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NOTE

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Genetic diversity of cytochrome P450 2A with different metabolic activities in domestic cats

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ABSTRACT. Knowledge of genetic polymorphisms of cytochrome P450 (CYP), the most important xenobiotic metabolizing enzyme, is very limited in cats. Preliminarily, we investigated genetic polymorphisms in CYP2A13, one of the major CYP isoforms in the liver and lung. Four synonymous and three non-synonymous polymorphic variants were identified in feline CYP2A13 in domestic cats in Japan, without an obvious major type. Metabolic parameters, Km and Vmax, of coumarin hydroxylation of CYP2A13 were shown to range within two times for the identified non-synonymous polymorphic variants by using heterologous coexpression system in *Escherichia coli*. The results confirmed the polymorphic nature of CYP2A13 as a basis for effective application of medicines and prevention of adverse reactions in treatment of domestic cats.

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 medical 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 [1]. Genetic polymorphism of CYP is the representative factor for variation of sensitivity to medical drugs and toxicants [11]. Cytochrome P450 2A6 (CYP2A6) accounts for about 4% of total hepatic P450 that is involved in the metabolism of toxic xenobiotics such as coumarin, nicotine, and aflatoxin B1 as well as medical drugs such as cyclophosphamide [2, 4]. Human CYP2A13 is predominantly expressed in the respiratory tract and is significantly involved in nicotine metabolism [10] and in the activation of aflatoxin B1 to carcinogenic derivatives [3]. It was reported that single nucleotide polymorphisms (SNPs) predominantly occurred in 3 polymorphic CYPs, CYP2D6, CYP2A6 and CYP2B6, in humans [8].

Cats are popular companion animals; however, knowledge about the CYPs involved in biotransformation of xenobiotics is limited despite the fact that there are remarkable species differences in drug metabolism. In a previous study, we found that CYP2A13 is one of the major CYP isoforms in the liver and lung as well as its hydroxylation activity of coumarin and other substrates using heterologous coexpression system in *Escherichia coli* (*E. coli*) [6, 7]. Therefore, in this study, we aimed to determine genetic polymorphisms of CYP2A13 and their effects on metabolic activity in cats.

Gonad samples from 18 male (testes) and 11 female (ovaries) cats (*Felis catus*) that had been obtained 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). Direct DNA sequencing of PCR products of CYP2A13 variants with genomic DNA (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). We randomly selected 25 genomic DNA for analysis among 29 genomic DNA for each exon of CYP2A13. Additionally, some cDNA samples were used for direct sequencing as described previously. Heterologous coexpression of CYP2A13 isoforms in *E. coli* was carried out as previously described [7]. Wild-type feline cytochrome P450 reductase (POR) was coexpressed with CYP2A13 [9]. POR is required for electron transfer from NADP to P450 in the hydroxylation of substrates by P450. For obtaining variants of CYP2A13, PCR products that were prepared with cDNA were modified using a KOD-Plus

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Table 1. Identified variants of cytochrome P450 2A13 (CYP2A13) in cats

	Position	Nucleotide change	Amino acid change	Allele frequency	Comments
*1A	-	-	-	24 / 50	
*1B	225 (Exon2)	GGGCC (G>A) CGGCG	-	5 / 50	Synonymous
*1C	444 (Exon3)	GAGCG (C>G) ATCCA	-	2 / 50	Synonymous
*1D	870 (Exon6)	AAGAA (C>T) CTGGT	-	17 / 50	Synonymous
*1E	1410 (Exon9)	CAAGA (C>T) ATTGA	-	20 / 50	Synonymous
*2	49 (Exon1)	TGACA (A>G) TAATG	I17V	6 / 50	
*3	429, 430 (Exon3)	AAGCG (CA>TG) GCATC	S144G	10 / 50	
*4	742 (Exon5)	ACTTC (A>G) TAACC	I248V	17 / 50	

Table 2. Apparent kinetic parameters for metabolism of coumarin in polymorphic variants of CYP2A13

	Km	Vmax	Vmax/Km
*1A	5.1 ± 0.7	23.0 ± 1.3[a)]	4.5 ± 0.3[a)]
*2	3.9 ± 0.7	20.0 ± 1.4[ab)]	4.3 ± 0.3[a)]
*3	4.0 ± 0.4	28.0 ± 1.1[a)]	6.6 ± 0.4[b)]
*4	2.9 ± 0.3	13.7 ± 0.5[b)]	4.5 ± 0.4[a)]

Different letters on the shouluder 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 variants (N=4, mean ± SEM).

Mutagenesis Kit (Toyobo). Metabolic activity of recombinant CYP2A13 was measured with coumarin, a substrate specific also for human CYP2A as described previously [7]. Primers used in PCR, DNA sequencing and mutagenesis are listed in Table S2. Quantitative 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).

Overall, eight polymorphic CYP2A13 variants including three non-synonymous and four synonymous variants were identified in 29 cats (Table 1). We tentatively named the major variant CYP2A13*1A (DDBJ Accession No. LC458470). The synonymous types identified were named CYP2A13*1B–*1E following the position of the mutation in order. Five out of eight SNPs were clustered in the first three exons (Fig. S1). No SNPs were identified in the six putative substrate recognition sites (SRSs) and heme-binding region.

Using a heterologous coexpression system of CYP2A13 in *E. coli* [6, 7], activity for hydroxylation of coumarin (8 concentrations between 0.3125 and 15 μM) by non-synonymous polymorphic CYP2A13 was determined (Fig. S2). We used feline POR instead of human POR, which was used in previous experiments [6, 9]. Coumarin is known as a specific substrate for CYP2A6 in humans and cats [6, 7]. Based on a Michaelis-Menten plot, Vmax and Km for each of the polymorphic CYP2A13 variants were determined (Table 2). Significant differences were observed for some pairs in Vmax, although Km values only tended to be different in these variants. The range was almost 2 times (2.1 for Vmax and 1.8 for Km as maximum) and Vmax/Km was smaller (1.5, Table 2).

This is the first study to confirm the polymorphic nature of CYP2A13, which must be involved in the metabolism of drugs and other chemicals in the liver and lung of cats [6, 7]. Although we tentatively determined the wild type in feline CYP2A13, we found the second major variant (CYP2A13*2) in 29 cats. The frequency of CYP2A13*2 was 75% of that of the first major variant (CYP2A13*1A). Naturally, however, the distribution might be different in a study with more cat samples. Just recently, we reported seven non-synonymous CYP3A131 variants with only one synonymous variant in cats [9]. The polymorphic status in other CYP isoforms should be studied in cats.

When coumarin was used as a substrate in the heterologous expression system in *E. coli*, the variation in enzymatic activity of variants ranged within 2 times (Table 2). This is somewhat different to the situation in human CYP2A6. CYP2A6 polymorphism has been extensively studied with nicotine as a substrate in relation to nicotine addiction [5]. According to "CYP2A6 allele nomenclature, 2014; https://www.pharmvar.org/htdocs/archive/cyp2a6.htm", 42 well-characterized allelic variants and some haplotypes that are uncharacterized have been identified. Activities of most CYP2A6 variants are decreased or absent of 35 variants that were provided for their enzyme information [5]. The absence of CYP2A6 protein synthesis is rather minor, including CYP2A6*2, *4, *20 and *27 [5, 8]. Although CYP2A6*12 showed decreased expression in human liver samples, it is very difficult to study its expression in cats because fresh liver samples from many healthy companion cats were practically unavailable. Naturally, additional study is needed to find other polymorphic variants in feline CYP2A13. Also, substrates other than coumarin should be used.

In summary, the present study confirmed the polymorphic nature of CYP2A13 with quantitatively different hydroxylation activities in cats. This is the first study about polymorphism in a CYP2A isoform in cats. Although none of the 3 non-synonymous

types identified in this study showed a great change in their hydroxylation activity, further study can reveal a critical polymorphic CYP2A13 that is important for drug treatment.

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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