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SUPPLEMENT ARTICLE

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Population variability in animal health: Influence on dose–exposure–response relationships: Part I: Drug metabolism and transporter systems

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Abstract

There is an increasing effort to understand the many sources of population variability that can influence drug absorption, metabolism, disposition, and clearance in veterinary species. This growing interest reflects the recognition that this diversity can influence dose–exposure–response relationships and can affect the drug residues present in the edible tissues of food-producing animals. To appreciate the pharmacokinetic diversity that may exist across a population of potential drug product recipients, both endogenous and exogenous variables need to be considered. The American Academy of Veterinary Pharmacology and Therapeutics hosted a 1-day session during the 2017 Biennial meeting to explore the sources of population variability recognized to impact veterinary medicine. The following review highlights the information shared during that session. In Part I of this workshop report, we consider sources of population variability associated with drug metabolism and membrane transport. Part II of this report highlights the use of modeling and simulation to support an appreciation of the variability in dose–exposure–response relationships.

1 | INTRODUCTION

As clinical pharmacologists, we recognize that within the context of a well-designed and carefully executed study, pharmacokinetic (PK) variability is not something to be avoided but rather is something that needs to be described if we are to appreciate the behavior of a therapeutic agent within the intended patient population. Interindividual variability in the dose–exposure–response relationship may be a consequence of differences in the genetic expression of a particular drug metabolizing enzyme or transporter (genetic polymorphisms), inherent differences in physiological attribute (such as percent body fat or intestinal surface area), diet, pregnancy, lactation, environment, disease, or age. There is also the potential for drug–drug interactions (DDIs) that can affect both drug PK and pharmacodynamics (PD). These potential sources of population variability are summarized in Figure 1.

For many of these factors, there is also the involvement of epigenetic regulation of gene expression (i.e., including induced and reversible alterations in heritable traits (Maggert, 2012)). Indeed, epigenetic changes have been implicated in the regulation of drug metabolizing enzymes and transporters (Ingelman-Sundberg et al., 2013; Kim, Han, Burckart, & Oh, 2014) and in some cases can potentially serve as alternative therapeutic targets (Hamm & Costa, 2011, 2015).

Because of its relationship to therapeutic outcomes, there is an increasing effort to understand the many sources of population variability that can influence drug absorption, metabolism, distribution, and clearance in veterinary species. In recognition of the importance of understanding how dose, efficacy, and toxicity may be influenced by endogenous (related to the animal) and exogenous factors

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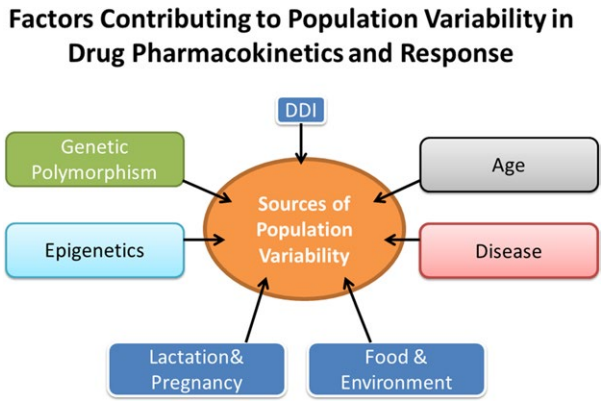

FIGURE 1 Variables that can influence the dose–exposure–response relationship to therapeutic agents

(related to variables that are outside of the animal and its physiology or pathology), a day was dedicated to the topic of population variability in animal health at the 2017 Biennial meeting of the American Academy of Veterinary Pharmacology and Therapeutics (AAVPT). Part I of this workshop report provides a summary of discussions on drug metabolism and active transport mechanisms.

This manuscript summarizes information shared during the Population Variability session of the 2017 AAVPT Biennial meeting. Therefore, sources of variability or species-specific polymorphisms that were not discussed at the meeting (e.g., other species, sources of PD variability, epigenetics, environmental, and others) are not included in this report. The objective for publishing this report was to showcase some of the known sources of population PK variability within a single document that helps us to recognize our current accomplishments and highlights the need for a concerted effort to increase our knowledge in this area. To that end, it should be appreciated that we have provided only a reflection of the biological principles foundational to reasons for observed population variability and the factors potentially influencing the in vivo processing of a chemical entity.

One of the most important take-home messages from the discussions during this session was that there continue to be many scientists who do not consider it important to define conditions that can lead to distinctly different dose–exposure–response relationships, even though many of the presentations clearly identified how some factors could have clinical relevance. Thus, while a growing number of veterinary pharmacologists recognize the need to describe sources of population variability and work to insure that this information is disseminated, there remain tremendous gaps in terms of information, research funding, education, and the communication of potential consequences of ignoring the presence of genotypic and phenotypic subpopulations. This huge gap in our understanding of pharmacogenetics is illustrated in the recently published article by Kongara (2018) in which the pharmacogenetics of opioid analgesics in dogs was reviewed.

It is in the spirit of fostering more work in this area and greater utilization of the available tools to support our population understanding that the Meeting Summary, Parts I and II, have been written.

2 | DRUG METABOLISM POLYMORPHISMS IN DOGS AND CATS

Polymorphisms in genes encoding important drug metabolizing enzymes are likely to explain high interindividual PK variability observed with some commonly used drugs in dogs and cats (Court, 2013a, 2013b; Martinez et al., 2013; Mosher & Court, 2010). Furthermore, inter-breed differences in the prevalence of these polymorphisms could account for some of the breed-related adverse drug reactions, ranging from unexpectedly high systemic or local tissue drug concentrations and toxicity to low drug concentrations and lack of efficacy. Accordingly, an understanding of the prevalence and consequences of these genetic differences is essential for enabling the extrapolation of the PK, safety, and effectiveness data determined during veterinary drug development from small homogenous research animal populations to the much larger and genetically heterogeneous companion animal populations that will be receiving these drugs in the clinic.

Unfortunately, although considerable research has been conducted on drug metabolism polymorphisms in humans and in some laboratory animal species (primarily rodents), relatively little work has been reported for similar studies in dogs and even less information is available for cats. Hopefully, the information contained in this section will underscore the importance of generating more information for veterinary species, especially considering the ability to integrate this kind of information into the *in silico* models (as described in the meeting report #2) where there is the opportunity to explore the population variability in dose–exposure relationships across the target species population.

Published studies regarding drug metabolism polymorphisms in dogs and cats were reviewed previously in 2010 (Mosher & Court, 2010). Most of the studied polymorphisms were in genes encoding the cytochrome P450 (CYP) enzymes, which are critical to the efficient elimination of most drugs in humans, and likely in dogs and cats. Some studies also explored polymorphisms in the canine and feline genes encoding the thiopurine methyltransferase (TMPT) enzymes that detoxify some important mercatopurine cancer and immunosuppressant drugs (Mosher & Court, 2010). Two 2013 reviews provided updated information on canine cytochrome P450 polymorphisms (Court, 2013a) and pharmacokinetic variability in cats (Court, 2013b). Most of this information is summarized in Table 1, as well as new information that have been published since those reviews. Readers are encouraged to consult those reviews and the associated references for more in-depth information. The following provides an update on research studies that have been newly identified since those reviews were published.

2.1 | CYP2B11 polymorphism in dogs

Mutation of the gene encoding *CYP2B11* has been hypothesized as the cause of slow recovery from several injectable anesthetic drugs (propofol and thiopental) and slower liver metabolism of propofol in greyhounds (Hay Kraus, Greenblatt, Venkatakrishnan, & Court,

TABLE 1 Polymorphisms of cytochrome P450 (CYP) and P450 oxidoreductase (POR) enzymes in dogs

Enzyme	Polymorphisms	Impact	Breed predisposition	References
CYP1A2	Premature stop codon (p.R373X)	No enzyme protein or activity		Mise, Hashizume, and Komur (2008), Mise et al. (2004), Tenmizu, Endo, Noguchi, and Kamimura (2004), Kamimura (2006); Tenmizu, Noguchi, and Kamimura (2006), Tenmizu, Noguchi, Kamimura, Ohtani, and Sawada (2006)
		About twofold increase in phenacetin plasma concentrations		Whiterock, Morgan, Lentz, Orcutt, and Sinz (2012)
		Up to 17-fold increase in plasma concentrations of a test compound in homozygous deficient dogs		Tenmizu, Noguchi, Kamimura et al. (2006), Tenmizu, Noguchi, Kamimura, Ohtani, et al. (2006)
			Highest allele frequencies in Irish Wolfhounds (42%) and Beagles (15%–39%) in 1,158 dogs tested from 29 breeds	Mise et al. (2004), Tenmizu et al. (2004), Whiterock, Delmonte, Hui, Orcutt, and Sinz (2007), Aretz and Geyer (2011), Scherr, Lourenco, Albuquerque, and Lima (2011)
CYP2B11	Non-synonymous SNP (p.R74C) in exon 2	Probable reduction of CYP activity based on computational prediction	Allele frequency of 8% in 100 dogs tested from 12 breeds. Mainly in Labrador Retrievers	Wenker (2009)
	Synonymous SNP (c.966G>A) in exon 7	Unclear. Possible effect on mRNA splicing	Allele frequency of 65% in 100 dogs tested from 12 breeds. Highest in Collies (100%)	
			Allele frequency of 38% in 106 dogs tested from four breeds. Highest in Uruguayan Cimarrons (92%)	Gagliardi, Llambí, and Arruga (2015)
	5 linked variants in 3′-untranslated region of mRNA (CYP2B11-H2)	~30%–40% reduction in luciferase-3′UTR reporter gene expression	Highest allele frequencies in Silken Windhounds (87%) and Scottish Deerhounds (78%) in 1,976 dogs tested from 66 breeds	Data presented by a coauthor (MHC) at the 20th Biennial Symposium of the American Academy of Veterinary Pharmacology and Therapeutics, Potomac, MD, May 21–24, 2017
	Single SNP in 3′-untranslated region of mRNA (CYP2B11-H3)	~60%–80% reduction in luciferase-3′UTR reporter gene expression	Highest allele frequencies in Greyhounds (59%) and Pembroke Welsh Corgis (30%) in 1,983 dogs tested from 66 breeds	
CYP2C41	Gene copy number variation	No enzyme protein or activity	Gene present in 7 of 30 Beagles (23%) and 2 of 10 mixed-breed dogs (20%). Not studied in other breeds	Blaisdell, Goldstein, and Bai (1998), Graham et al. (2003)
CYP2D15	p.S186G, I250F, I307V (WT2)	Recombinant enzyme showed lower activity for some substrates	Discovered and studied in Beagles; breed frequencies unknown	Paulson et al. (1999)
	p.S186G, I250F, I307V, I338V, K407E (V1)	No effect on recombinant enzyme activity		
	p.S186G (CYP2D15*2)			
	p.I250F, I307V (CYP2D15*3)			
CYP3A12	5 nonsynonymous SNPs: p.T309S, R421K, K422E, N423K, M452T (CYP3A12*2)	No effect on recombinant enzyme activity		

(Continued)

TABLE 1 (Continued)

Enzyme	Polymorphisms	Impact	Breed predisposition	References
POR	Single nonsynonymous SNP in exon 13 (POR-H2)	~40% reduction in CYP2B11 enzyme activity when variant POR-H2coexpressed with CYP2B11 (versus POR-H1)	Highest allele frequencies in Rottweilers (51%) and Doberman Pinschers (19%) in 1,968 dogs tested from 66 breeds	Data presented by a coauthor (MHC) at the 20th Biennial Symposium of the American Academy of Veterinary Pharmacology and Therapeutics, Potomac, MD, May 21–24, 2017
	2 nonsynonymous SNPs in exons 9 and 13 (POR-H3)	~60% reduction in CYP2B11 enzyme activity when variant POR –H3 coexpressed with CYP2B11 (versus POR-H1)	Highest allele frequencies in Scottish Deerhounds (36%) and Greyhounds (35%) in 1968 dogs tested from 66 breeds	

2000; Sams, Muir, Detra, & Robinson, 1985; Zoran, Riedesel, & Dyer, 1993). Two *CYP2B11* polymorphisms were reported as part of a doctoral thesis (Wenker, 2009). The study involved sequencing the *CYP2B11* exon coding regions using DNA from 100 dogs representing 12 diverse breeds. An exon 2 polymorphism (p.R74C) was found mainly in Labrador Retrievers and predicted by computational methods to disrupt enzyme function. Another polymorphism without a breed predilection was found in exon 7 (c.966G>A). This did not change the amino acid sequence, but was proposed by the author (without evidence) to alter mRNA splicing. No additional mutations were discovered by sequencing the eight greyhound DNA samples evaluated in that study, suggesting that the putative greyhound *CYP2B11* mutation may be located outside of the protein coding region. The existence of the exon 7 polymorphism was confirmed in a more recent study (Gagliardi et al., 2015).

2.2 | CYP2B11 and POR polymorphisms in greyhounds

Data from several (unpublished) studies were presented at the meeting by a coauthor (MHC) investigating the molecular genetic causes of slow oxidative drug metabolism in greyhounds, focusing on the *CYP2B11* and P450 oxidoreductase (*POR*) genes. POR is an essential enzyme that is required for efficient electron transfer from NADPH to all CYPs including CYP2B11.

Six different variants (mainly SNPs) were identified in the mRNA 3′-untranslated region (3′-UTR) of the *CYP2B11* gene using DNA samples from 13 greyhounds. Five of these variants were linked forming a single variant allele (CYP2B11-H2), while the other SNP was unique (CYP2B11-H3). Sequencing of almost 2,000 dogs from 66 breeds showed that the CYP2B11-H2 allele was relatively common among most breeds tested, while the CYP2B11-H3 allele was restricted to much fewer breeds with the highest allele frequency (59%) in greyhounds. In vitro functional studies were also performed by constructing luciferase-3′UTR reporter gene plasmids for each variant and the reference CYP2B11-H1 sequence, transfecting into cells, and measuring luciferase activity. The results indicated a moderate 30%–40% decrease in gene expression (relative to CYP2B11-H1) for the CYP2B11-H2 variant, but a much larger 60%–80% decrease in gene expression for the CYP2B11-H3 variant. These results suggest that the CYP2B11-H3 variant may contribute to slower CYP2B11 metabolism in greyhounds. This will need to be confirmed by study of CYP2B11 activity differences in *CYP2B11* genotyped dogs.

Two nonsynonymous (amino acid sequence changing) SNPs were also identified in exons 9 and 13 of the *POR* gene by sequencing greyhound DNA samples. These SNPs were found as either a single exon 9 SNP (POR-H2) or linked exon 9 and 13 SNPs (POR-H3). Sequencing of almost 2,000 dogs from 66 breeds showed relatively low allele frequencies for both POR-H2 and POR-H3 among most breeds tested. However, POR-H2 was quite common in Rottweiler (51%) and Doberman Pinscher (19%). POR-H3 was also quite common in Scottish Deerhounds (36%) and greyhounds (35%). In vitro functional studies were also performed by coexpressing recombinant

CYP2B11 with the reference POR-H1 enzyme and comparing with the variant POR-H2 and POR-H3 enzymes. The results indicated a moderate ~40% decrease in CYP2B11 enzyme activity for POR-H2, but a larger ~60% decrease in CYP2B11 enzyme activity for POR-H3 compared with POR-H1. These results suggest that the POR-H3 variant may contribute to slower CYP2B11 metabolism in greyhounds. This will need to be confirmed by studying CYP2B11 activity differences in *POR* genotyped dogs.

2.3 | CYP2D15 polymorphism in dogs

Like human CYP2D6, the canine CYP2D isoform (CYP2D15) is highly polymorphic, containing at least five common amino acid mutations (Paulson et al., 1999). The effect of these mutations on drug metabolism and PK is currently largely unknown. Prior work has demonstrated that celecoxib is mainly metabolized by CYP2D15 in dogs, and so CYP2D15 polymorphisms could explain the highly variable and bimodal distribution of plasma PK in a research beagle population (Paulson et al., 1999). A similar multimodal distribution in the plasma PK of civacoxib in research beagles was also attributed to CYP2D15 polymorphism (Jeunesse et al., 2013). Note that this conclusion is based on the structural similarities of civacoxib and celecoxib as direct experimental evidence is lacking.

With respect to the potential clinical ramifications of this genetic variability, recent work with the analgesic tramadol has shown that O-desmethyltramadol (M1), the active metabolite, is formed exclusively by CYP2D15 (Perez Jimenez, Mealey, Grubb, Greene, & Court, 2016). Consequently, CYP2D15 polymorphisms could potentially affect the analgesic properties of this drug, with less analgesia in CYP2D15 poor metabolizers. That said, there currently is no direct proof that any CYP2D15 polymorphism accounts for clinically relevant variability in the metabolism of any drug approved for use in dogs.

2.4 | CYP polymorphism in cats

Little has been published to date regarding CYP polymorphisms in cats. A study by Tanaka et al. (2005) identified a nonsynonymous SNP in the gene encoding feline CYP2E2. However, there appeared to be minimal effect of this SNP on the enzyme activity of recombinant CYP2E2. Furthermore, few clinically important drugs are metabolized by CYP2E enzymes, at least in humans and other species.

Although no other studies have reported to date on feline CYP polymorphisms, there is substantial evidence for high variability in the metabolic clearance of some clinically important drugs in cats that is likely explained by genetic variation (Court, 2013b). One important example is clopidogrel, a platelet aggregation inhibitor used widely in cats to prevent arterial thromboembolism with cardiomyopathy (Hogan et al., 2015). In humans, the efficacy of clopidogrel is greatly reduced in individuals with a mutation in the CYP2C19 gene, which reduces formation of the clopidogrel active metabolite (Lewis & Shuldiner, 2017). High variability has also been reported in the effects of clopidogrel on platelet function in different cats (Teuber & Mischke, 2016). Unpublished data using a newly developed assay (Lyngby, Court, & Lee, 2017) were presented at the meeting by a coauthor (MHC) showing over 10-fold variation in active metabolite concentrations among 19 cats given clopidogrel. Consequently, a polymorphism in the feline CYP2C ortholog could explain this variability, and variant genotyping may ultimately be used to identify cats that are resistant to clopidogrel treatment.

3 | TRANSPORTER POLYMORPHISMS

The number of drugs and toxins that are identified as substrates for one or more membrane transporters continue to increase, allowing the assumption that active carrier-mediated transport is a rule rather than an exception. Efflux transporters belonging to the family of (ATP-binding cassette) ABC transporters are ancient, being found in all living species ranging from bacteria where such efflux pumps confer resistance to various classes of antibiotics to mammalian species, including humans. In human pharmacotherapy, efflux transporters were first associated with multidrug resistance to cytostatic agents employed in the treatment of cancers. During the last decade, numerous drugs have been identified as substrates for efflux transporters and are now recognized as one of the major drivers of population variability in drug disposition and elimination.

3.1 | Companion animals

Drug transporters can directly modulate drug absorption, distribution, and excretion and can indirectly modulate drug metabolism. Drug transporter function can be corrupted either intrinsically (i.e., genetic polymorphisms) or extrinsically (i.e., drug-drug interactions) resulting in either decreased drug efficacy or increased risk of toxicity in affected patients. Therefore, it should not be surprising that polymorphisms in drug transporters can result in inter-patient variability in drug safety and efficacy.

Two superfamilies of drug transport proteins having well-described roles in human drug disposition are the ATP-binding cassette (ABC) superfamily and the solute carrier (SLC) superfamily (DeGorter, Xia, Yang, & Kim, 2012). Altered function of these transporters because of polymorphisms (human) or genetic modifications (rodent models) can lead to clinically significant changes in drug disposition (Kerb, 2006). Whether one can extrapolate data from human or mouse studies and apply it universally to companion animal species is unknown. However, we are aware of several examples whereby drug transporter polymorphisms in companion animals have been linked to extreme, even fatal, differences in drug disposition.

3.1.1 | ATP-binding cassette (ABC) superfamily

Although there are over 40 members of the ABC protein superfamily, polymorphisms that affect drug disposition have been identified in only two of these transporters in companion animals: P-glycoprotein (P-gp, which is encoded by the ABCB1 gene [formerly known as the Multidrug Resistance 1 gene MDR1]), and the

Breast Cancer Resistance Protein (BCRP, which is encoded by the ABCG2 gene; Mealey, 2013). Both P-gp and BCRP are membrane-spanning proteins that function as transmembrane efflux pumps. They are expressed on epithelial cell membranes in a variety of mammalian tissues and are thought to decrease the extent of oral drug absorption (apical border of intestinal epithelial cells), enhance drug elimination (biliary canalicular or renal tubular epithelial cells), or limit drug distribution to so-called sanctuary sites (endothelial cells at blood–brain barrier, blood–retina barrier, testes and placenta; DeGorter et al., 2012). Based on their tissue distribution and drug/toxin efflux capability, P-gp and BCRP are presumed to provide a protective function by decreasing systemic or localized organ exposure to potentially toxic xenobiotics. It is not unreasonable therefore to expect that a defect in P-gp or BCRP transporter proteins can lead to reduced (or absent) transporter function and excessive levels of exposure to drugs that are normally effluxed by these proteins. Indeed, this has been confirmed to be the case in dogs and cats presenting with polymorphisms in either P-gp or BCRP (Mealey, 2013).

3.1.2 | ABCB1 (P-glycoprotein)

Examples of P-gp substrates include many antiparasitic drugs (ivermectin, selamectin, milbemycin, etc.), anticancer drugs (doxorubicin, vincristine, vinblastine, paclitaxel, and others), loperamide, and acepromazine (Mealey, 2013).

A nonsense polymorphism (encoding a premature stop codon) has been described in both dogs (Mealey, Bentjen, Gay, & Cantor, 2001) and cats (Mealey & Burke, 2015). The ABCB1 polymorphism in dogs consists of a four base-pair deletion mutation that occurs at the 5′ end of the gene such that protein synthesis is terminated before even 10% of the protein product is synthesized (Mealey et al., 2001). Thus, dogs with two mutant alleles (homozygous for this mutation) exhibit a P-gp null phenotype while heterozygotes, dogs with one mutant allele and one wild-type allele (ABCB1 mutant/normal) have an intermediated phenotype for which its influence on transporter function appears to be somewhat drug dependent (Deshpande, Hill, Mealey, Chambers, & Gieseg, 2016; Mealey, Fidel, et al., 2008; Mealey, Greene, et al., 2008). Affected dogs include many herding breeds, with more than 50% of dogs of certain breeds carrying a mutant allele (Gramer et al., 2011; Kawabata, Momoi, Inoue-Murayama, & Iwasaki, 2005; Mealey & Meurs, 2008; Mealey, Munyard, & Bentjen, 2005; Neff et al., 2004). A two base-pair deletion mutation has been identified in cats. The resulting frame shift causes truncation after about half the protein is synthesized (Mealey & Burke, 2015). Approximately 4% of cats carry this nonsense polymorphism.

The brain is among the sanctuary sites dependent upon P-gp activity. Dogs and cats that lack functional P-gp are exquisitely sensitive to neurological toxicity caused by P-gp substrates such as macrocyclic lactone antiparasitic agents (Mealey et al., 2001; Nelson, Carsten, Bentjen, & Mealey, 2003) the tranquilizer acepromazine (Deshpande et al., 2016), and the over-the-counter antidiarrheal drug loperamide (Mealey, Fidel, et al., 2008; Mealey, Greene, et al., 2008; Sartor, Bentjen, Trepanier, & Mealey, 2004). The latter can be reversed by the opioid antagonist naloxone, but there are no antidotes available for macrocyclic lactones or acepromazine.

Biliary excretion of P-gp substrate drugs is also markedly different in animals with the previously described ABCB1 genotypes. Marked differences in biliary excretion of a radiolabeled P-gp substrate (^{99m}Tc-sestamibi) was observed when comparing dogs with normal P-gp to dogs with defective P-gp (Coelho et al., 2009). ^{99m}Tc-sestamibi is essentially undetectable in gallbladders of MDR1 (mutant/mutant) dogs but is highly concentrated in gallbladders of ABCB1 (normal/normal) dogs. Dogs that are heterozygous have an intermediate phenotype regarding biliary 99mTc-sestamibi excretion. Lack of biliary drug excretion greatly enhances total exposure of affected dogs and cats to P-gp substrates such as vincristine and doxorubicin (Mealey, Fidel, et al., 2008; Mealey, Greene, et al., 2008). These drugs are significantly more likely to cause myelosuppression in dogs with the MDR1 mutation than in wild-type dogs. For this reason, lower than typical doses of vincristine, doxorubicin, and vinblastine should be given to dogs with P-gp dysfunction to avoid severe toxicity. A clinical study comparing vincristine-induced myelosuppression in normal cats and cats with the ABCB1 deletion mutation is currently underway.

3.1.3 | ABCG2 (Breast Cancer Resistance Protein, BCRP)

The efflux transporter BCRP/*AGCG2*, localized in the alveolar epithelium of the mammary gland, is the major driver of the excretion of drugs and xenobiotics into milk. BCRP (*ABCG2*) was first described in human patients when common treatment protocols used in the therapy of breast cancer apparently failed (hence the name breast cancer resistance protein). BCRP is a so-called half-transporter (contains one transmembrane domain and one nuclear binding fold; Dean, Hamon, & Chimin, 2001) which distinguishes it from other ABC transporters such as P-gp, which is a full transporter (contains two transmembrane domains and two nuclear binding folds; Dean, Hamon & Chimin, 2001). BCRP expression has been identified at the blood–brain barrier and central nervous system (endothelia cells), lung, liver, intestines, and kidney, as well as in the testis and prostate, uterus, placenta mammary gland in humans and animals (Fletcher, Williams, Henderson, Norris, & Haber, 2016; Jemnitz, Veres, Tugyi, & Vereczkey, 2010).

The ATP-binding domain is critical to BCRP function because ATP binding provides the energy needed to pump substrates against a concentration gradient. Individuals with the ABCG2 421C>A genotype exhibit decreased ABCG2 expression and function as compared to individuals with the wild-type allele. This defect in ABCG2 function has been associated with enhanced oral bioavailability of substrate drugs and has been linked with altered PK and/or increased toxicity when substrate drugs such as gefitinib, irinotecan, and sulfasalazine have been administered to affected individuals (Cusatis & Sparreboom, 2008; Cusatis et al., 2006). Thus, it is likely that functional changes in ABCG2 are also likely to have therapeutic ramifications in veterinary patients.

Although polymorphisms in canine ABCG2 have not been described, this is not the case in cats. The feline ABCG2 sequence has been determined and the consensus amino acid sequence has some key differences when compared to that of 10 other mammalian species, including humans (Ramirez et al., 2011). There are four feline-specific amino acid changes in conserved regions of ABCG2. One of the amino acid changes, like the previously mentioned human variant ABCG2 421C>A, occurs within the ATP-binding domain. A glutamate (polar) residue is shifted to a methionine (nonpolar) residue. Similar to what occurs in humans with ABCG2 421C>A, feline ABCG2-mediated efflux is defective compared to wild-type human ABCG2 (Ramirez et al., 2011). These four amino acid changes are not polymorphisms but rather appear to be present in all cats. For this reason, cats are expected to experience greater sensitivity to ABCG2 substrate drugs as compared to wild-type individuals in other species.

Where this has had the greatest clinical impact in cats is the retinal toxicity that has been observed in some individuals following the administration of certain fluoroquinolones. ABCG2 is a key component of the blood–retina border (Asashima et al., 2006; Hornof, Toropainen, & Urtti, 2005) and as such restricts the entry of fluoroquinolones. Accumulation of photo reactive fluoroquinolones in feline retinal tissue can result in retinal degeneration and blindness—a devastating adverse drug reaction that has not been reported in other species despite the wide use of fluoroquinolones in many species (Ford, Dubielzig, Giuliano, Moore, & Narfström, 2007).

3.1.4 | Solute carrier (SLC) superfamily

Unlike the ABC transporter system, the SLC transporter system does not rely directly on ATP hydrolysis and largely, but not exclusively act as uptake transporters. Transport can be either passive (movement down the concentration gradient) or active (where it is coupled with a transport system that relies upon an electrochemical potential difference created by pumping ions in to out of the cell; Colas, Ung, & Schlessinger, 2016; Nigam, 2015). Polymorphisms in several members of the SLC superfamily of transporters have been documented to cause interindividual variability in drug disposition (DeGorter et al., 2012). As with the ABC family transport family, SLC transporters are expressed along the body's functional "barrier" tissues including intestine, brain capillary endothelial cells, placenta, liver, and kidney as well as other tissues.

Polymorphisms of SLC transporters in companion animals have not yet been reported (personal comment by KMealey).

3.2 | Food-producing animals

In contrast to human and companion animal species, the characterization of efflux transporters in farm animal species remains incomplete, with few identified polymorphisms (Lindner, Halwachs, Wassermann, & Honscha, 2013; Schrickx & Fink-Gremmels, 2008). Rather for food-producing species, most investigations have focused on the risk of drug residues in milk. Along these lines, the focus has been on polymorphisms associated with BCRP/*AGCG2*.

It should be noted that the examples of drug substances included in this section reflect information deduced from the original publications, irrespective of current drug use, and licensing policies in the USA.

3.2.1 | Dietary modulators of BCRP

Driven by the hypothesis that drug resistance in the treatment of cancers could be influenced by the co-administration of BCRP-inhibitors, numerous compounds were tested for their ability to modulate BCRP transport functions (for a recent review, see Peña-Solórzano, Stark, König, Sierra, & Ochoa-Puentes, 2017). The most consistent results were determined with individual flavonoids, including quercetin, silymarin, daidzein and other polyphenols, as well as the natural stilbenoid resveratrol. These compounds are present in numerous consumable plants (fruits and vegetables), and their prevalence in the daily diet may account for much of the clinically observed diet-dependent population variability in drug disposition.

Of special veterinary interest is the presence of flavonoids in the soy beans typical added to feed concentrates. Indeed, Perez et al. (2013) demonstrated that the soy isoflavones genistein and daidzein inhibit BCRP activity both in vitro and in vivo and that this inhibition results in lower levels of danofloxacin in the milk of lactating ewes (note that fluoroquinolones are typical BCRP substrates). While this kind of modulation in excretion pattern would be desirable in terms of residue avoidance, it would also impair the therapeutic efficacy of such a drug when given for the treatment of mastitis.

3.2.2 | Drugs and toxins acting as modulators of BCRP activity

Veterinary drugs identified as inhibitors of BCRP include the sulfoxide metabolites of triclabendazole (Barrera et al., 2012; Lifschitz, Virkel, Ballent, Sallovitz, & Lanusse, 2009). Co-administration of triclabendazole and ivermectin (a P-gp substrate) to sheep resulted in a threefold higher plasma concentration of ivermectin and a prolongation of its systemic elimination. Co-administration of triclabendazole (due to its flukicidal activity) and broad-spectrum endectocides such the macrocyclic lactones is a common practice in ruminants. A comparable interaction was described by Barrera et al. (2013) demonstrating the inhibitory effects of triclabendazole metabolites on BCRP. This inhibitory activity was found to modulate the excretion of danofloxacin and moxidectin (both BCRP substrates) into the milk of lactating ewes. Monepantel, another anthelmintic used in cattle, was also identified as a BCRP substrate, and its active metabolite MNPSO2 reached a milk-to-plasma ratio of 6.75 (Mahnke et al., 2016).

3.2.3 | Polymorphisms of BCRP in bovines

In bovines, the primary polymorphism of interest has been that of the SNP AGCG2_49. This is considered a reliable marker (quantitative

trait locus) for milk yield (Olsen et al., 2007). In investigations of Chinese Holstein-Frisian cows (Yue et al., 2011), two mutations in the nucleotide binding domain were detected in exon 6: one at position 45599 (A→C transversion) and the other at 45610 (A→C). Statistical analyses revealed that such mutations are associated with a phenotype that shows higher milk yield, lower milk protein concentrations, and lower somatic cell counts in the first and second lactation. Further investigations identified a single nucleotide exchange in exon 14, encoding a substitution of tyrosine-581 to serine (Y581S) within the transmembrane domain region of BCRP. The Y581 allele could be associated with higher milk yield and fat and protein concentrations in dairy milk and is therefore classified also as a *gain-of-function* polymorphism.

The BCRP-mediated increase in milk production is likely to be accompanied by an increase in the concentration of drugs and xenobiotics in milk. Indeed, Otero et al. (2013) could show an approximately 2.5-fold increase in the lactogenic excretion of danofloxacin in Y581S cows, despite comparable plasma drug concentrations as compared to the wild-type cows. The same authors showed that Y581S cows excreted endogenous substrates such as riboflavin and nutrient-derived substrates (such as uric acid and enterolactone) into milk at a higher rate than did the wild-type cows (Otero et al., 2015).

These findings demonstrate the dual and opposing activities of BCRP in that it influences milk yield and composition in a desirable manner while increasing the risk for undesirable contamination of milk with residues of drugs and toxins. Today, reliable in vitro models are available, consisting of transfected cells (MDCK cells expressing bovine BCRP; Wassermann, Halwachs, Lindner, Honscha, & Honscha, 2013), or HC11 cells (originating from mice for a first characterization of BCRP substrates). These in vitro systems are now used to screen veterinary pharmaceuticals and common feed contaminants that may be BCRP substrates. Considering the high impact of BCRP on typical PK variables, this information should be considered in the modeling and simulation of population variabilities.

4 | PHENOCONVERSION

An additional wrinkle is that factors other than genetic polymorphisms can significantly alter dose–exposure–response relationships. This has been termed phenoconversion, (Shah & Smith, 2015a; Shah et al., 2016). Phenoconversion is a phenomenon whereby the presence of intrinsic and/or extrinsic factors alters a phenotype in a manner that differs from what would be expected based on genotype (i.e., a genotype–phenotype mismatch). Thus, phenoconversion can significantly impact the analysis and interpretation of genotype-focused clinical outcomes.

Because of this phenomenon, it is evident that an appreciation of the genetic variants associated with population variability in dose–exposure–response relationships constitutes only one of many determinants of PK and PD variability. For example, the Flockhart Cytochrome P450 Drug Interaction Table of the Indiana University contains a compilation of enzyme inducers and inhibitors that can influence predictions that are based solely on the pharmacogenetic information. It is also interesting to see the recent discussion of "inflammation-induced phenoconversion," with down-regulation of the CYP enzymes in inflammation and disease (Shah & Smith, 2015b). This phenomenon underscores the importance of using both top-down (to monitor drug PK across an actual patient population) and bottom-up (to appreciate expected dose–exposure relationships based upon known genetic variants in metabolism, transporter function, formulation, dose and anatomical characteristics) approaches when trying to define clinically relevant population variability in drug dose–exposure–response relationships.

5 | CONCLUDING THOUGHTS

There are many endogenous and exogenous factors that can influence animal (or human) health, drug dose–exposure–response relationships, and the residues present in edible tissues of food-producing animals. For this reason, it is essential that each species be viewed as a diverse pool of individuals, despite their shared commonality of breed and age. The question posed to each of us is how to encourage the generation of information necessary to characterize these factors so that we can determine if and when there are identifiable variables that may affect safe and effective drug use? Are young practitioners trained to appropriately apply this information once it is available? What can we do to optimize the use of our available therapeutic arsenal?

It is our hope that the information conveyed in this Meeting Report will promote an active pursuit of information to fill the gaps in our understanding as it applied to veterinary medicine and ultimately, to integrate this appreciation into safety and effectiveness evaluations, residue depletion assessments, and into veterinary prescribing practices.

In Part II of this meeting summary, modeling and simulation is explored as a tool that can be applied for improving our understanding of population variability in animal health.

CONFLICT OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTION

Although this was a collaborative effort, the following were primary areas of contributions for the individual authors: MNM contributed to abstract, introduction, phenoconversion, conclusions, decision on manuscript contents; MHC contributed to drug polymorphisms in dogs and cats; JF-G contributed to transporter polymorphisms food-producing animals; KLM contributed to transporter polymorphisms companion animals; All authors have read and approved of the contents in this final manuscript and the subsequent revision.

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