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NOTE

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Genetic diversity of cytochrome P450 1A2 with different metabolic activities in domestic cats

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ABSTRACT. Knowledge of genetic polymorphisms of metabolizing enzymes of medical drugs and xenobiotics including cytochrome P450 (CYP) is very limited in cats. We investigated polymorphisms in CYP1A2, one of the major CYP isoforms in the feline liver. Wild-type and three non-synonymous polymorphic variants, but no synonymous variant, were identified in feline CYP1A2 in 50 alleles of domestic cats in Japan. Metabolic parameters, Km and Vmax, of ethoxyresorufin hydroxylation by CYP1A2 were shown to range within two times for identified non-synonymous variants by using a heterologous coexpression system. The results confirmed the polymorphic nature of CYP1A2 as a basis for effective application of medicines and prevention of adverse reactions in the treatment of domestic cats as well as for hereditary disorders.

KEY WORDS: cytochrome P450, domestic cat, polymorphism, xenobiotic

Cytochrome P450 monooxygenase (CYP or P450) is the most important enzyme that is involved in the biotransformation of a broad range of endogenous and exogenous chemicals including drugs. It has been reported that there is considerable interindividual variability in response to drugs in humans and possibly in other animals including dogs since many years ago [3]. Genetic polymorphism of CYP is the representative factor for variation of sensitivity to medical drugs and toxicants [14]. Cytochrome P450 1A2 (CYP1A2) is one of the most important CYP isoforms in the biotransformation of broad clinical therapeutics [6]. CYP1A2 is also important for bioactivation of a number of procarcinogens including polycyclic aromatic hydrocarbons, mycotoxins and other chemicals. Furthermore, this enzyme metabolizes a large number of essential endogenous compounds including retinols, melatonin, steroids, uroporphyrinogen and arachidonic acids [5, 12]. In dogs, CYP1A2 premature stop codon polymorphism is well known [3].

Unless adequate approved drugs for companion animals are not available, drugs approved for humans can be prescribed for off-label use for veterinary medicine at the discretion of a veterinarian. Thus, they are also used in companion animals, of which the domestic cat, *Felis catus*, is one of the most common. However, little is known about the CYPs that are involved in the biotransformation of xenobiotics in cats despite their remarkable species differences in drug metabolism.

CYP1A2 is the CYP isoform that metabolizes the second largest number of drugs in humans [8]. Previously, we reported the amount of transcripts, metabolic activity and interactions with other drugs of feline CYP1A2 [7]. However, there are few reports on genetic polymorphism of CYP isoforms in the cat. Therefore, the aim of this study was to determine genetic polymorphism of CYP1A2 and their metabolic activity in cats.

Gonad samples from 18 male (testes) and 11 female (ovaries) cats (*Felis catus*) that had been obtained in spay surgery in local animal hospitals in Japan from 2010 to 2016 were used in this study with permission from the animal owners (Table S1). Following the company's instructions, genomic DNA and total RNA of gonads were extracted with TRI Reagent (Molecular Research Center, Cincinnati, OH, U.S.A.), and cDNA was prepared from total RNA with PrimeScript Reverse Transcriptase (Takara, Osaka, Japan). In order to address SNPs (single nucleotide polymorphisms), direct DNA sequencing of PCR products of CYP1A2 variants with cDNA and genomic DNA (Chromosome B3, ENSFCAG00000000344) (BigDye Terminator v3.1 Cycle Sequencing Kit, Thermo Fisher, Waltham, MI, U.S.A.) was repeated with an ABI310 sequencer (Thermo Fisher) from the PCR step (KOD-Plus-Neo, Toyobo, Osaka, Japan). The sequences of all of the exons coding CYP1A2 was analyzed. Heterologous

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Table 1. Identified variants of cytochrome P450 1A2 (CYP1A2) in cats

	Position	Nucleotide change	Amino acid change	Allele frequency	Comments
*1	-	-	-	38 / 50	
*2	680 (Exon1)	TGCGT (A>C) CTCCG	Y227S	9 / 50	SRS2
*3	799 (Exon1)	AGGAG (C>A) ACTAC	H267N	11 / 50	
*4	1229 (Exon5)	GCAGG (T>C) CAATC	V410A	2 / 50	
*5	1381 (Exon6)	TGGCC (A>G) AGTGG	K461E	3 / 50	

Mutation site of CYP1A2*2 is located in SRS2 (possible substrate-recognition site 2).

Table 2. Apparent kinetic parameters for metabolism of ethoxyresorufin in polymorphic variants of CYP1A2

	Km	Vmax	Vmax/Km
*1	0.99 ± 0.09[a)]	66.9 ± 2.2[ac)]	67.7 ± 2.2[a)]
*2	1.29 ± 0.13[a)]	58.2 ± 2.2[c)]	45.3 ± 1.6[b)]
*3	1.25 ± 0.15[a)]	85.0 ± 4.0[a)]	68.8 ± 3.8[ac)]
*4	1.42 ± 0.11[b)]	82.2 ± 2.5[a)]	63.9 ± 1.8[ab)]
*5	1.22 ± 0.12[a)]	96.4 ± 3.6[b)]	78.2 ± 3.5[c)]

Different letters on the shoulder of each figure indicate significant difference to other letters (P<0.05). Vmax (pmol/min/pmol P450). Km (μM). Values are average of four different preparation of recombinant alleles of CYP1A2 (N=4, mean ± SEM).

coexpression of CYP1A2 isoforms in *E. coli* was carried out as previously described [7, 10]. Wild-type feline cytochrome P450 reductase (POR) (DDBJ Accession No. LC416293) was coexpressed [10]. For obtaining variants of CYP1A2 and POR, PCR products that were prepared with cDNA were modified using a KOD-Plus Mutagenesis Kit (Toyobo). Metabolic activity of recombinant CYP1A2 was measured with 7-ethoxyresorufin as described previously [7]. Primers used in PCR, DNA sequencing and mutagenesis are listed in Table S2. Results are presented as means ± SEM. Significance of differences among groups was determined by one-way ANOVA followed by the Tukey-Kramer test (P<0.05). Kinetic parameters were determined by nonlinear regression fitting of the concentration velocity curve with the Michaelis-Menten equation using GraphPad Prism version 7 (GraphPad Software, San Diego, CA, U.S.A.).

Overall, five non-synonymous variants including wild type were identified in 50 alleles from 29 domestic cats in Japan (Table 1). Surprisingly, no synonymous variant was found. The major variant with the highest frequency of 38/50 was determined as wild type (CYP1A2*1). The second and third highest frequencies were 11/50 (CYP1A2*3) and 9/50 (CYP1A2*2), respectively. CYP1A2*2 contained non-synonymous SNPs in the possible substrate-recognition site (SRS) 1 (Fig. S1).

Using a heterologous coexpression system of CYP1A2 in *E. coli* [7], activity for hydroxylation of ethoxyresorufin (8 concentrations between 0.078 and 5 μM) by polymorphic CYP1A2 variants was determined (Fig. S2). We used feline POR instead of human POR, which was used in previous experiments [7]. Based on a Michaelis-Menten plot, Vmax and Km for each of the polymorphic CYP1A2 variants were determined (Table 2). Significant differences were observed for some pairs in both Vmax and Km. However, the range was relatively small, 1.4–1.7 times (1.4 for Km, 1.7 for Vmax and Vmax/Km as maximum) (Table 2).

This is the first study to confirm the presence of a polymorphism of the CYP1A2 isoform that could affect metabolism of drugs and other chemicals in the liver and other tissues of cats by using a heterologous coexpression system with feline POR. The most surprising finding was that a synonymous variant was not found despite identifying four non-synonymous variants in this study. In human CYP1A2, 145 synonymous variants and 337 non-synonymous variants have been identified by using the Genome Aggregation Database (http://gnomad.broadinstitute.org/about). Thus, this very high ratio of non-synonymous to synonymous mutations (ds/dn) suggests the necessity of many types of CYP1A2 for adaptation to various environments, although more extensive analysis using a much larger number of samples from cats obtained worldwide is needed. It was reported that human CYP1A2, a major enzyme catalyzing the metabolism of both endogenous and exogenous agents, may play a role in the etiology of carcinogenesis and various other disorders including diabetes and neuropsychiatric disorders [4, 11]. Just recently, we reported seven non-synonymous CYP3A131 variants with only one synonymous variant in cats [10]. The polymorphic status in other CYP isoforms should be studied in cats.

It is well known that CYP1A2 premature stop codon (c.1117C>T; R383X) with a complete lack of enzyme activity is highly prevalent in certain dog breeds including Beagle and others studied [14]. The area under the curve of phenacetin was doubled in mutant dogs. When ethoxyresorufin was used as a substrate in a heterologous expression system in *E. coli*, the variation of enzymatic activity of polymorphic variants was relatively low and the ranges of some kinetic parameters were less than 1.7. It is thought that these coding variants of feline CYP1A2 may contribute to, but are not likely to be the major cause of, interindividual differences in the clearance of CYP1A2 substrates. Shah *et al.* [9] reported EROD activity by the liver microsomes of cats for experiment (0.075 ± 0.003 μM for males, 0.13 ± 0.05 μM for females: n=5), although extent of contribution of CYP1A1 in this

reaction was unknown. It was reported that 10 non-synonymous variants in humans, including CYP1A2*3, CYP1A2*4 and CYP1A2*6, of 30 haplotype-defined non-synonymous variants (https://www.pharmvar.org/, last update on November 12, 2007) were associated with diminished activity of CYP1A2 [1, 13]. The corresponding non-synonymous variants were not found in cats in this study. While there is relatively few non-synonymous variants with marked changes in enzymatic activity in human CYP1A2, we were not able to find non-synonymous variants of feline CYP1A2 that seriously affected the activity in this study. Though coding variants may affect the amount of transcripts of CYP1A2 such as CYP1A2*1F with higher inducibility [2], it is difficult to obtain fresh liver samples from household companion cats. Further study is required to investigate metabolic activity with other substrates in addition to detecting other polymorphic variants in feline CYP1A2.

In summary, the present study revealed for the first time the polymorphic nature of CYP1A2 with changes in hydroxylation activity in cats. Although none of the four non-synonymous types identified in this study showed a great change in their hydroxylation activity, further study is needed to find a possible critical polymorphic CYP1A2 that is important for drug treatment. Four non-synonymous variants were identified without any synonymous variant, suggesting an essential role of CYP1A2 for adaptation to various environments and survival of cats.

CONFLICT OF INTEREST. None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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