPPGPWRL	PFLGNFFLVD	FEQSHLEVQL	FVKKYGNLFS	LELGDISAVL	ITGLPLIKEA	100
dCYP2J2	1:	MLAAMGSLAA	TLWAVLHRT	LLLGAVALF	FADFLKRRR	KNYPPGPVPL	PFVGNFFHLD	FEQSHLKLQR	FVKKYGNVFS	VQMGDMPLVV	VTGLPLIKEV	100
fCYP2J2	1:	MLAAMGSLAA	AVWAVLHRT	VLLGAVAFLE	FADFLKRRR	KNYPPGPVPL	PFVGNFFHLD	FERSHLQLQR	LVEKYGNIFS	LQLGDMSLVL	ITGLPLIKEV	100
pCYP2J33	1:	MTQALGSLAE	ALWTALHPT	LLLGAVTFLF	FADFLKRRR	KNYPPGPLRL	PFVGNLFHLD	FEKAHLSLQR	FVKKYGNIFS	LDLCAISAVV	VTGLPLIKEV	100
pCYP2J34	1:	MTPALGLAE	ALWTALRPT	LLLGAVALF	FADFLKRRR	KNYPPGPPLR	PFLGNFFHLD	VEKGHLALQR	FVKKYGNIFS	LDSSVFSSVV	ITGLPLIKEA	100
pCYP2J35	1:	MLGAVGFLAE	VFGTALGPSA	LLLSAVAFLE	VADILKRRR	KNYPPGPLRL	PFVGNFFHLD	FEQWHLSLQR	FVKKYGNVLS	LDLGAFFSSVV	ITGLPLIKEA	100
pCYP2J91	1:	MLAPMGSLAE	VLCTALHPLT	FLLSAVALI	FADFLKRRR	KNYPPGPLSL	PFVGNFFHLD	FEKGHLALQR	FVKKYGNLFS	LDLFGDLPSVV	VTGLPLIKEV	100
pCYP2J93	1:	MPLALGSLAE	ALWTALRPT	LLLGAVAFLE	FADFLKRRR	KNYPPGPPLR	PFIGNLFHLD	LDKGHLALQR	FVKKYGNVFS	LDLFGALSSVV	ITGLPLIKEA	100
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<b>SRS-1</b>												
hCYP2J2	101:	LIHMDQNFNG	RPVTPMREHI	FKKNGLIMSS	GQAWKEQRRF	TLTALRNFLG	GKKSLEERIQ	EEAQHLTEAI	KEENGQPFDP	HFKNINNAVSN	IICSITFGGR	200
dCYP2J2	101:	LVDQNFQFVN	RPITPIREVR	FKNSGLIMSS	GQIWKEQRRF	TLATLKNFGL	GRKSIEERIQ	EEAHLIQA	EEENGQPFNP	HFKNINNAVSN	IICSITFGGR	200
fCYP2J2	101:	LVDQNFQFVN	RPTPIRIGRI	FKNNGLIMSN	GQTWKEQRRF	TLATLKNFGL	GKKSLEECIQ	EEARNLIQA	KEENGQPFNP	HFKNINNAVSN	VICSITFGGR	200
pCYP2J33	101:	LVHQNQKFAN	RPIPIQDRV	FKNGGVVTS	GQVWKEQRRF	TLTTLRNFLG	GKKSLEERIQ	EEAQYLTQAI	GEENGQPFNP	QPKISNAVSN	IICSITFGGR	200
pCYP2J34	101:	FVHQDQHFAN	RMIPIQERV	FKKNGLIMSN	GQVWKEQRRF	ALTTLRNFG	GKKSLEERIQ	EEAQYLIQA	GEENGQPFNP	HFKNINNAVSN	IICSITFGGR	200
pCYP2J35	101:	LVHQDQNFVN	RPIN--LNQV	FKKNGLIMSN	GQVWKEQRRF	ALTTLRNFG	GKKSLEERIQ	EEAQYLIQAV	REENGQPFDP	HFKNINNAVSN	IICSITFGGR	198
pCYP2J91	101:	LVHHDQNFNM	RPIVPIREHV	FQKNGLIMSS	GQVWKEQRRF	TLTTLRNFG	GKKSLEERIQ	EEAQYLIQAV	REENGQPFDP	HFKNINNAVSN	IICSITFGGR	200
pCYP2J93	101:	FVHQDKNFSN	RPVPIQQRV	FKDKGVMSN	GQVWKEQRRF	ALTTLRNFG	GKKSLEERIQ	EEAQYLIQA	GEENGQPFNP	HFKNINNAVSN	IICSITFGGR	200
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<b>SRS-2</b>												
hCYP2J2	201:	FEYQDSWFQQ	LLKLLDEVTV	LEASKTCQLY	NVFPWIMKFL	PGPHQTLFSN	WEKLLKLFVSH	MIDKHKRDWN	PAETRDIFDA	YLKEMSKHTG	NPTSSFHEEN	300
dCYP2J2	201:	FEYQDEQFQE	LLRLLDEVTC	LETSMRCQLY	NVFPWIKFL	PGPHQKLFND	WEKLLKLFIAH	MTENHRRDWN	PAEPRDFIDA	YLKEMEKNRG	NATSSFHEEN	300
fCYP2J2	201:	FDYEDAQFQE	LLRLLDDVTF	QEASKRCQLY	NVFPWIMKFL	PGPHQTLFSN	WEKLLKLFVAQ	MIENHRRDWN	PDQTRDFIDA	YLKEIEKNRG	NATSSFHEEN	300
pCYP2J33	201:	FDYQDDQFQE	LLRLLREVTH	LQTLWCQLF	NVFPWIMKFL	PGPHQTLFSD	WEKLEMFIA	VIENHRRDWN	PAEARDFIDA	YLQIEKNRG	NATSSFHEEN	300
pCYP2J34	201:	FDYQDDQFQE	LLRLLDEVTC	QHTSVQVQLY	NMFPWIMKFL	PGPHQTLFSN	WEKLIQIFVAC	VIENHRRDWN	PAEARDFIDA	YLQIEKHKG	NATSSFHEEN	300
pCYP2J35	199:	FDYQDDQFQE	LLRLLDEVTC	LPKSVRVQLF	NVFPWIMKFL	PGPHQTLFSN	WEKLIQIFVAC	VIENHRRDWN	PAEARDFIDA	YLQIEK--G	SSPSVFNEEN	296
pCYP2J91	201:	FDYQDDQFQE	LLRLLDEVTC	QEPSTQCQFY	NVFPWIMKFL	PGPHQTLFSN	WEKLMKFVAR	MIENHRRDWN	PAEARDFIDA	YLQIEKHKG	DATSSFHEEN	300
pCYP2J93	201:	FDYQDNQFQE	LLKLLDEVMC	LQTSVWCQIY	NIIPWIMKFL	PGPHQTLFSN	WEKLMKFVAH	VIENHRRDWN	PAEARDFIEA	YLQIEKHKG	DATSSFHEEN	300
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<b>SRS-3</b>												
hCYP2J2	301:	LICSTLDLFF	AGTETTSTTL	RWALLYMALY	PEIQEKVQAE	IDRVIGQSQ	PSTAARESM	YTNNAVIHEVQ	RMGNIIPLVN	PREVTVDTTL	AGYHLPGKTM	400
dCYP2J2	301:	LIYSTLDLFF	AGTETTSTTL	RWGLLYLALN	PEIQEKVQAE	IDRVIGQSQ	PGLAVRESMP	YTNNAVIHEVQ	RMGNIIPLVN	PREVTDGTTT	AGYHLPGKTM	400
fCYP2J2	301:	LIYSTLDLFF	AGTETTSTTL	RWGLLYLALY	PEIQEKVQAE	IDRVIGQSHI	PSTAARESM	YTNNAVIHEVQ	RMGNIIPLVN	PREVIDDTTL	AGYHLPGKTM	400
pCYP2J33	301:	LICSTLDLFF	AGTETTSTTL	RWGLLYMALH	PEIQEKVQAE	IDRVLGQSQ	PSTAARESM	YTNNAVIHEVQ	RMGNIIPLVN	PREVEDTTL	AGYHLPGKTM	400
pCYP2J34	301:	LIFTTLDLFF	AGTETTSTTL	RWSLLYMALY	PEIQEKVQAE	IDRVLGQSQ	PSTAARESM	YTNNAVIHEVQ	RMGNIIPLVN	PREVAEDTTL	AGYHLPGKTM	400
pCYP2J35	297:	LICSTLDLFF	AGTETTSTTL	RWGLLYMALY	PEIQEKVQAE	IDRVLGQSQ	PSTAARESM	YTNNAVIHEVQ	RMGNIIPLVN	PREVAEDTTL	AGYHLPGKTM	396
pCYP2J91	301:	LIFTTLDLFF	AGTETTSTTL	RWGLLFMAMY	PEIQEKVQAE	IDRVLGQSQ	PSIAARECM	YTNNAVIHEVQ	RMGNIIPMNV	PREAAEGTTL	AGYHLPGKTM	400
pCYP2J93	301:	LICSTLDLFF	AGTETTSTTL	RWGLLYMALY	PEIQEKVQAE	IDRVLGQSQ	PSTAARESM	YTNNAVIHEVQ	RMGNIIPLVN	PREVAEDTTL	AGYHLPGKTM	400
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<b>SRS-4</b>												
hCYP2J2	401:	ILTNLTALHR	DPEWATPDT	FNPDFLENG	QFKKREAFMP	FSIGKRACLG	EQLARTELF	FFTSLLMQKFT	FRPPDNEKLS	LKFRMGITTS	PVSHRLCAVP	500
dCYP2J2	401:	IVTNLTALHR	DPAEWATPDT	FNPDFLENG	QFKKREAFMP	FSIGKRVCLG	EQLARSELFI	FFTSLLVQKFT	FRPPDNEKLS	LEFRTGLTIS	PVSHRLCAVP	500
fCYP2J2	401:	IVTNLTALHR	DPEWATPDR	FNPDFLENG	QFKKREAFMP	FSIGKRVCLG	EQLAKSELFI	FFTSLLVQKFT	FRPPDNEKLS	LKFRMGITTS	PVSHRLCAVP	500
pCYP2J33	401:	IMTNLTALHR	DPEWATPDT	FNPDFLENG	QFKKREAFMP	FSIGKRACLG	EQLARTELF	FFTSLLVQKFT	FRPPDNEKLS	LKFRMGITTS	PVSHRLCAVP	500
pCYP2J34	401:	ILTNLTALHR	DPAEWATPNI	FNPDFLENG	QFKKREAFMP	FSIGKRACLG	EQLARTELF	FFTSLLVQKFS	FRPPDNEKLS	LKFRVGLTIS	PVSHRLCAVP	500
pCYP2J35	397:	VLINLTALHR	DPAEWATPDT	FNPDFLENG	QFKKREAFMP	FSIGKRACLG	EQLARTELF	FFTSLLVQKFS	FRPPDNEKLS	LKFRVGLTIS	PVSHRLCAVP	496
pCYP2J91	401:	ILTNLTALHR	DPAEWATPDT	FNPDFLENG	QFKKREAFMP	FSIGKRACLG	EQLARTELF	FFTSLLVQKFS	FRPPDNEKLS	LKFRMGITTS	PVSHRLCAVP	500
pCYP2J93	401:	VITNLTALHS	DPEWATPDT	FNPDFLENG	QFKKREAFMP	FSIGKRACLG	EQLARTELF	FFTSLLVQKFS	FRPPDNEKLS	LKFRMGITTS	PVSHRLCAVP	500
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<b>SRS-5</b>												
hCYP2J2	501:	QV										502
dCYP2J2	501:	RS										502
fCYP2J2	501:	RS										502
pCYP2J33	501:	RA										502
pCYP2J34	501:	RA										502
pCYP2J35	497:	RA										498
pCYP2J91	501:	RA										502
pCYP2J93	501:	RA										502
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<b>SRS-6</b>												

**Fig. 1.** Amino acid sequences deduced from dog, cat, and pig CYP2J cDNAs. Amino acid sequences of dog (d), cat (f), pig (p), and human (h) CYP2Js were aligned using ClustalW program. The broken line above the sequences indicates the putative heme-binding region characteristic of P450 proteins, and the solid lines show the six putative substrate recognition sites (SRSs). Asterisks and dots under the sequences indicate identical amino acids and conservatively changed amino acids, respectively.

concentrations of proteins in membrane preparations were determined as previously described for the CYP2J protein (Omura and Sato, 1964) and NADPH-P450 reductase (Phillips and Langdon, 1962; Iwata et al., 1998).

**Measurement of Drug-Metabolizing Activities.** The activities of recombinant proteins and liver microsomes for terfenadine *t*-butyl hydroxylation, ebastine primary hydroxylation and secondary carebastine formation, and astemizole *O*-demethylation were determined using high-performance liquid chromatography with a UV detector system with a reversed-phase  $C_{18}$  column (5  $\mu$ m, 150  $\times$  4.6 mm) as described previously (Uehara et al., 2014; Uehara et al., 2015; Uehara et al., 2016; Uehara et al., 2018). Briefly, the incubation mixtures consisted of liver microsomes (0.10 mg/mL) or recombinant CYP2J proteins (20 pmol equivalent/mL), 100 mM potassium phosphate buffer (pH 7.4), an NADPH-generating system (0.25 mM NADP<sup>+</sup>, 2.5 mM glucose 6-phosphate, and 1 unit/ml glucose-6-phosphate dehydrogenase), and substrate (1.0–100  $\mu$ M of ebastine or astemizole, 10  $\mu$ M terfenadine) in a final volume of 0.25 mL. After incubation at

37°C for 10–30 minutes, reactions were terminated by the addition of 0.25 ml of ice-cold acetonitrile. After centrifugation at 900g for 5 minutes, the supernatant was analyzed. Kinetic parameters were calculated from a curve based on Michaelis-Menten or Hill equations fitted by nonlinear regression (mean  $\pm$  standard error) using Prism (GraphPad Software; La Jolla, CA).

## Results

**Identification of CYP2J cDNAs.** The following CYP2J cDNAs were successfully isolated by RT-PCR using the gene-specific primers designed based on the CYP2J gene sequences found in the genomes by BLAT: dog and cat CYP2J2 from liver and pig CYP2J33, CYP2J34, CYP2J35, CYP2J91, and CYP2J93 from liver and small intestine. These CYP2Js were named by the P450 Nomenclature Committee

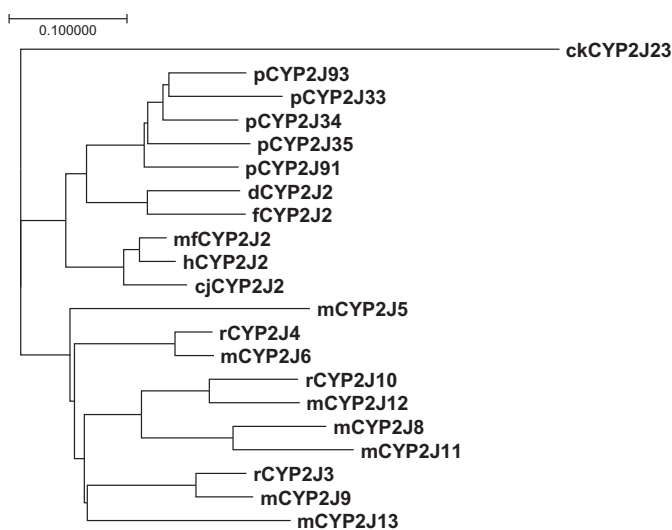
TABLE 1

Sequence identities of dog, cat, and pig CYP2Js compared with human CYP2J2. Dog, cat, and pig CYP2J amino acid and cDNA sequences (coding region) were compared using BLAST.

P450	cDNA (%)	Amino Acids (%)
Dog CYP2J2	85	79
Cat CYP2J2	85	80
Pig CYP2J33	84	77
Pig CYP2J34	83	78
Pig CYP2J35	83	77
Pig CYP2J91	84	80
Pig CYP2J93	83	78

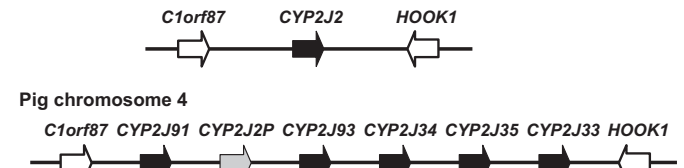
(Nelson, 2009) and had an open reading frame of 502 amino acid residues, except for pig CYP2J35, which had 498 residues. The primary sequence structures were characteristic of P450 proteins, i.e., the presence of six substrate recognition sites (SRSs) (Gotoh, 1992) and a heme-binding region (Fig. 1). The amino acid sequences deduced from the CYP2J cDNAs were highly identical (77%–80%) to human CYP2J2 (Table 1). Phylogenetic analysis of the CYP2J amino acid sequences from humans, cynomolgus macaques, marmosets, pigs, dogs, cats, rats, and mice revealed CYP2J clusters for primates, pigs, dogs/cats, and rats/mice (Fig. 2). The CYP2J cDNA sequences identified in the current study were deposited in GenBank under the accession numbers ON164796, ON164791, ON164801, ON164802, ON164803, ON164800, and ON164804 for cat CYP2J2; dog CYP2J2; and pig CYP2J33, CYP2J34, CYP2J35, CYP2J91, and CYP2J93, respectively.

**Genome and Gene Structure.** The genomic locations of the newly identified CYP2J genes were analyzed with dog, cat, pig, and human genome data using BLAT and Sequence Viewer. The analysis indicated that the CYP2J genes had the same directions and were in the same locations in the genomes of these species, and six pig CYP2J genes (including one pseudogene LOC100525291) formed a gene cluster (Fig. 3). Gene structures were similar among the dog, cat, pig, and human CYP2Js. Dog, cat, and human CYP2J2 genes were approximately 42.0, 31.5, and 33.1 kb, respectively. Among the pig CYP2J genes, the largest was CYP2J91 (>35.6 kb) and the smallest was CYP2J35 (~23.7 kb).



**Fig. 2.** Phylogenetic tree of CYP2J amino acid sequences created using the neighbor-joining method for CYP2J amino acid sequences from humans (h), cynomolgus macaques (mf), marmosets (cj), dogs (d), cats (f), pigs (p), rats (r), and mice (m). The scale bar indicates 0.1 amino acid substitutions per site for distance measurement. Chicken (ck) CYP2J23 was used as the outgroup.

Human chromosome 1  
Dog chromosome 5  
Cat chromosome C1

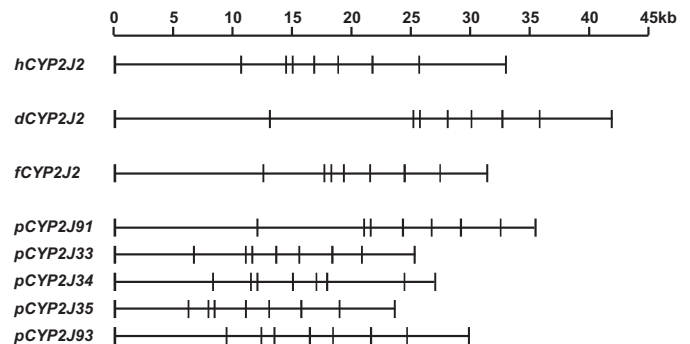


**Fig. 3.** Genomic structure of CYP2J genes. The dog, cat, pig, and human genomes were analyzed using BLAT and Sequence Viewer. The pig CYP2J genes formed a gene cluster in the genome, unlike cat, dog, or human CYP2J2. The locations in the genome and directions of these CYP2J genes were similar among dogs, cats, pigs, and humans. Black and gray arrows, respectively, indicate functional and pseudogenized CYP2J genes, while white arrows show non-CYP2J genes. The sizes of the genes and the distances between them are not proportional to actual measurements.

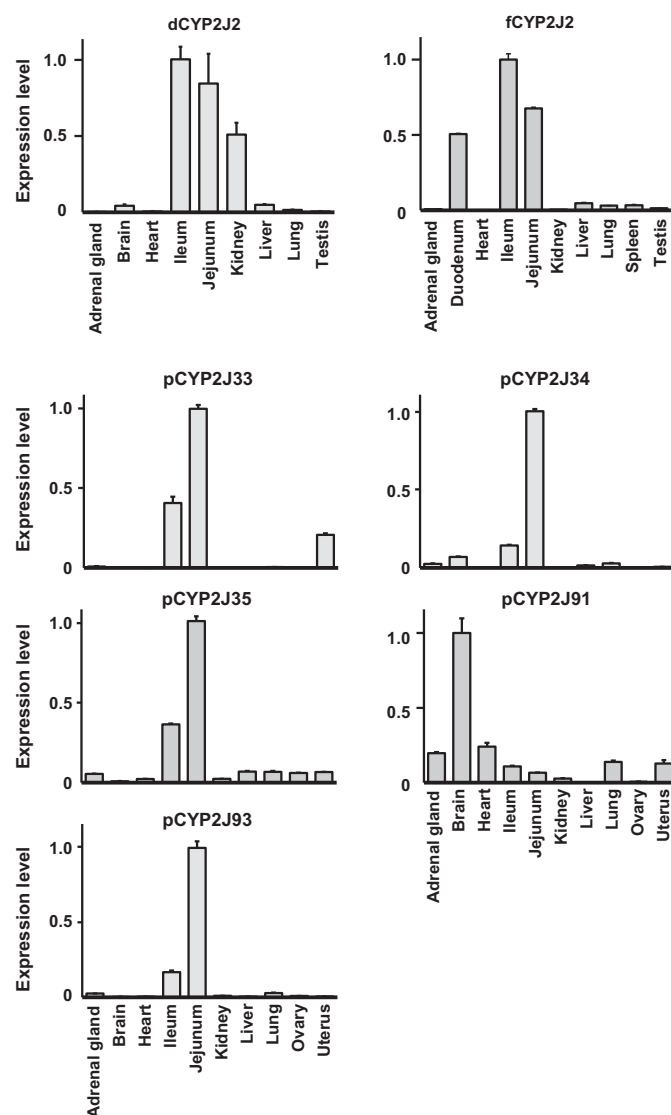
The exon sizes were well conserved in all these CYP2J genes (Fig. 4), i.e., 163, 150, 161, 177, 142, 188, and 139 bp for exons 2–8, respectively, except for exon 2 (157 bp) and exon 6 (136 bp) of pig CYP2J35, which resulted in six amino acid residues fewer than the other CYP2Js. All dog, cat, pig, and human CYP2J genes begin with the dinucleotide GU and end with AG, consistent with the consensus sequences for splice junctions in eukaryotic genes.

**Tissue Expression.** Expressions of dog, cat, and pig CYP2J mRNAs were evaluated using real-time RT-PCR in nine tissue types for dogs and ten tissue types for cats and pigs. Among the tissue types analyzed, cat CYP2J2 mRNA was predominant in small intestine (duodenum, jejunum, and ileum) with lower expression in liver (Fig. 5). A similar expression pattern was observed for dog CYP2J2 mRNA, with most abundant expression in the small intestine, followed by kidney and liver (Fig. 5). In pigs, all the CYP2J mRNAs (except for CYP2J91) also showed predominant expression in the small intestine, with more abundant expression in jejunum than ileum, contrasting with dog and cat CYP2J2 mRNAs, which were more abundant in the ileum than jejunum (Fig. 5). Among the pig CYP2J mRNAs, CYP2J35 mRNA was the most abundant in the small intestine, followed by CYP2J93 and CYP2J33 mRNAs (Fig. 6). Pig CYP2J35 mRNA was also the most abundant of the pig CYP2J mRNAs in the liver, kidney, and lung (Fig. 6). In contrast, pig CYP2J91 mRNA was expressed preferentially in the brain (Fig. 5) and had the highest expression level in the brain out of all the pig CYP2J mRNAs (Fig. 6).

**Drug-Metabolizing Capabilities of CYP2Js.** The drug oxidation activities of recombinant dog CYP2J2; cat CYP2J2; and pig CYP2J33, CYP2J34, CYP2J35, CYP2J91, and CYP2J93 were investigated and compared with those of human CYP2J2. All recombinant dog CYP2J2;



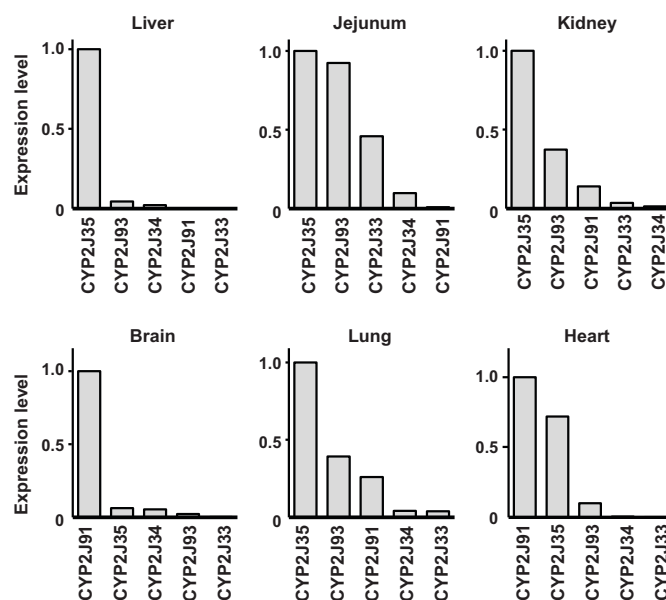
**Fig. 4.** Gene structures of CYP2J genes. The coding region of each CYP2J cDNA sequence was aligned with the genome using BLAT to determine the gene structures for dog (d), cat (f), pig (p), and human (h) CYP2J genes.



**Fig. 5.** Tissue expression patterns of CYP2J mRNAs. The expression levels of dog (d), cat (f), and pig (p) CYP2J mRNAs were measured using real-time RT-PCR in the adrenal gland, brain, duodenum, heart, ileum, jejunum, liver, lung, kidney, ovary, spleen, and testis/uterus. Expression levels of each CYP2J mRNA were normalized using the expression level of 18S rRNA and represent the average  $\pm$  S.D. from three independent amplifications. The most abundant expression level was arbitrarily set to 1, and all other expression levels were adjusted accordingly.

cat CYP2J2; and pig CYP2J33, CYP2J34, CYP2J35, CYP2J91, and CYP2J93 showed terfenadine *t*-butyl hydroxylation activities (4–28 nmol/min/nmol CYP2J) roughly comparable with that of human CYP2J2 (20 nmol/min/nmol CYP2J2). The ebastine primary hydroxylation and secondary carebastine formation activities of recombinant CYP2J enzymes were measured at substrate concentrations of 1.0, 10, and 100  $\mu$ M (Fig. 7). Under the present fixed conditions, human CYP2J2; dog CYP2J2; and pig CYP2J34 and CYP2J35 efficiently oxidized ebastine to the secondary carebastine at low substrate concentrations of 1.0 and 10  $\mu$ M. The rates of ebastine primary hydroxylation by pig CYP2J33 and CYP2J93 increased with the substrate concentrations, but those by cat CYP2J2 and pig CYP2J91 were apparently saturated at substrate concentrations approaching 10  $\mu$ M.

Kinetic analyses of astemizole *O*-demethylation using Michaelis-Menten or Hill equations found that for recombinant proteins, the

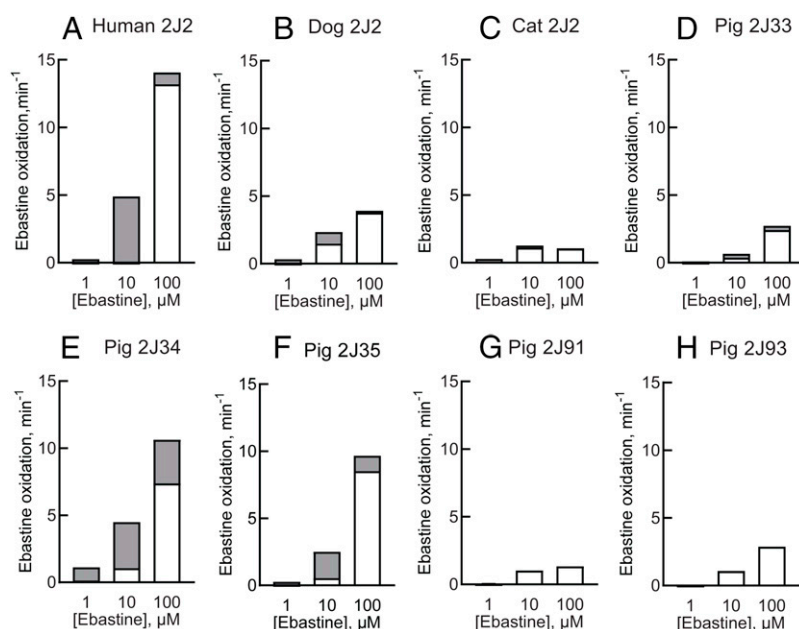


**Fig. 6.** Expression levels of pig CYP2J mRNAs in brain, jejunum, kidney, liver, lung, and heart. Averaged quantitative values of expression levels were compared among pig CYP2J mRNAs in the brain, jejunum, kidney, liver, lung, and heart. Pig CYP2J35 mRNA was most abundantly expressed in liver, kidney, jejunum, and lung, although pig CYP2J93 mRNA was also abundantly expressed in jejunum. The most abundant expression was arbitrarily set to 1, to which all other values were adjusted.

apparent  $K_m$  values were in the range of  $\sim$ 1–13  $\mu$ M, whereas the estimated intrinsic clearance ( $V_{max}/K_m$ ) values were in the range of  $\sim$ 0.2–1  $\mu$ M $^{-1}$ min $^{-1}$  (Table 2; Fig. 8). The plots of velocity versus substrate concentration exhibited sigmoidal relationships on kinetic analysis of dog CYP2J2, cat CYP2J2, and pig CYP2J33 (Fig. 8), which indicated an allosteric interaction (apparent positive cooperativity) between astemizole and CYP2J enzymes; data were analyzed using the general allosteric model and the Hill equation. For human liver microsomes, the  $K_m$  value of astemizole *O*-demethylation was 4.5  $\mu$ M, similar to that for human CYP2J2 recombinant protein, suggesting that human CYP2J2 is predominantly involved in astemizole *O*-demethylation in human liver. In contrast, the Hill coefficient ( $n$ ) values for human, dog, cat, and pig liver microsomes were 2.2, 1.2, 2.8, and 2.3, respectively, for astemizole *O*-demethylation (Table 2). The calculated  $S_{50}$  ( $K_m$ ) and  $V_{max}$  values were, respectively, 30  $\mu$ M and 0.14 nmol/min/mg for dog liver microsomes, 12  $\mu$ M and 0.11 nmol/min/mg for cat liver microsomes, and 36  $\mu$ M and 0.31 nmol/min/mg for pig liver microsomes, implying the possible involvement of additional P450 enzymes because of these higher  $K_m$  values than those for recombinant CYP2J enzymes. Overall, these results suggest that dog CYP2J2; cat CYP2J2; and pig CYP2J33, CYP2J34, CYP2J35, CYP2J91, and CYP2J93 all exhibit catalytic function toward typical human CYP2J2 probe substrates terfenadine, ebastine, and astemizole.

## Discussion

Human CYP2J2 expressed in small intestine is an important drug-metabolizing enzyme in the first-pass metabolism of drugs such as ebastine and astemizole (Hashizume et al., 2002; Matsumoto et al., 2002). In the present study, we found seven CYP2J genes in the dog, cat, and pig genome (Fig. 2), namely, dog CYP2J2; cat CYP2J2; and pig CYP2J33, CYP2J34, CYP2J35, CYP2J91, and CYP2J93, and the corresponding CYP2J cDNAs were identified. These CYP2Js showed high amino acid sequence identities to human CYP2J2 (Table 1) and

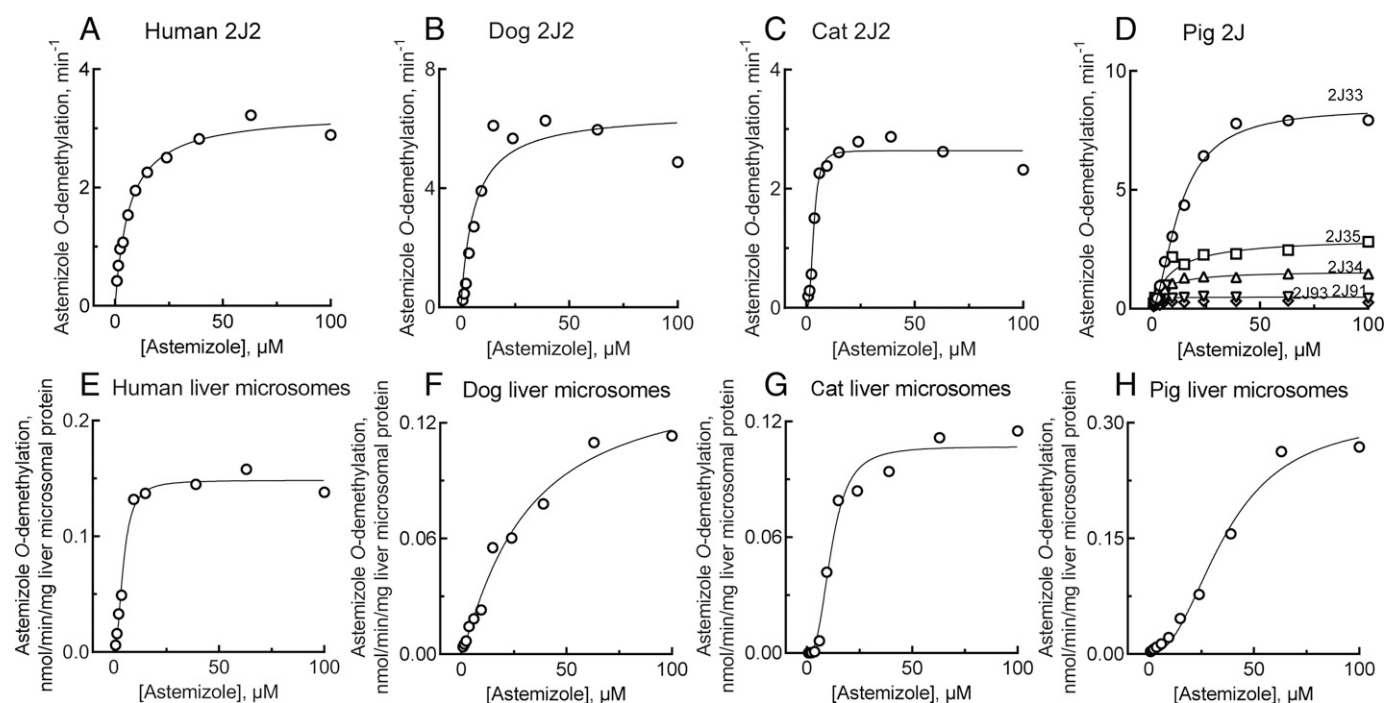


**Fig. 7.** Ebastine oxidation by recombinant P450 2J proteins. Ebastine primary hydroxylation (white) and secondary carebastine formation (gray) by recombinant human (A), dog (B), cat (C), and pig CYP2J proteins (D–H) at substrate concentrations of 1, 10, and 100  $\mu\text{M}$  were determined at 37°C for 10 minutes.

exhibited the primary sequence characteristics of P450s (Fig. 1). All the *CYP2J* genes contained conserved exon-intron structures (Fig. 4) and were located in corresponding genomic regions in humans, dogs, cats, and pigs; moreover, the pig *CYP2J* genes formed a gene cluster (Fig. 3). Therefore, the molecular properties of CYP2Js are generally similar in dogs, cats, pigs, and humans.

Dog, cat, and pig CYP2J mRNAs (except for pig CYPJ91 mRNA) were preferentially expressed in small intestine, among the tissue types analyzed, but were also expressed in liver and heart at lower levels (Fig. 5). The relatively low levels of dog, cat, and pig CYP2J mRNAs

in the heart contrasted with human CYP2J mRNA, which is abundant in human hearts (Wu et al., 1996; Delozier et al., 2007). This discrepancy might be accounted for by the transactivation mechanism of the *CYP2J* genes, which has not been fully investigated even for the human *CYP2J2* gene. In humans, *CYP2J2* contains a TATA-less promoter and putative binding sites for transcription factors (specificity protein-1 and activator protein-1) near the 5'-flanking region, which might be involved in *CYP2J2* transcription (Murray, 2016). Disrupting one of the specificity protein-1 binding sites by single nucleotide polymorphism (2J2\*7) reduces transcription and enzyme activity of human CYP2J2,



**Fig. 8.** Astemizole *O*-demethylation by recombinant CYP2J proteins (A–D) and liver microsomes (E–H) from humans, dogs, cats, and pigs. The kinetic parameters under the present conditions are shown in Table 2.

TABLE 2  
Kinetic parameters of astemizole *O*-demethylation by recombinant CYP2J enzymes and liver microsomes  
Kinetic parameters were calculated by nonlinear regression analysis (mean ± S.E., n = 12 substrate concentrations in the range 1.0–100 μM).

Enzyme Source	<i>K<sub>m</sub></i> or <i>S</i> <sub>50</sub>	Hill Coefficient	<i>V</i> <sub>max</sub>	<i>V</i> <sub>max</sub> / <i>K<sub>m</sub></i> ( <i>S</i> <sub>50</sub> )
Recombinant P450	(μM)		(min <sup>−1</sup> )	(mL/min/nmol)
Human CYP2J2	6.5 ± 0.7		3.3 ± 0.1	0.51
Dog CYP2J2	5.7 ± 1.0	2.5 ± 0.9	5.8 ± 0.3	1.0
Cat CYP2J2	3.3 ± 0.5	2.8 ± 1.1	2.6 ± 0.2	0.79
Pig CYP2J33	13 ± 1	1.7 ± 0.1	8.5 ± 0.3	0.65
Pig CYP2J34	5.3 ± 2.1		1.6 ± 0.2	0.30
Pig CYP2J35	8.8 ± 2.2		3.0 ± 0.2	0.34
Pig CYP2J91	0.82 ± 0.12		0.49 ± 0.01	0.60
Pig CYP2J93	2.0 ± 0.5		0.33 ± 0.01	0.17
Liver microsomes			(nmol/min/mg)	(μL/min/mg)
Human	4.5 ± 0.4	2.2 ± 0.3	0.15 ± 0.01	33
Dog	30 ± 9	1.2 ± 0.2	0.14 ± 0.02	4.7
Cat	12 ± 1	2.8 ± 0.6	0.11 ± 0.01	9.2
Pig	36 ± 7	2.3 ± 0.7	0.31 ± 0.05	8.6

and has been reported to be associated with the risk of cardiovascular diseases (Xu et al., 2013; Murray, 2016). Five *CYP2J* genes were found in pigs, unlike in dogs, cats, and humans. Multiple copies of *CYP2J* genes have been found in other species, such as mice (Nelson et al., 2004). Among the pig *CYP2J* mRNAs, *CYP2J35* mRNA, followed by *CYP2J93* mRNA, was the most abundant in livers, small intestines, kidneys, and lungs (Fig. 6). It would be of great interest to investigate the transactivation mechanisms of dog, cat, and pig *CYP2J* genes and the genetic variants at the upstream region.

In small intestines, dog and cat *CYP2J* mRNAs were expressed in jejunum and ileum at comparable levels (Fig. 5). Similarly, human *CYP2J2* protein is expressed in the gastrointestinal tract, including both jejunum and ileum (Zeldin et al., 1997), in contrast to human *CYP3A*, *CYP2C*, and *CYP2D6* proteins, which are more abundant in the proximal region than the distal region of the small intestine (Paine et al., 2006). Pig *CYP2J33*, *CYP2J35*, and *CYP2J93* mRNAs were the most profusely expressed mRNAs in jejunum (Fig. 6) and were more abundantly expressed in jejunum than ileum (Fig. 5). Such abundant expression of *CYP2J* mRNAs in small intestines suggests roles for *CYP2Js* in first-pass metabolism in dogs, cats, and pigs, similar to humans. Pig *CYP2J91* mRNA was preferentially expressed in the brain (Fig. 5), where it was the most abundant pig *CYP2J* mRNA (Fig. 6). Similarly, mouse *CYP2J8* and *CYP2J9* are abundantly expressed in the brain (Qu et al., 2001; Graves et al., 2013). Because *CYP2Js* are involved in the synthesis of epoxyeicosatrienoic acids, which play protective roles in inflammation and vasodilation (Solanki et al., 2018), *CYP2Js* in the brain might play roles in neuroprotective mechanisms. In contrast, a lower expression of *CYP2J* mRNAs in hearts might suggest only the minor protective roles of *CYP2Js* in this tissue for dogs, cats, and pigs, although further investigation is needed.

Dog and cat *CYP2J2* enzymes showed lower activities than human *CYP2J2* for ebastine oxidation (Fig. 7). Dog *CYP2J2* contains the residues A143 and C158, which are different from the T143 and R158 residues of human *CYP2J2*, and the substitution T143A was also found in cat *CYP2J2* (Fig. 1). In human *CYP2J2*, the alleles *CYP2J2*\*2 and *CYP2J2*\*3 possess the substitutions T143A and R158C, respectively, resulting in decreased enzyme activity (King et al., 2002). The decreased activities of dog and cat *CYP2J2* compared with human *CYP2J2* might be accounted for by the substitutions T143A and R158C.

Pig *CYP2J34* and *CYP2J35* enzymes showed activities roughly comparable to those of human *CYP2J2* toward all the substrates analyzed, i.e., ebastine and astemizole (Figs. 7 and 8). Although, pig *CYP2J34* and *CYP2J35* showed high ebastine oxidation rates compared to human *CYP2J2* (Fig. 7), pig *CYP2J33* mediated astemizole *O*-demethylation

even more efficiently than human *CYP2J2* (Fig. 8). Among the pig *CYP2Js*, *CYP2J91* showed the lowest activities toward the substrates analyzed in the present study (Figs. 7 and 8). Compared with human *CYP2J2*, pig *CYP2J91* contained the substitution L378M, which is located in SRS-6 (Fig. 1). Molecular docking analysis predicted that residues L378 and G486 are important for recognition and positioning of the substrate in the active site of the enzyme (Xia et al., 2014).

Pig *CYP2J35* contained 498 amino acid residues—four residues fewer than the other pig *CYP2Js*—resulting in substitutions or deletions of four residues in SRS-1 (Fig. 1). Of these four residues, the substitution P115L (human *CYP2J2*\*10 allele) decreases metabolic activity (King et al., 2002), and the residues M116 and R117, among others, play roles in binding and orientation of the substrate in the active site of human *CYP2J2*, as predicted by molecular docking simulations (Li et al., 2008). The latter study indicated additional residues important for substrate binding and orientation, including F56, L83, G222, L229, I376, G486, L487, and T488, which were found to be substituted in dog, cat, and pig *CYP2Js* (Fig. 1). Some substitutions were located in substrate recognition sites (SRS-1, SRS-4, SRS-5, or SRS-6), which are important for enzyme function (Gotoh, 1992; Lafite et al., 2007; Xu and Chen, 2020); these substitutions included I127V in pig *CYP2J33* and *CYP2J93*, F310V in pig *CYP2J93*, I376V and I487L in dog *CYP2J2*, and I487P in all the pig *CYP2Js* except *CYP2J33* (Fig. 1). These amino acid substitutions likely influence enzyme function, resulting in catalytic activities different from those of human *CYP2J2*. Taken together, the results of the current study indicated potentially more substantial contributions of pig *CYP2J33/34/35* than of pig *CYP2J91/93* to *CYP2J*-dependent drug metabolism in pigs.

In humans, *CYP2J2* exists in various forms, partly due to genetic polymorphisms (Xu et al., 2013; Murray, 2016). Similarly, in dogs, cats, and pigs, genetic variants have been identified, and some of them influence enzyme function (Puccinelli et al., 2011; Martinez et al., 2013; Lee et al., 2019); nonetheless, their relevance to enzyme function largely remains to be determined. It would be of great interest to investigate genetic variants of *CYP2J* in dogs, cats, and pigs.

In conclusion, all seven dog, cat, and pig *CYP2Js* identified in the present study were highly identical to human *CYP2J2* and were functional enzymes able to metabolize the typical human *CYP2J2* substrates ebastine, astemizole, and terfenadine. Except for pig *CYP2J91* mRNA, all the newly characterized *CYP2J* mRNAs were expressed predominantly in small intestine, just as human *CYP2J2* is. Among the pig *CYP2Js* investigated, *CYP2J93* and *CYP2J35* mRNAs were more abundant than other pig *CYP2J* mRNAs in small intestines, and *CYP2J35* showed metabolic properties similar to those of human

CYP2J2. Therefore, dog, cat, and pig CYP2Js likely play roles as drug-metabolizing enzymes in small intestines, just as human CYP2J2 does.

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## Authorship Contributions

*Participated in research design:* Uno, Yamazaki.

*Conducted experiments:* Uno, Murayama.

*Contributed new reagents or analytic tools:* Uno, Ijiri, Kawaguchi, Yamato, Teraoka, Mizukawa, Nakayama, Ikenaka, Ishizuka.

*Performed data analysis:* Uno, Asano, Shiraishi, Yamazaki.

*Wrote or contributed to the writing of the manuscript:* Uno, Yamazaki.

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