Title

Identification and quantification of domestic feline cytochrome P450 transcriptome across multiple tissues

Journal

Journal of Veterinary Pharmacology and Therapeutics, 42(1)

ISSN

0140-7783

Authors

Visser, Marike
Weber, Kristina L
Lyons, Leslie A
et al.

Publication Date

2019

DOI

10.1111/jvp.12708

Peer reviewed

HHS Public Access

Author manuscript

J Vet Pharmacol Ther. Author manuscript; available in PMC 2020 January 01.

Published in final edited form as:

J Vet Pharmacol Ther. 2019 January ; 42(1): 7–15. doi:10.1111/jvp.12708.

Identification and Quantification of Domestic Feline Cytochrome P450 Transcriptome across Multiple Tissues

Marike Visser[1], **Kristina L. Weber**[2], **Leslie A. Lyons**[3], **Gonzalo Rincon**[4], **Dawn M. Boothe**[5], and **Dawn A. Merritt**[6]

[1]Global Therapeutics Research, VMRD, Zoetis, Kalamazoo, MI

[2]Bioinformatics Field Applications Support, Pacific Biosciences, Menlo Park, CA

[3]Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, MO

[4]Genetics R&D, VMRD, Zoetis, Kalamazoo, MI

[5]Department of Anatomy, Physiology, and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, AL

[6]Lab Sciences, VMRD, Zoetis, Kalamazoo, MI

Abstract

Understanding of cytochrome P450 (CYP) isoform distribution and function in the domestic feline is limited. Only a few studies have defined individual CYP isoforms across metabolically relevant tissues, hampering the ability to predict drug metabolism and potential drug-drug interactions. Using RNA-sequencing (RNA-seq), transcriptomes from the 99 Lives Cat Genome Sequencing Initiative databank combined with experimentally acquired whole transcriptome sequencing of healthy, adult male (n=2) and female (n=2) domestic felines, expression of 42 CYP isoforms were identified in 20 different tissues. Thirty-seven of these isoforms had not been previously reported in cats. Depending on the tissue, three to twenty-nine CYP isoform transcripts were expressed. The feline genome annotations did not differentiate *CYP2E1* and *2E2* genes, demonstrating poor annotation for this gene using the reference genome. Since the majority of the sequences are based on automated pipelines, complete cDNA sequences for translation into CYP protein sequences could not be obtained. This study is the first to identify and characterize 37 additional CYP isoforms in feline tissues, increasing the number of identified CYP from the previously reported seven isoforms to 42 across 20 tissues.

Corresponding author: Marike Visser; marike.visser@zoetis.com.
DR MARIKE VISSER (Orcid ID: 0000-0002-4233-7491)
DR DAWN A MERRITT (Orcid ID: 0000-0001-9974-2888)

Conflict of interest: The authors do not have any conflict of interest to declare.

Author Contribution Statement: Marike Visser and Kristina Weber collected experimental samples and completed data analysis. Leslie Lyons provided 99 Lives sample data for inclusion in analysis. Gonzalo Rincon, Dawn M Boothe, and Dawn A. Merritt provided input on study design and organized study execution.

Keywords

feline; RNA-seq; cytochrome P450; 99 Lives Cat Genome Sequencing Initiative

Introduction

Cytochrome P450 (CYP) constitutes the major enzyme family catalyzing oxidative metabolism of drugs and lipophilic xenobiotics. This family also serves an important role in homeostasis, with the synthesis of steroid hormones, prostaglandins, bile acids, and eicosanoids. In humans, 57 functional genes and 58 pseudogenes, are clustered into 18 families and 44 subfamilies based on their amino acid homologies(Zanger & Schwab, 2013). This contrasts with the 44 CYP identified in the canine transcriptome(Visser et al., 2017). CYP isoforms can be classified based on function into CYPs involved in the synthesis or metabolism of sterols, xenobiotics, fatty acid, eicosanoid, vitamin and unknown(Guengerich et al., 2016). Significant CYP families involved in xenobiotic or drug metabolism include CYP1A, CYP2B, CYP2C, CYP2D, CYP2E, and CYP3A. These CYP have been implicated in drug-drug interactions (DDI), acting as a substrate, inhibitor or inducer to alter the pharmacokinetics of concurrently used drugs(Danielson, 2002).

Differences between the canine, feline and human CYP profile include gene expression, *in vitro* microsomal clearance, and enzyme kinetics of probe substrates(Bogaards et al., 2000; Graham et al., 2003; Lee et al., 2015; Nishimuta et al., 2013; Okamatsu et al., 2016). Commonly used human CYP probe substrates report limited CYP2C activity and gender differences in felines, with the rate of CYP2D6 metabolism greater in females than males, and an inverse relationship reported in CYP3A(Shah et al., 2007). A comparison between human, equine, canine and feline CYP probe substrates found significantly higher activity of coumarin in humans compared to felines, higher tolbutamide activity in human and equine compared to cat, higher dextromethorphan O-demethylase and chlorzoxazone 6-hydroxylase activity in equine compared cat, and higher testosterone 6B-hydroxylase activity in humans compared to cat(Chauret et al., 1997). Compared to the canine CYP repertoire and function, only a few individual feline CYP isoforms have been described. Reported differences between canine and feline, include feline *CYP2B6* expression in lung and duodenum but not in the liver(Okamatsu et al., 2017) and duplication of *CYP2E*(Tanaka et al., 2005). Additional feline CYP previously described include *CYP2A13*(Okamatsu et al., 2015), *3A131, 3A132*(Honda et al., 2011), and *2D6*(Komatsu et al., 2010). While probe substrates suggest differences between the various species, the limited information regarding CYP expression in the feline limits the ability to predict drug efficacy and safety.

Next generation sequencing accurately sequences the entire genome or transcriptome of an organism. RNA-sequencing (RNA-Seq) has proven to be a powerful tool for quantifying gene expression and transcripts, discovering alternative splicing sites, detecting gene fusions, mapping transcription pathways, and the detection of single nucleotide variations(Chen et al.,2011; Marguerat & Bahler, 2010). This technology has been used to map cell response to feline immunodeficiency virus(Ertl & Klein, 2014), identify orthologues to genes in the published genome(Hong et al., 2011) and determine a missense

mutation in *HES7* leading to short tails in Asian domestics cats (Lyons et al., 2016; Xu et al., 2016). Currently, a large scale, community research project entitled “99 Lives” is underway, which uses whole genome and transcriptome sequencing to identify genetic variation between feline breeds, identify mutations of health concern and provide individual feline sequences for clinical research (Aberdein et al., 2017; Leslie A Lyons, 2016; Mauler et al., 2017; Oh et al., 2017).

The quantification of gene expression from short read RNA-seq data depends on binning reads by their transcript of origin. This may be done by aligning to a de novo transcript assembly or to a reference genome annotated with genes and transcripts. Annotated reference genomes allow annotated genes to be overlaid with other features including non-coding transcripts and variants (Wit et al., 2012), as well as homologous regions to be compared across individuals and species. With the availability of publicly available reference genomes via NCBI and Ensembl, alignment or mapping to the reference genome is generally the method of choice, particularly when comparing gene expression between individuals. Each sample’s sequenced reads are compared to the whole genome assembly and aligned to the position of greatest similarity. If a read matches to multiple places on the reference, it is discarded since one cannot identify which is the correct location. Once aligned, reads are binned by gene or transcript based on unique sequence (such as exon-exon junctions or exons unique to a single gene or transcript) or random assignment to one of the pool of genes or transcripts to which they match. The shorter the read, the higher the probability of a non-unique assignment, which is a source of error in RNA-seq based gene expression measures.

Recently, RNA-seq was used to characterize the CYP transcriptome in canine liver, lung, duodenum, kidney and whole blood (Visser et al., 2017). The purpose of this study was to identify and quantify CYP expression in five tissues collected from the domestic feline and combine the sequenced transcriptomes with 99 Lives transcriptomes to characterize CYP isoform expression in multiple tissues.

Methodology

Subjects had served as a control group for a separate study. All protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at Zoetis. Procurement of samples for the 99 Lives Project was in accordance with University of Missouri IACUC protocols 8787, 9178, and 9056.

Sample collection

Male (n=2) and female (n=2) healthy, adult domestic felines were euthanized via an intravenous injection of sodium pentobarbital; blood (n=4), and sections of liver (n=4), kidney (n=4), lung (n=4), duodenum (n=4), were collected. Blood (PAXgene Blood RNA Tube, PreAnalytiX, Hombrechtikon Switzerland) and tissues (RNA*later*®, Qiagen, Valencia, CA, USA) were kept frozen at −80°C until processing. Liver and kidney total RNA was isolated using the RNeasy Mini protocol (Qiagen®), lung and duodenum isolated using the Fibrous RNeasy protocol (Qiagen®) and blood using PAXgene Blood RNA Kit, PreAnalytiX (Qiagen®). RNA integrity number (RIN) (Bioanalyzer 2100, Agilent

Technologies, Santa Clara, CA, USA) and cDNA quality (NanoDrop 8000, ThermoScientific, Wilmington, DE, USA) were evaluated before cDNA library preparation (Schroeder et al., 2006). The RIN relies on 18S to 28S ribosomal subunit ratios plus other features of the electrophoresis trace to quantify RNA integrity on a 1–10 scale and was utilized to assess the quality of the RNA prior to cDNA library preparation. cDNA library preparation was completed utilizing the TruSeq® Stranded mRNA sample preparation protocol (Illumina ®, San Diego, CA, USA). Libraries were quantified (Quanti-IT® High-Sensitivity DNA Assay Kit, ThermoScientific, Grand Island, NY, USA) and submitted for sequencing (Eureka Genomics®, Hercules, CA, USA).

99Lives Cat Genome Sequencing Initiative

Transcriptomes from a panel of 32 tissues were provided by the 99Lives project at the University of Missouri. Samples, frozen at −80°C, were derived from 15 cats, collected between 2010 and 2015, suffering from a variety of health conditions including: congenital myasthenic syndrome (occipital brain), hydrocephalus (lung, pancreas, heart, muscle, ear cartilage, spinal cord, and thymus), polycystic kidney disease (kidney, testes, and cerebellum), progressive retinal atrophy (retina, salivary gland, and bone marrow), seizures (temporal lobe, parietal lobe, hippocampus, cerebellum, and liver), tail abnormality (skin and retina), carrier of LPL deficiency (skin and kidney), carrier of Bengal and Persian progressive retinal atrophy (kidney, spleen, uterus, and ear tip), and whole fetuses from healthy cats. Total RNA was extracted using Qiagen RNeasy Mini protocol, and NGS libraries prepared using the Illumina TruSeq® Stranded mRNA sample preparation protocol. Libraries were sequenced on an Illumina HiSeq 2500 (v4), 2 × 125bp to a read depth of approximately 121M reads/sample at HudsonAlpha Institute for Biotechnology (Huntsville, AL).

RNA-seq Analysis and Statistics

RNA-seq analysis was performed using CLC Genomics Workbench 9.0 (Qiagen ®). Quality checks of RNA sequencing reads included statistics on the number of reads per tissue sample, base quality score, read length, GC content, and most frequent 15-mers. Reads were mapped to the most recent feline annotated genome (Felis catus 8.0, NCBI release 103) from NCBI (Cunningham et al., 2015; Curwen et al., 2004). Transcript per million (TPM) was used to quantify transcripts, and data with a TPM> 2 were included, as sequencing depth and number of replicates precluded characterization of very lowly abundant transcripts and 2 TPM represents a conservative cutoff for disambiguating gene expression from transcriptional noise (Wagner et al., 2013). To maximize the likelihood of detecting all CYP in the genome, orthologues of human, ferret and feline CYP annotations were identified via ENSEMBL BioMart, a data mining tool allowing for comparison between the annotated genome of organisms. An extensive literature search was also done to ensure that all known feline CYP were accounted. *CYP2B6* was not present in the annotated feline genome, and the sequence information was found in GenBank (ncbi.gov, KU198409.1) and included in the genome (Okamatsu et al., 2017). Each gene was checked in ENSEMBL and NCBI, and described including the ENSEMBL and NCBI identification, whether the gene was modeled based on automated pipeline or provided by an outside source requiring additional curation, and reported chromosomal location (Table 1).

Results

Data from both healthy and diseased cats was analyzed

The number of paired reads/sample averaged 46.4million (M) with liver having the lowest tissue total (144M) and kidney having the largest (248M). Greater than 90% of reads mapped to the feline reference genome or all tissues, with an average of 95.6% and 80% mapping uniquely. The genome annotations accounted for greater than 85% of mapped reads (i.e., less than 15% reads mapping to intergenic regions) for most samples with an exception for kidney (17.5% mapped to intergenic regions) and neural tissue such as spinal cord (16.1%) and brain (up to 21%). A total of 42 CYP were identified across 20 tissues (Table S1). The numbers of biological replicates varied between the samples, ranging from six for kidney, brain, and liver to only one for the spinal cord, testes, uterus, heart, muscle, pancreas, salivary gland, thymus and bone marrow. The highest CYP isoform transcript expression was liver (n=5), with 29 individual CYP identified, and the lowest was pancreas (n=1) with only three CYP isoforms transcripts identified (Figure 1a). Based on CYP function (Table 1), the spinal cord contained the highest number of isoforms involved in sterol synthesis. The liver contained the highest number of xenobiotic isoforms. The tissues with the highest number of eicosanoid isoforms were liver, cartilage, skin, lung, kidney, and duodenum. Vitamin synthesis isoforms were highest in cartilage, retina and fetal tissue. Summation of the CYP TPM shows that liver has the greatest count, with the majority designated for sterol and xenobiotics. The pancreas and muscle had the lowest CYP TPM count (Figure 1b).

The five highest expressed CYP in the liver are *CYP2E1/2>3A132*>2A13>*2D6>3A131* (Figure 2). In the duodenum, *CYP3A131* expression was the highest, followed by *CYP2C41>4B1>2B6>2J2* (Figure 3).. *CYP2B6* expression in the lung was estimated to be 43% of the CYP content, with *CYP4B1>2F2>2S1>39A1*, making up the remaining four highest expressed CYP mRNA transcripts. \ (Figure 4). The blood only expressed a total of 6 CYP isoforms, with *CYP4F2* expression the highest, followed by *CYP2J2>4V2>4B1>51A1>4F3* (Figure 5). The kidney had a variety of CYP transcripts *CYP4A11>4F2>4F3>39AA1>27A1*(Figure 6).

Discussion

This study is the first to characterize global CYP expression in the domestic cat, providing insight into different tissue CYP transcript profile at multiple sites in the body. Whole transcriptome sequencing from multiple sources was combined to increase the number of reported feline CYP isoforms from seven to 42. A major CYP xenobiotic family, CYP2C, was also identified and described for the first time.

The nomenclature utilized in this report is based upon the currently publically available nomenclature on ENSEMBL. This is based upon completed referenced sequences and automated annotations. Complete sequencing and submission of the sequences to the CYP nomenclature committee is necessary to improve the naming and association of the CYPs presented in this article(Nelson, 2006; Nelson, 2009).

All of the recognized major drug metabolizing CYP families (CYP1A, 2A, 2B, 2C, 2D, and 3A) were identified in at least one tissue. Previous reports on *CYP2B6* expression found negligible transcript and protein expression in the liver, but transcript and protein expression were predominant in the lung and detectable in the small intestine(Okamatsu et al., 2017). RNA-seq found negligible transcript expression in the liver and blood, with the highest expression in the lung followed by kidney and duodenum. Drugs requiring CYP2B6 for metabolism can be expected to have slower metabolism in the feline compared to the canine due to the lack of CYP2B6 in the liver. Reported CYP2B6 substrates include drugs such as methadone(Kharasch & Stubbert, 2013), cyclophosphamide(Bachanova et al., 2015), propofol, diazepam, midazolam, ketamine, S-medetomidine, R-medetomidine, warfarin, and phenytoin(Martinez et al., 2013). Pharmacokinetic/pharmacodynamics studies of CYP2B6 substrates are necessary to determine the clinical impact of this CYP difference between cats and dogs.

The CYP3A family consists of *CYP3A131* and *3A132*, with previous reports of *CYP3A131* mRNA transcript expression in small intestine and liver, and limited *CYP3A132* expression only in liver(Honda et al., 2011). RNA-Seq results indicate *CYP3A131* expression was present in all five experimentally acquired tissues examined, with the majority of expression in liver and duodenum. In contrast to reported results, *CYP3A132* expression was higher in the liver than *CYP3A131* transcript expression and was present in the remaining four tissues, but to a lesser extent than *CYP3A131*(Okamatsu et al., 2016). The two isoform transcripts are reported to be 94.4% identical and the amino acid sequences are 90% identical. This may have resulted in a mismatch when the sequences were mapped to the reference genome, or poor annotations resulting in overlap. Due to the limited annotations available and the wide variety of tissues used, a stringent TPM cut-off and a use of unique matches only, may have resulted in some sequences being dropped. The CYP3A family is a highly significant xenobiotic metabolizing family in both humans and canines due to the described large enzyme pocket allowing a variety of substrates to be metabolized(Ekins et al., 2003). The impact of different isoforms on drug metabolism has not been established, but different human and canine isoforms have diverse drug metabolism for the CYP3A family(Court, 2013) and whether this is also true in feline CYP3A needs to be explored further.

CYP2E has been reported to be duplicated in the literature (named *CYP2E1* and *CYP2E2*), but annotation in ENSEMBL and NCBI does not consistently differentiate these isoforms. As each copy has been described to have different tissue distributions (liver, kidney, lung, stomach, gastric gland, pyloric gland, small intestine, and pancreas for CYP2E1, and liver and mononuclear cells for 2E2(Tanaka et al., 2005)), ambiguity between them could lead to misunderstanding of function. In ENSEMBL the isoform is denoted as *CYP2E1*, but in NCBI the same gene is denoted at *CYP2E2*. This lack of consistent and complete annotation prevents proper identification of two separate isoform mRNA transcripts if present.

CYP2C21 and *CYP2C41*is reported for the first time in the feline. Detection of these two isoforms was based on the automated ENSEMBL annotation prediction algorithm using homology in genome sequence(Curwen et al., 2004). Therefore the individual transcripts have not been independently sequenced and protein content verified, key components

necessary to determine the impact on drug metabolism. The two CYP2C isoforms were detected in moderate abundance across several animals, compared to the canine CYP2C family where only a small percentage of canines report to expressed both isoforms(Court, 2013; Martinez et al., 2013). Substrates reported to be metabolized by CYP2C in humans include celecoxib, omeprazole, phenytoin, tolbutamide, warfarin, diazepam, lansoprazole, and voriconazole (Ingelman-Sundberg, et al., 2007; Zhang et al., 2016). However, humans have four CYP2C isoforms compared to the two detected in felines, and inferences regarding substrate metabolism should be made with caution.

CYP families important for the regulation of inflammation, steroid and vitamin D synthesis, and regulation of vascular resistance were prominent in blood and kidney. Biological replicates are essential to improve the accuracy of any prediction, and therefore no inferences regarding CYP transcript quantity can be made when there are only a few biological replicates present. In the literature, reports are conflicting regarding the minimum number of biological replicates necessary, which is based on the type and the purpose of the analysis such as detecting physiological gene expression versus differential gene expression based on site or disease state.

This is the first global overview of CYP expression in the feline and characterized CYP expression across a variety of tissues. While several CYP isoforms were described, mRNA expression does not correlate to protein expression, requiring further studies for complete isoform sequencing and protein quantification. Studies have shown that the same CYP isoform in different species will have variable enzyme kinetics, leading to sub-therapeutic concentrations or toxicity(Kimble et al., 2014). Compared to the canine genome annotations, the accuracy and depth of feline annotation is extremely limited, leading to discrepancies between the two databases and the need for manual curation of the genome. Additional research into protein quantification and CYP phenotyping will improve the prediction of drug metabolism and decrease the risk of drug-drug interactions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors would like to thank Sarah Corum for her technical expertise.

Funding was provided in part by the National Center for Research Resources R24 RR016094/OD010928, the University of Missouri – Columbia Gilbreath-McLorn Endowment and the Winn Feline Foundation (MTW13-010) (LAL) and Zoetis Inc®.

References

Aberdein D, Munday JS, Gandolfi B, Dittmer KE, Malik R, Garrick DJ. … Consortium L. 2017; A FAS-ligand variant associated with autoimmune lymphoproliferative syndrome in cats. Mammalian Genome. 28(1–2):47–55. [PubMed: 27770190]

Bachanova V, Shanley R, Malik F, Chauhan L, Lamba V, Weisdorf DJ, … Lamba JK. 2015; Cytochrome P450 2B6*5 Increases Relapse after Cyclophosphamide-Containing Conditioning and Autologous Transplantation for Lymphoma. Biol Blood Marrow Transplant. 21(5):944–948. DOI: 10.1016/j.bbmt.2015.02.001 [PubMed: 25677220]

Bogaards JJ, Bertrand M, Jackson P, Oudshoorn MJ, Weaver RJ, van Bladeren PJ, Walther B. 2000; Determining the best animal model for human cytochrome P450 activities: a comparison of mouse, rat, rabbit, dog, micropig, monkey and man. Xenobiotica. 30(12):1131–1152. DOI: 10.1080/00498250010021684 [PubMed: 11307970]

Chauret N, Gauthier A, Martin J, Nicoll-Griffith DA. 1997; In Vitro Comparison of Cytochrome P450-Mediated metabolic Acivtities in Human, Dog, Cat, and Horse. Drug Metab Disp. 25(10):1130–1136.

Chen G, Wang C, Shi T. 2011; Overview of available methods for diverse RNA-Seq data analyses. Sci China Life Sci. 54(12):1121–1128. DOI: 10.1007/s11427-011-4255-x [PubMed: 22227904]

Court MH. 2013; Canine cytochrome P-450 pharmacogenetics. Veterinary Clinics of North America: Small Animal Practice. 43(5):1027–1038. DOI: 10.1016/j.cvsm.2013.05.001 [PubMed: 23890236]

Cunningham F, Amode MR, Barrell D, Beal K, Billis K, Brent S, … Flicek P. 2015; Ensembl 2015. Nucleic Acids Research. 43(Database issue):D662–669. DOI: 10.1093/nar/gku1010 [PubMed: 25352552]

Curwen V, Eyras E, Andrews TD, Clarke L, Mongin E, Searle SM, Clamp M. 2004; The Ensembl automatic gene annotation system. Genome research. 14(5):942–950. [PubMed: 15123590]

Danielson PB. 2002; The cytochrome P450 superfamily: biochemistry, evolution and drug metabolism in humans. Current Drug Metabolism. 3(6):561–597. [PubMed: 12369887]

Ekins S, Stresser DM, Williams JA. 2003In vitro and pharmacophore insights into CYP3A enzymes. Trends on Pharmacological Sciences. :161–166.

Ertl R, Klein D. 2014; Transcriptional profiling of the host cell response to feline immunodeficiency virus infection. Virology journal. 11(1):52. [PubMed: 24642186]

Graham MJ, Bell AR, Crewe HK, Moorcraft CL, Walker L, Whittaker EF, Lennard MS. 2003; mRNA and protein expression of dog liver cytochromes P450 in relation to the metabolism of human CYP2C substrates. Xenobiotica. 33(3):225–237. DOI: 10.1080/0049825021000048782 [PubMed: 12637241]

Guengerich FP, Waterman MR, Egli M. 2016; Recent structural insights into cytochrome P450 function. Trends in Pharmacological Sciences. 37(8):625–640. [PubMed: 27267697]

Honda K, Komatsu T, Koyama F, Kubota A, Kawakami K, Asakura H, … Teraoka H. 2011; Expression of two novel cytochrome P450 3A131 and 3A132 in liver and small intestine of domestic cats. Journal of Veterinary Medical Science. 73(11):1489–1492. [PubMed: 21712641]

Honda K, Komatsu T, Koyama F, Kubota A, Kawakami K, Asakura H, … Teraoka H. 2011; Expression of two novel cytochrome P450 3A131 and 3A132 in liver and small intestine of domestic cats. Journal of Veterinary Medical Science. 73(11):1489–1492. [PubMed: 21712641]

Hong LZ, Li J, Schmidt-Kuntzel A, Warren WC, Barsh GS. 2011; Digital gene expression for non-model organisms. Genome Research. 21(11):1905–1915. DOI: 10.1101/gr.122135.111 [PubMed: 21844123]

Ingelman-Sundberg M, Sim SC, Gomez A, Rodriguez-Antona C. 2007; Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoepigenetic and clinical aspects. Pharmacology and Therapeutics. 116(3):496–526. DOI: 10.1016/j.pharmthera.2007.09.004 [PubMed: 18001838]

Kharasch ED, Stubbert K. 2013; Role of cytochrome P4502B6 in methadone metabolism and clearance. The Journal of Clinical Pharmacology. 53(3):305–313. [PubMed: 23361846]

Kimble B, Li KM, Valtchev P, Higgins DP, Krockenberger MB, Govendir M. 2014; In vitro hepatic microsomal metabolism of meloxicam in koalas (Phascolarctos cinereus), brushtail possums (Trichosurus vulpecula), ringtail possums (Pseudocheirus peregrinus), rats (Rattus norvegicus) and dogs (Canis lupus familiaris). Comparative Biochemistry and Physiology: Toxicology & Pharmacology. 161:7–14. DOI: 10.1016/j.cbpc.2013.12.002 [PubMed: 24345479]

Komatsu T, Honda K, Kubota A, Kitazawa T, Hiraga T, Teraoka H. 2010; Molecular cloning and expression of cytochrome P450 2D6 in the livers of domestic cats. Journal of Veterinary Medical Science. 72(12):1633–1636. [PubMed: 20661004]

Lee JY, Lee SY, Lee K, Oh SJ, Kim SK. 2015; Determination of species-difference in microsomal metabolism of amitriptyline using a predictive MRM-IDA-EPI method. Chemico-Biological Interactions. 229:109–118. DOI: 10.1016/j.cbi.2015.01.024 [PubMed: 25623954]

Lyons, LA. 99 Lives Cat Whole Genome Sequencing Initiative Update, nearly there!. Paper presented at the Plant and Animal Genome XXIV Conference; 2016.

Lyons LA, Creighton EK, Alhaddad H, Beale HC, Grahn RA, Rah H, … Gandolfi B. 2016; Whole genome sequencing in cats, identifies new models for blindness in AIPL1 and somite segmentation in HES7. BMC Genomics. 17(1):265.doi: 10.1186/s12864-016-2595-4 [PubMed: 27030474]

Marguerat S, Bahler J. 2010; RNA-seq: from technology to biology. Cellular and Molecular Life Sciences. 67(4):569–579. DOI: 10.1007/s00018-009-0180-6 [PubMed: 19859660]

Martinez MN, Antonovic L, Court M, Dacasto M, Fink-Gremmels J, Kukanich B, … Trepanier L. 2013; Challenges in exploring the cytochrome P450 system as a source of variation in canine drug pharmacokinetics. Drug Metabolism Reviews. 45(2):218–230. DOI: 10.3109/03602532.2013.765445 [PubMed: 23432217]

Mauler DA, Gandolfi B, Reinero CR, O'Brien DP, Spooner JL, Lyons LA. 99 Lives C. 2017; Precision Medicine in Cats: Novel Niemann-Pick Type C1 Diagnosed by Whole-Genome Sequencing. Journal of Veterinary Internal Medicine. 31(2):539–544. DOI: 10.1111/jvim.14599 [PubMed: 28233346]

Nelson, DR. Cytochrome P450 protocols. Springer; 2006. Cytochrome P450 Nomenclature, 2004; 1–10.

Nelson DR. 2009; The cytochrome p450 homepage. Hum Genomics. 4(1):59–65. [PubMed: 19951895]

Nishimuta H, Nakagawa T, Nomura N, Yabuki M. 2013; Species differences in hepatic and intestinal metabolic activities for 43 human cytochrome P450 substrates between humans and rats or dogs. Xenobiotica. 43(11):948–955. [PubMed: 23593983]

Oh A, Pearce JW, Gandolfi B, Creighton EK, Suedmeyer WK, Selig M, … Lives C. 2017; Early-Onset Progressive Retinal Atrophy Associated with an IQCB1 Variant in African Black-Footed Cats (Felis nigripes). Scientific Reports. 7:43918.doi: 10.1038/srep43918 [PubMed: 28322220]

Okamatsu G, Kawakami K, Komatsu T, Kitazawa T, Uno Y, Teraoka H. 2016Functional expression and comparative characterization of four feline P450 cytochromes using fluorescent substrates. Xenobiotica. :1–11.

Okamatsu G, Komatsu T, Kubota A, Onaga T, Uchide T, Endo D, … Teraoka H. 2015; Identification and functional characterization of novel feline cytochrome P450 2A. Xenobiotica. 45(6):503–510. DOI: 10.3109/00498254.2014.998322 [PubMed: 25547627]

Okamatsu G, Komatsu T, Ono Y, Inoue H, Uchide T, Onaga T, … Teraoka H. 2017; Characterization of feline cytochrome P450 2B6. Xenobiotica. 47(2):93–102. DOI: 10.3109/00498254.2016.1145754 [PubMed: 26956662]

Shah SS, Sanda S, Regmi NL, Sasaki K, Shimoda M. 2007; Characterization of cytochrome P450-mediated drug metabolism in cats. Journal of Veterinary Pharmacology and Therapeutics. 30(5): 422–428. DOI: 10.1111/j.1365-2885.2007.00902.x [PubMed: 17803734]

Tanaka N, Shinkyo R, Sakaki T, Kasamastu M, Imaoka S, Funae Y, Yokota H. 2005; Cytochrome P450 2E polymorphism in feline liver. Biochimica et Biophysica Acta. 1726(2):194–205. DOI: 10.1016/j.bbagen.2005.08.004 [PubMed: 16168565]

Visser M, Weber K, Rincon G, Merritt DA. 2017Use of RNA-seq to determine variation in canine cytochrome P450 mRNA expression between blood, liver, lung, kidney and duodenum in healthy beagles. Journal of Veterinary Pharmacology and Therapeutics. :1–8.

Wagner GP, Kin K, Lynch VJ. 2013; A model based criterion for gene expression calls using RNA-seq data. Theory in Biosciences. 132(3):159–164. [PubMed: 23615947]

Wit P, Pespeni MH, Ladner JT, Barshis DJ, Seneca F, Jaris H, … Palumbi SR. 2012; The simple fool's guide to population genomics via RNA-Seq: an introduction to high-throughput sequencing data analysis. Molecular Ecology Resources. 12(6):1058–1067. [PubMed: 22931062]

Xu X, Sun X, Hu XS, Zhuang Y, Liu YC, Meng H, … Luo SJ. 2016; Whole Genome Sequencing Identifies a Missense Mutation in HES7 Associated with Short Tails in Asian Domestic Cats. Scientific Reports. 6:31583.doi: 10.1038/srep31583 [PubMed: 27560986]

Zanger UM, Schwab M. 2013; Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacology and Therapeutics. 138(1):103–141. DOI: 10.1016/j.pharmthera.2012.12.007 [PubMed: 23333322]

Zhang HF, Wang HH, Gao N, Wei JY, Tian X, Zhao Y, … Qiao HL. 2016; Physiological Content and Intrinsic Activities of 10 Cytochrome P450 Isoforms in Human Normal Liver Microsomes. Journal of Pharmacology and Experimental Therapeutics. 358(1):83–93. DOI: 10.1124/jpet.116.233635 [PubMed: 27189963]

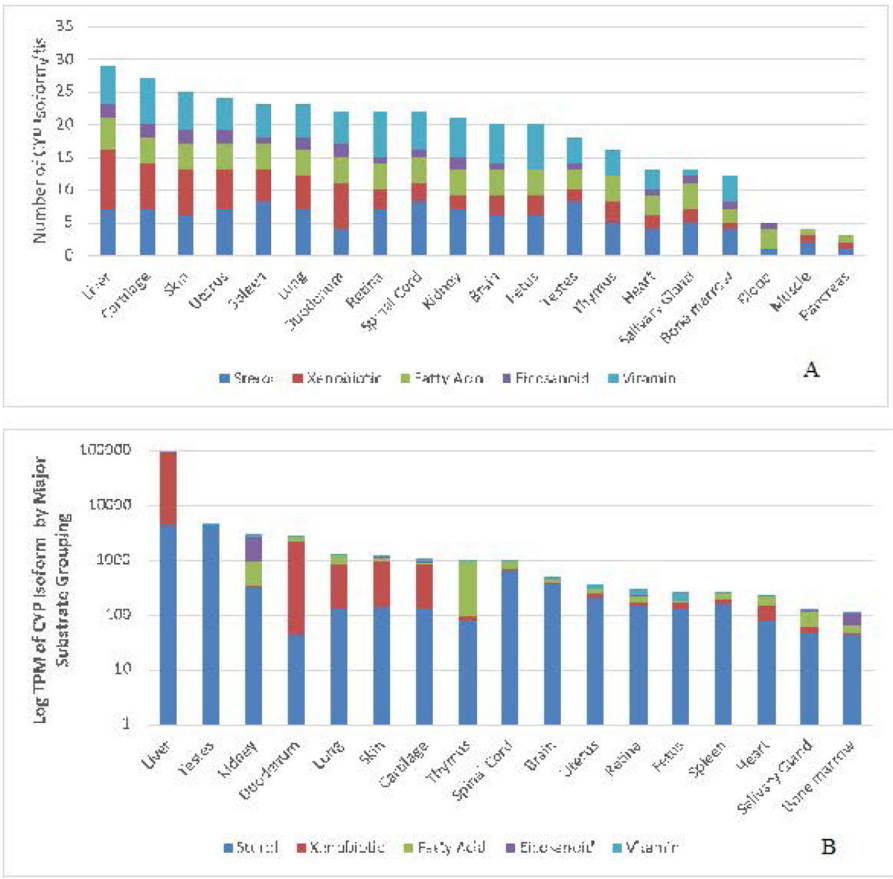

Figure 1.

(A) CYP Isoform expressed within a tissue by major human substrate category(B): Sum of the average TPM expression per tissue as reported major human substrate category

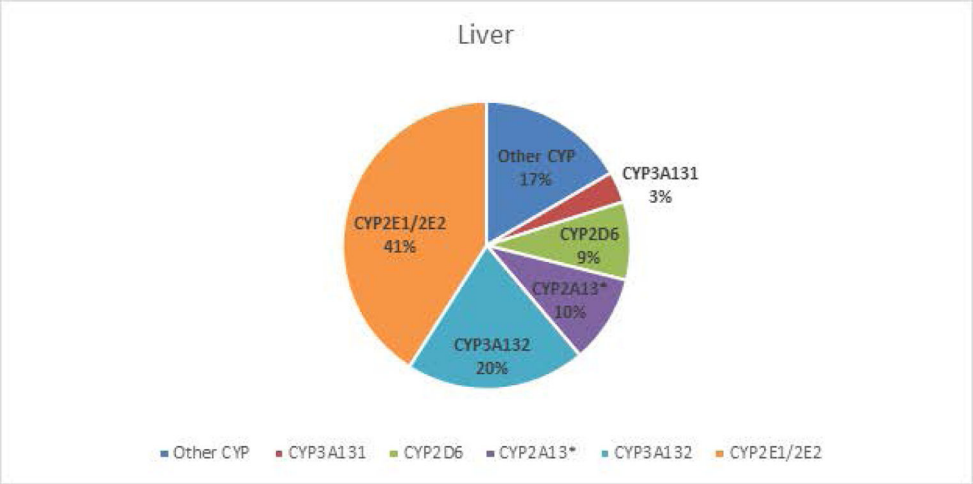

Figure 2.

RNA-seq expression of CYP in liver. Other include: *CYP4V2, 8B1, 1A2, 1A1, 2C41, 27A1, 4F6, 51A1, 4F, 7A1, 2U1, 2C21, 39A1, 7B1, 2J2, 20A1, 4X1, 2F1, 26A1,* and *21A2*

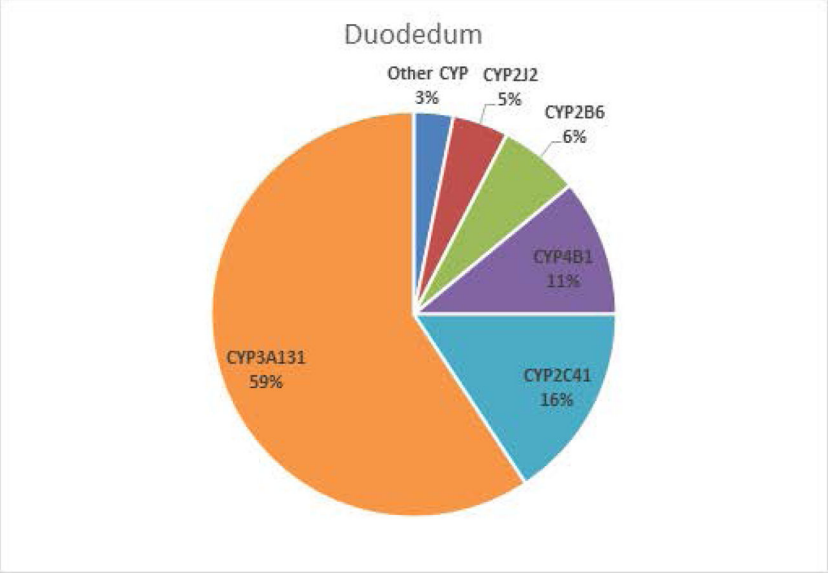

Figure 3.

RNA-seq expression in duodenum. Other CYP include *CYP21A2, 4F6, 3A132, 51A1, 2C21,* and *20A1*

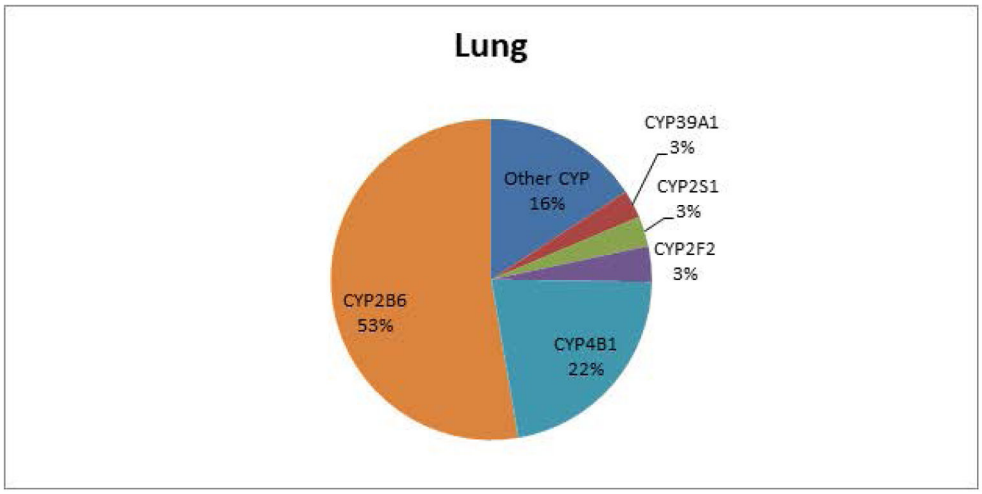

Figure 4.

Expression pattern of RNA-seq data in lung. Other CYP include *CYP4X1, 4V2, 1A1, 51A1, 7B1, 2J2, 21A2, 26B1, 1B1, 20A1, 2U1, 27A1, 4F3, 2A13, 2C21,* and *3A131.*

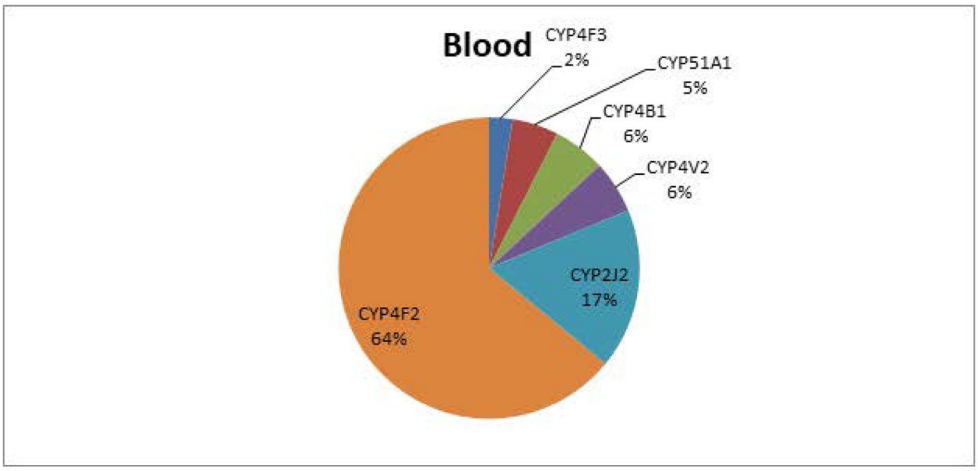

Figure 5.
Expression pattern of RNA-seq CYP transcripts in blood.

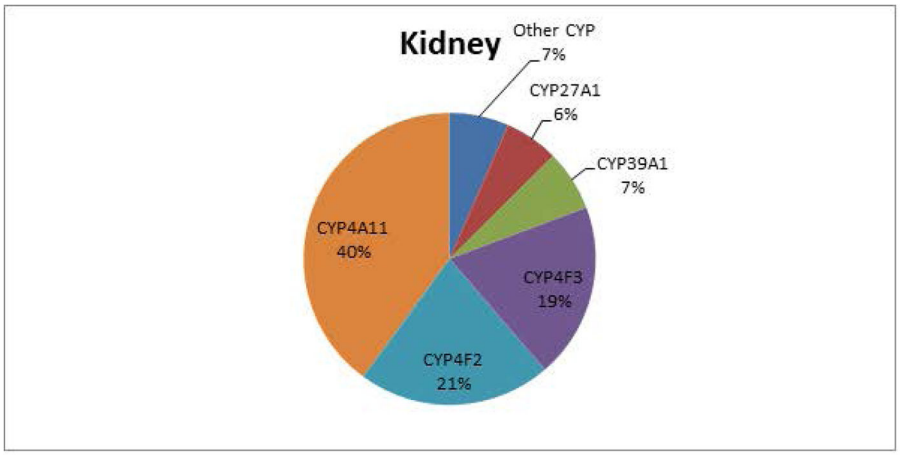

Figure 6.

Expression pattern of RNA-seq data in kidney. Other CYP include *CYP 24A1, 20A1, 51A1, 4B1, 2E1/2, 42, 7B1, 27B1, 1B1, 8B1, 46A1, 4X1,* and *2U1.*

J Vet Pharmacol Ther. Author manuscript; available in PMC 2020 January 01.

Table 1

Identified feline CYP. including chromosome and location and reported function in humans based on major substrate category. In addition to the chromosome location, the strand direction is noted if an orthologue was used. N/A indicates that the location could not be determined.

	CYP	RefSeq Status	Ensembl ID/ NCBI ID	Chromosome	Begin	End	Strand
Sterol	11A1	model	ENSFCAG00000010871/ LOC101085980	B3	31,931,481	31,945,909	F
	17A1	provisional	ENSFCAG00000001254	D2	61,263,913	61,270,481	R
	19A1	model	ENSFCAG00000023859/ LOC101094932	B3	53,128,736	53,169,889	F
	1B1	model	ENSFCAG00000026376/ LOC101099873	A3	109,723,884	109,728,015	F
	21A2	model	ENSFCAG00000004522/ LOC101094596	B2	31,162,007	31,164,510	F
	27A1	model	ENSFCAG00000007063/ LOC101091962	C1	203,282,910	203,316,872	F
	39A1	model	ENSFCAG00000011052/ LOC101100564	B2	44,730,707	44,827,141	R
	46A1	model	ENSFCAG00000001352/ LOC101093112	B3	142,276,527	142,302,008	F
	51A1	model	ENSFCAG00000025479/ LOC101083499	A2	94,639,749	94,660,854	R
	7A1	model	ENSFCAG00000008003/ LOC101099612	F2	10,297,970	10,308,851	R
	7B1	model	ENSFCAG00000010211/ LOC101086873	F2	15,509,007	15,682,083	R
	8B1	model	ENSFCAG00000028583/ LOC101082004	C2	148,923,327	148,924,832	F
Xenobiotics	1A1	provisional	ENSFCAG00000002016	B3	31,598,216	31,608,403	F
	2E1/2E2	provisional	ENSFCAG00000013762	D2	88,026,552	88,044,313	F
	1A2	provisional	ENSFCAG00000000344	B3	31,577,004	31,582,681	R
	2A13	model	ENSFCAG00000008557/ LOC101089816	E2	13,685,451	13,696,149	R
	2B6	model	ENSFCAG00000030256/ LOC105259649	Scaffold JH408948.1	598	1,209	R
	2C21-like	model*	ENSFCAG00000031987	D2	54,751,959	54,780,874	R
	2C41-like	model	ENSFCAG00000028217/ LOC101089396	D2	54,700,618	54,735,297	F
	2D6	provisional	ENSFCAG00000026110/ NM_001195406.1	B4	135,474,347	135,479,443	R
	3A131	provisional	ENSFCAG00000026022[#]/ NM_001246278.2	E3	6,884,885	6,993,231	F
	3A132	provisional	ENSFCAG00000023135[#]/ NM_001246271.1	E3	7,047,493	7,053,069	F
Fatty Acid	2J2	model	ENSFCAG00000011145/ LOC101097746	C1	49,023,851	49,053,419	R
	2U1	model*	ENSFCAG00000015335	B1	115,544,814	115,568,973	R
	4A11	model	ENSFCAG00000039117/ LOC101083281	C1	37,271,155	37,287,368	R

	CYP	RefSeq Status	Ensembl ID/ NCBI ID	Chromosome	Begin	End	Strand
	4B1	model	ENSFCAG00000002762/ LOC101096660	C1	37,228,490	37,248,565	F
	4F22	model	ENSFCAG00000028754/ LOC101089989	A2	10,536,589	10,568,498	R
	4V2	model	ENSFCAG00000001547/ LOC101097556	B1	17,071,356	17,098,301	F
Eicosanoid	4F3	model*	ENSFCAG00000008623	A2	11,725,594	11,744,125	F
	4F3/16-like*	model	ENSFCAG00000032109/ LOC101091202	B3	72,007,006	72,007,999	F
Vitamin	2R1	model	ENSFCAG00000011383/ LOC101093889	D1	71,137,315	71,159,177	R
	24A1	model*	ENSFCAG00000002452	A3	8,324,792	8,340,756	F
	26A1	model	ENSFCAG00000007248/ LOC101086422	D2	53,364,341	53,369,822	F
	26B1	model	ENSFCAG00000026867/ LOC101100712	A3	88,811,764	88,831,815	R
	26C1	model*	ENSFCAG00000009933	D2	53,352,177	53,360,021	F
	27B1	model*	ENSFCAG00000014701	B4	84,114,381	84,120,093	R
	27C1	model	ENSFCAG00000013628/ LOC101096908	C1	111,832,886	111,843,855	F
	20A1	model	ENSFCAG00000022927/ LOC101088261	C1	189,508,704	189,594,624	F
	2F1	model	ENSFCAG00000027930/ LOC101087438	E2	13,725,196	13,735,767	F
	2G1	model	ENSFCAG00000019378/ LOC101089311	E2	13,650,037	13,660,082	F
	2S1	model	ENSFCAG00000013094/ LOC101086936	E2	13,483,944	13,495,653	R
	2W1	model	ENSFCAG00000011410/ LOC101088802	E3	925,044	932,868	F
	4A24	model*	ENSFCAG00000037991	C1	37,283,763	37,304,624	R
	4X1	model	ENSFCAG00000007168/ LOC101096906	C1	37,363,074	37,404,124	F

Model: based on RefSeq automated pipeline; model*: based on ENSEMBL automated pipeline; provisional: RefSeq record has not yet been subject to individual review by an NCBI staff or outside collaborators.

[#]Discrepancy between ENSEMBL and NCBI databases.