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REVIEW

Personalized medicine: going to the dogs?

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Abstract
Interindividual variation in drug response occurs in canine patients just as it does in human patients. Although canine pharmacogenetics still lags behind human pharmacogenetics, significant life-saving discoveries in the field have been made over the last 20 years, but much remains to be done. This article summarizes the available published data about the presence and impact of genetic polymorphisms on canine drug transporters, drug-metabolizing enzymes, drug receptors/targets, and plasma protein binding while comparing them to their human counterparts when applicable. In addition, precision medicine in cancer treatment as an application of canine pharmacogenetics and pertinent considerations for canine pharmacogenetics testing is reviewed. The field is poised to transition from single pharmacogene-based studies, pharmacogenetics, to pharmacogenomic-based studies to enhance our understanding of interindividual variation of drug response in dogs. Advances made in the field of canine pharmacogenetics will not only improve the health and well-being of dogs and dog breeds, but may provide insight into individual drug efficacy and toxicity in human patients as well.

Introduction

Researchers in the field of human pharmacogenetics are often surprised and even amused by the notion of pharmacogenetics or individualized medicine for companion animals, such as dogs. However, interindividual variation in drug response, whether manifesting as unexpected toxicity or failure to respond, occurs in canine patients just as it does in their human counterparts. Practicing veterinarians have recognized the former, unexpected drug toxicity, phenotype for decades, especially if the adverse drug reaction tended to occur in a specific dog breed, such as macrocyclic lactone toxicity in Collies (Jerram 1985) and prolonged thiopental recovery in Greyhounds (Sams et al. 1985). Many of these breed-related adverse drug reactions or breed "sensitivities" to certain drugs were described decades before the first pharmacogene mutation was identified (Jerram 1985; Sams et al. 1985). Some of the most important canine pharmacogenetic discoveries are based on the observations of these astute veterinarians in private practice. In fact, it is likely because these breed-related drug sensitivities were widely recognized that "DNA testing" seemed to be readily accepted by veterinarians in 2004 when the first canine pharmacogenetic test became commercially available (Mealey et al. 2001).

Undoubtedly, the field of canine pharmacogenetics lags behind its human counterpart. There are a myriad of reasons for this including the relative paucity of funding available for companion animal research and the relatively recent availability of the canine genome sequence (Lindblad-Toh et al. 2005) relative to the human genome. Despite these handicaps, the past two decades have witnessed life-saving discoveries in canine pharmacogenetics. Information gleaned from human pharmacogenetics and rodent pharmacogene knockout models has been fundamental in canine pharmacogenetic research. As summarized in this review, important pharmacogenes for dogs include the usual suspects described for people—those that encode drug-metabolizing enzymes, drug transporters, and drug targets, whether those targets are intended or unintended. This review highlights the most well-characterized canine pharmacogenes, compares them to their human counterparts, if applicable, and describes recognized gene variants and their clinical consequences in affected dogs.

Canine pharmacogenetics has probably passed the "low hanging fruit" phase of discovery, where a phenotype would yield an obvious candidate gene that could then be sequenced and a mutation identified. Currently, the field is

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such that the past pharmacogenetic approach, association of a drug response with a single genetic difference, is being replaced by a pharmacogenomic-based approach, investigating the full spectrum of genes involved in a drug response. It is reasonable to anticipate that many new canine pharmacogene variants will be discovered in the near future.

Drug transporters

Drug transporters are highly conserved among mammals and can dramatically affect the disposition of substrate drugs in both human and veterinary patients. The most important drug transporters are members of either the ATP-binding cassette (ABC) superfamily or the solute carrier (SLC) family of transport proteins. ABC and SLC transporters are often expressed at tissue barriers, where they function to transport xenobiotics out of the body or away from sensitive tissues. For example, ABC transporters are expressed on the luminal surfaces of enterocytes, brain capillary endothelial cells, renal tubules, and biliary canalicular cells, where the proteins function as efflux pumps to prevent intestinal absorption, brain penetration or enhance renal or biliary excretion of xenobiotics. Among 66 human pharmacogenes listed as "VIPs" or very important pharmacogenes, six are transporters, including three ABC transporters and three SLC transporters (Whirl-Carrillo et al. 2012). In human patients, the disposition (i.e., absorption, distribution, metabolism, and/or excretion) of dozens of drugs is affected by these six transporters including drugs used for treating cancer, diabetes, hypercholesterolemia, pain, depression, fungal and viral infections, and other diseases.

Polymorphisms in these genes can alter the efficacy and safety of substrate drugs in affected patients [reviewed in (Cascorbi 2006; Cascorbi and Haenisch 2010; Roth et al. 2012)]. For example, people with the C421A variant of ABCG2 have 300% higher plasma concentrations of the anticancer drug diflomotecan (Sparreboom et al. 2004) compared to people with the wild-type ABCG2 genotype. This variant is present in 30–60% of Asians and 5–10% of whites and African-Americans. This and other ABCG2 polymorphisms affect the disposition of drugs including tyrosine kinase inhibitors, statins, valproic acid, and others (Heyes et al. 2018). Similarly, polymorphisms in certain SLC transporters affect the disposition of statins, HIV-1 protease inhibitors, mycophenolic acid, and thiazolidinedione antihyperglycemic agents (Roth et al. 2012).

Both ABC and SLC transporters have been described in dogs (Bleasby et al. 2006), but information on the SLC transporters is very limited in dogs. Much more is known about certain ABC transporters, specifically ABCB1, which encodes the multidrug transporter *P*-glycoprotein. By virtue of its expression at the blood–brain barrier, intestinal barrier, and at hepatic and renal excretory sites, *P*-glycoprotein plays a critical role in protecting mammals from xenobiotic exposure. This has been vividly illustrated using *Abcb1* knockout mice which have 20–50 times higher brain concentrations of *P*-glycoprotein substrate drugs than wild-type mice exposed to the same dose (Schinkel et al. 1995). While some polymorphisms in human ABCB1 have been reported, their impact on *P*-glycoprotein function, and consequently the disposition of substrate drugs, is only moderate (Ma and Lu 2011).

A specific polymorphism of canine ABCB1, ABCB1-1Δ, also known as the MDR1 mutation and ABCB1:c.227_230delATAG, illustrates why ABCB1 is without a doubt the single most "VIP" in dogs. ABCB1-1Δ consists of a four base-pair deletion that generates several premature stop codons resulting in a severely truncated, nonfunctional protein (Mealey et al. 2001). Relative to ABCB1 wild-type dogs, those dogs harboring ABCB1-1Δ experience exquisite sensitivity to *P*-glycoprotein substrate drugs including ivermectin and related macrocyclic lactones (Mealey 2008), vincristine (Mealey et al. 2008a), loperamide (Mealey et al. 2008b), doxorubicin, and other drugs (Mealey 2004). This sensitivity is a direct result of the blood–brain barrier defect (Fig. 1) and/or deficient biliary excretion (Fig. 2) that occurs when those tissues are devoid of functional *P*-glycoprotein. Using a radiolabeled *P*-glycoprotein substrate, Figs. 1 and 2 illustrate the markedly different concentrations of substrate drugs achieved in the brain and bile of ABCB1 wild-type and ABCB1-1Δ homozygous dogs (Mealey et al. 2008b). It should be simple for the reader to predict the variability in drug response between dogs with the respective genotypes. Thus, it is considered standard of care in the veterinary profession to perform ABCB1-1Δ genotyping prior to treatment with many *P*-glycoprotein substrate drugs in any dog at risk for harboring ABCB1-1Δ. As discussed below, ABCB1-1Δ genotyping services should be performed by a laboratory with qualified experts capable of providing appropriate veterinary pharmacogenetic treatment recommendations (i.e., a veterinary clinical pharmacologist).

What might be surprising to those more familiar with human pharmacogenomics is the high frequency of ABCB1-1Δ in the canine population (Fig. 3). Seventy-five percent of Collies and 50% of Australian Shepherds and English Shepherds have at least one mutant allele (Neff et al. 2004; Mealey and Meurs 2008). Other herding breeds, such as German Shepherds, Shetland Sheepdogs, Border Collies, and others, are at relatively high risk for having at least one ABCB1-1Δ allele (Mealey and Meurs 2008). ABCB1-1Δ has also been identified with a high frequency (30–50%) in some sighthounds (Silken Windhound and Long-haired Whippet) as well as numerous mixed breed dogs and other purebreds, albeit at a lower frequency (≤ 1%). The phylogeny

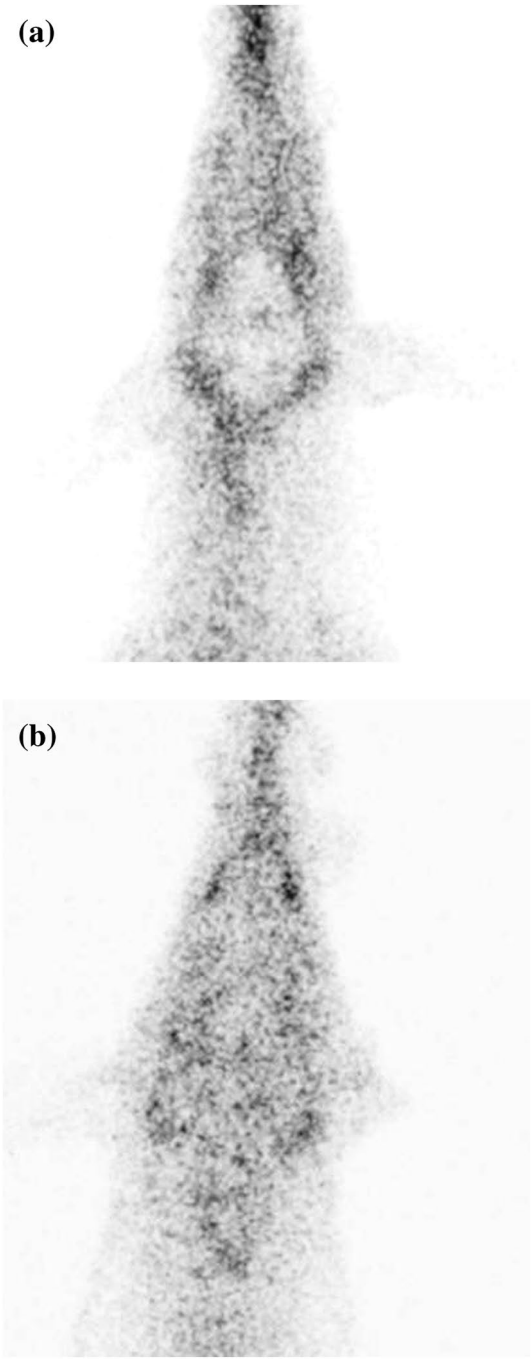

Fig. 1 99mTc-sestamibi nuclear scintigraphy. The ABCB1 wild-type dog (**a**) is essentially devoid of activity in the brain compared with surrounding tissue. In contrast, the ABCB1-1Δ dog (**b**) has similar activity in the brain compared with surrounding tissue due to lack of *P*-glycoprotein. Figure reproduced with permission from Mealey et al. (2008b)

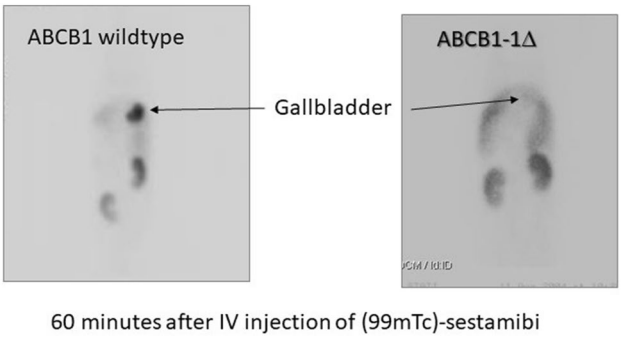

Fig. 2 99mTc-sestamibi nuclear scintigraphy. The ABCB1 wild-type dog (**a**) has an intensely concentrated area of activity within the gall bladder. In contrast, the ABCB1-1Δ dog (**b**) does not concentrate activity within the gallbladder. Figure reproduced with permission from Coelho et al. (2009)

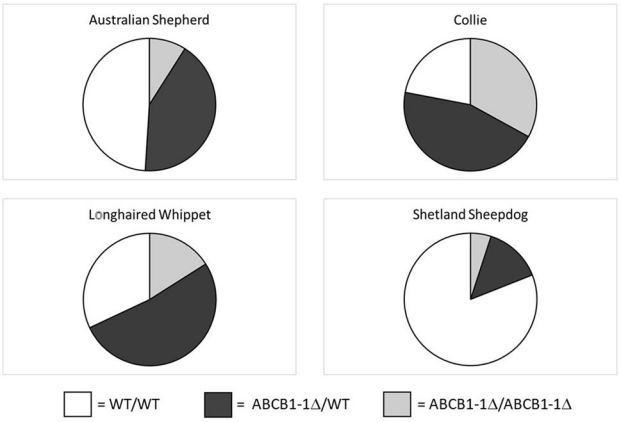

Fig. 3 Approximate ABCB1 genotype distribution based on samples from dog populations from North America, Europe, South America, and Asia. Compiled from: Neff et al. (2004); Mealey and Meurs (2008); Gramer et al. (2011); Monobe et al. (2015); Firdova et al. (2016)

of ABCB1-1Δ has been described in detail elsewhere (Neff et al. 2004).

Because of the sheer number of drugs that are substrates for canine *P*-glycoprotein and the high frequency of this mutation in several popular breeds, it is apparent why ABCB1 deserves its "Very Important Pharmacogene" status. Ongoing research in one of the author's (KLM) laboratories has identified several recently FDA-approved veterinary drugs that are *P*-glycoprotein substrates and, therefore, may pose a risk to affected dogs. This research is critical for the veterinary field, because, in contrast to the human drug development/approval process, assessment of whether or not a drug is a *P*-glycoprotein substrate is not required for veterinary drug approval. Finally, the ABCB1-1Δ mutation has contributed to our understanding of clinically important, often life-threatening, drug interactions involving canine *P*-glycoprotein (Mealey and Fidel 2015), because clinicians are now able to attribute clinical signs resulting from *P*-glycoprotein deficiency to a specific adverse drug event (Fig. 4).

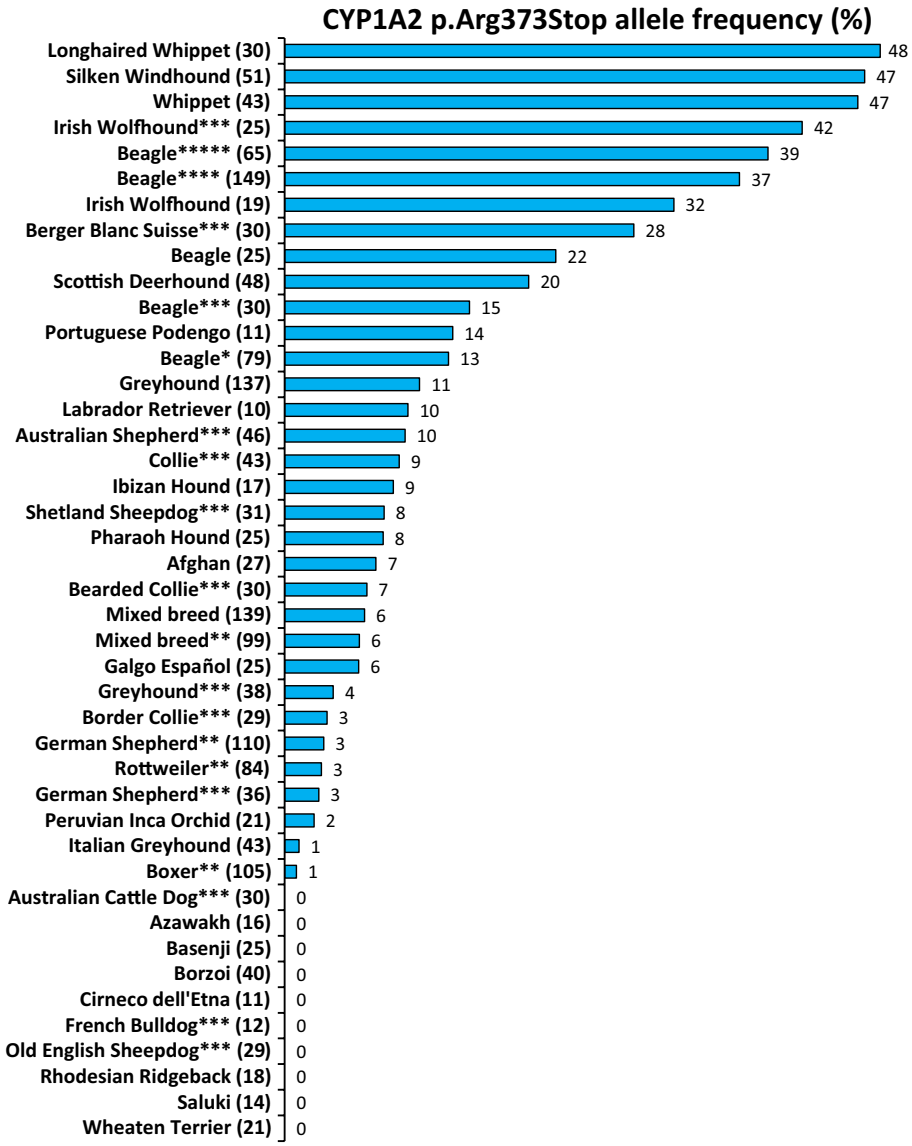

Fig. 4 Variation in CYP1A2 p.Arg373Stop allele frequencies (%) across different dog breeds. Each bar represents the allele frequency (number of variant alleles as a percent of total alelles) measured in the indicated dog breed. Shown after each breed name are the numbers of individual dogs that were sampled. Data are from dogs located in the USA [unpublished data, Court laboratory, Washington State University and *Whiterock et al. (2007)], Brazil [**Scherr et al. (2011)], Germany [***Aretz and Geyer (2011)], and Japan (****Mise et al. (2004b) and *****Tenmizu et al. (2004)]. Only data from breeds in which DNA from at least ten different dogs in that breed were sampled are shown. Other breeds in which at least one dog had the deficient allele included Springer Spaniel, Miniature Schnauzer, Dalmation, and Jack Russell Terrier

Drug-metabolizing enzymes

Cytochrome P450 enzymes

The cytochrome P450 (CYP) oxidation enzymes are critical to efficient drug metabolism and elimination in humans and dogs. Drug-metabolizing CYPs are primarily located in the liver and intestinal mucosa, where they are also an important determinant of drug systemic availability after oral administration. The enzymes are named based on gene sequence similarity and are grouped into families, subfamilies, and individual genes. CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4, and 3A5 are considered the most important drug-metabolizing CYPs in humans based on the identification of their role in the metabolism of the top 200 prescribed drugs in the USA (Williams et al. 2004). Evidence to date indicates that the canine orthologs of these enzymes, including CYPs 1A2, 2B11, 2C21, 2C41, 2D15, 3A12, and 3A26, are also likely to be significant for drug metabolism in dogs and so are discussed here.

CYP1A2

In humans, CYP1A2 constitutes 13–15% of liver CYP expression and metabolizes approximately 8–10% of clinical drugs, including theophylline, clozapine, and tizanidine (Zhou et al. 2010). Canine CYP1A2 constitutes approximately 11% of total CYPs measured (Uchida et al. 1990; Heikkinen et al. 2015) and has substantial overlap in metabolized drugs, including phenacetin and theophylline. Interindividual differences in expression and activity in human liver range from 10- to 200-fold and it is estimated that 35–75% of interindividual variability in CYP1A2 activity may be attributed to genetic factors (Gunes and Dahl 2008; Zhou

et al. 2010). To date, 29 human CYP1A2 variant alleles have been identified (Pharmacogene Variation Consortium 2018). However, a 2006 study assessing the genotype–phenotype relationship identified no single-nucleotide polymorphism (SNP) or haplotype in the CYP1A2 gene that could clearly predict metabolic phenotype (Jiang et al. 2006).

While CYP1A2 null alleles are very rare in humans, a common polymorphism in dogs results in a premature stop codon (c.1117 C > T; p.Arg373Stop) with complete loss of hepatic CYP1A2 protein and thus enzyme activity (Mise et al. 2004a, b; Tenmizu et al. 2004). This variant was first identified in research Beagle dog colonies in Japan. Genotyping revealed that 11–17% of the Beagles were homozygous for the mutant genotype and thus lacked CYP1A2 activity (Mise et al. 2004a; Tenmizu et al. 2004). Subsequent pharmacokinetic studies of several investigational drugs in genotyped Beagles found significantly higher plasma concentrations (up to 17 times greater) in homozygous mutant compared to wild-type dogs, with heterozygous dogs being intermediate (Tenmizu et al. 2006a, b). However, whether or not this polymorphism has an effect on the pharmacokinetics, pharmacodynamics, and safety of clinically used drugs in dogs is unknown. Human CYP1A2-selective probe drugs, such as phenacetin, caffeine, and melatonin, are not considered selective or robust probes for canine CYP1A2, since in vitro and in vivo metabolism of these probes show either no or small (albeit statistically significant) differences between wild-type and homozygous mutant dogs and dog-liver microsomes (Mise et al. 2008; Whiterock et al. 2012). Tizanidine has been proposed as an in vivo canine CYP1A2 probe substrate, but has yet to be fully validated and its sedative properties may limit its use (Locuson et al. 2015).

To date, 5 studies have reported the distribution of the CYP1A2 stop codon allele across a total of more than 1100 dogs, representing 15 unique breeds and mixed breed dogs with at least 10 dogs tested per breed group (Court 2013; Martinez et al. 2018). Unpublished data for an additional 843 dogs from one of the authors (MHC) extend those findings to 19 additional unique breeds (24 breed's total). Whippets and Whippet-derived breeds including Long-Haired Whippet and Silken Windhounds have the highest allele frequencies (47–48%). Several other breeds within the sighthound group including Irish Wolfhounds and Scottish Deerhounds also showed high allele frequencies (20–42%). Also of note is that differences were observed within some dog breeds sampled from different geographic locations. For example, Beagles in Japan, where the variant was initially discovered, had an allele frequency twofold higher (37–39%) than Beagles in the USA (13–22%) and Germany (15%). These differences may be related to population sample size and sampling bias, but also could reflect the isolation of these breeding populations after establishment followed by genetic drift. Interestingly, unlike the MDR1 deletion mutation, which is largely restricted to herding breeds, the CYP1A2 stop codon mutation is distributed across multiple breed types and geographic origins. This suggests that the mutation may have arisen relatively early during, or perhaps prior to, derivation of the more than 350 dog breeds established to date (Parker et al. 2017).

The clinical importance of the CYP1A2 premature stop codon mutation will ultimately depend on the degree to which a drug is dependent on CYP1A2 for clearance and frequency of the mutation in the canine population being treated. So far, there is insufficient evidence to recommend genetic testing for this mutation in dogs for clinical purposes.

CYP2B enzyme subfamily

Human CYP2B6 constitutes 1–10% of total hepatic CYP content and metabolizes approximately 8% of all clinical drugs including cyclophosphamide, bupropion, propofol, methadone, and efavirenz (Hedrich et al. 2016). Hepatic CYP2B6 expression varies by 250-fold among individuals (Hedrich et al. 2016). This may be explained in part by enzyme induction through exposure to constitutive androstane receptor (CAR) and pregnane-X receptor (PXR) ligands, as well as genetic polymorphisms. At present, 62 variant alleles of CYP2B6 have been identified in people (Pharmacogene Variation Consortium 2018). The most common and well-studied variant allele is CYP2B6*6, with allele frequencies ranging from 10 to 60%, depending on ethnicity. CYP2B6*6 causes a 50–75% decrease in hepatic protein expression compared to *1 (wild-type) due to erroneous mRNA splicing leading to protein truncation (Zanger and Klein 2013). Clinically, the CYP2B6*6 variant allele has been associated with reduced drug clearance causing serious adverse events in patients treated with efavirenz or methadone (Zanger and Klein 2013).

CYP2B11 (the canine orthologue of human CYP2B6; also referred to as canine CYP2B6) constitutes approximately 11% of total liver CYP content and is involved in the metabolism of several clinically important drugs used in veterinary medicine including propofol, ketamine, and tramadol (Martinez et al. 2013; Heikkinen et al. 2015; Perez et al. 2016). Despite amino acid sequence similarity to the human orthologue, some differences in substrate specificity between human CYP2B6 and canine CYP2B11 exist. For instance, canine CYP2B11 selectively metabolizes diazepam to nordiazepam (desmethyldiazepam) and temazepam to oxazepam, both of which are primarily catalyzed by human CYP3A4 (Shou et al. 2003).

A CYP2B11 polymorphism resulting in decreased hepatic drug metabolism has been hypothesized to contribute to prolonged recovery of certain dog breeds (primarily Greyhounds) following administration of injectable

anesthetics, including thiopental and propofol (Sams et al. 1985; Sams and Muir 1988; Zoran et al. 1993; Court et al. 1999; Hay Kraus et al. 2000). One study that sequenced the CYP2B11 exon-coding region in 100 dogs identified a rare nonsynonymous SNP (p.Arg74Cys) in exon 2 of CYP2B11 that was predicted to alter enzyme function by Polyphen-2. This variant was found in two of the 11 Labrador Retrievers, one of the 8 Pointers, one of the 11 mixed breed dogs, but not in any of the 8 Greyhounds tested (Wenker 2009). No other nonsynonymous variants were discovered. CYP2B11 sequencing of a further 13 Greyhound samples by two of the authors (MHC and SEM) also failed to identify any nonsynonymous mutations [preliminary findings reported in (Martinez et al. 2018)]. However, multiple SNPs were discovered in the CYP2B11 3′-untranslated region that were subsequently shown through in vitro experiments to decrease CYP2B11 reporter gene expression by up to 80%. One of these variants (CYP2B11-H3) showed the highest allele frequency in Greyhounds (approximately 60%) compared with other dog breeds and so may explain, in part, anesthetic drug sensitivity caused by lower CYP2B11 metabolism in this breed. However, additional evidence, such as showing an effect of the CYP2B11-H3 mutation on drug pharmacokinetics and pharmacodynamics in vivo, will be needed before genetic testing of Greyhound prior to anesthesia, can be recommended.

CYP2C enzyme subfamily

The human CYP2C subfamily contains four isoforms: CYP2C8, 2C9, 2C18, and 2C19. However, only CYP2C8, 2C9 and 2C19 play a significant role in drug metabolism accounting for approximately 5, 13, and 7% of metabolism, respectively, of clinically used drugs (Zanger and Schwab 2013). Collectively, the CYP2C subfamily constitutes approximately 24% of total hepatic CYP protein (Achour et al. 2014). CYP2C8 metabolizes paclitaxel and several antimalarial drugs. CYP2C9 metabolizes warfarin, phenytoin, losartan, tolbutamide, and most nonsteroidal anti-inflammatory drugs (NSAIDs). CYP2C19 metabolizes *S*-mephenytoin, proton pump inhibitors, such as omeprazole, and converts clopidogrel to the active metabolite (Zanger and Schwab 2013). Currently, there are 127 human variant alleles identified in the CYP2C subfamily (Pharmacogene Variation Consortium 2018). Many CYP2C9 substrates have a narrow therapeutic index such that individuals with the CYP2C9*2 or *3 allele (10–15% and 4–10% allele frequencies, respectively, in Caucasians) are at an increased risk for adverse drug reactions, such as tolbutamide-induced hypoglycemia, gastrointestinal toxicity from NSAIDs, or significant bleeding with warfarin anticoagulation therapy (Zanger and Schwab 2013). The CYP2C19*2 and *3 alleles are the most common cause of the CYP2C19 poor metabolizer phenotype (2–14% of the population) leading to no functional activation of the pro-drug clopidogrel and increased risk of adverse cardiovascular events (U.S. Food & Drug Administration 2010).

Dogs only have two CYP2C isoforms, CYP2C21 and CYP2C41 (Uchida et al. 1990; Blaisdell et al. 1998). CYP2C21 shares the highest protein homology (70%) with human CYP2C19, while CYP2C41 shares the highest protein homology with human CYP2C9 (75%) (Shou et al. 2003). CYP2C21 constitutes 21% of the total CYP content measured in the liver (Heikkinen et al. 2015). The absolute protein content of CYP2C41 in dog liver has not yet been reported. Little is known about the substrate specificity of CYP2C21 and CYP2C41. Diclofenac and *S*-mephenytoin are substrates of CYP2C21 and CYP2C41, although CYP2C21 is the more active of the two isoforms (Shou et al. 2003; Locuson et al. 2009). CYP2C21 and CYP2C41 also participate in the metabolism of tramadol and tramadol metabolites (Perez et al. 2016; Perez Jimenez et al. 2018), but no other substrates have been studied or identified.

Although no polymorphisms of CYP2C21 have been reported to date, a gene copy-number variation has been identified affecting CYP2C41. In the original study that cloned the CYP2C41cDNA from a single Beagle liver library, liver samples from many other dogs lacked detectable CYP2C41 mRNA expression (Blaisdell et al. 1998). Subsequent analysis using an allele-specific PCR genotyping test showed that the exon 7 region of the CYP2C41 gene was present in only two of 18 Beagles and two of 10 mixed breed dogs tested (approximately 16% of all dogs) (Blaisdell et al. 1998). Although no other CYP2C41 genotyping studies have been reported to date, another study provided indirect evidence for this polymorphism, showing that CYP2C41 mRNA was detectable by real-time PCR in only 6 of 11 Beagle liver samples (Graham et al. 2003). It is currently unclear whether the CYP2C41 gene is only partially (i.e., exon 7) or completely deleted. However, since the canine reference genome sequence (CanFam3.1) lacks any portion of the CYP2C41 gene, a large-scale deletion seems more likely. The clinical importance of CYP2C41 for drug metabolism in dogs that do possess this gene is currently unclear.

CYP2D enzyme subfamily

Human CYP2D6 constitutes approximately 4% of total CYP liver content, but it metabolizes approximately 20% of all clinically used drugs (Zanger and Schwab 2013; Achour et al. 2014). CYP2D6 has broad substrate specificity and metabolizes drugs from most therapeutic classes. Well-known CYP2D6 substrates include dextromethorphan, tramadol, codeine, metoprolol, debrisoquine, risperidone, and many tricyclic and second-generation antidepressants (Zanger and Schwab 2013). CYP2D6 is

the most widely studied polymorphic human drug-metabolizing CYP. Over 145 variant alleles are currently recognized (Pharmacogene Variation Consortium 2018), many of which substantially alter enzyme function. CYP2D6 is not inducible. A classic example of the importance of CYP2D6 metabolic phenotype is pain management with codeine, which relies on CYP2D6 for metabolism into the active metabolite, morphine. A poor metabolizer (approximately 5–10% of patients) will experience no analgesic activity because of insufficient morphine plasma concentrations, whereas an ultra-rapid metabolizer (approximately 1–2% of patients) is at risk of potentially fatal opioid intoxication due to excessive morphine plasma concentrations (Crews et al. 2014).

Canine CYP2D15 (the canine orthologue of human CYP2D6; also referred to as canine CYP2D6) constitutes approximately 17% of quantified hepatic CYP protein (Heikkinen et al. 2015). CYP2D15 selectively metabolizes several "classical" CYP2D6 substrates including dextromethorphan, tramadol, and metoprolol, but does not metabolize debrisoquine (Martinez et al. 2013; Perez et al. 2016). Similar to human CYP2D6, current evidence indicates that canine CYP2D15 is highly polymorphic (Roussel et al. 1998; Paulson et al. 1999). Using dog-liver cDNA clone libraries, two studies identified CYP2D15 mRNA sequence variants and evaluated effects on recombinant enzyme function (Roussel et al. 1998; Paulson et al. 1999). Four variants, CYP2D15WT2, 2D15V1, *2 and *3, contained two to five amino acid substitutions compared to the designated wild-type sequence (Sakamoto et al. 1995; Roussel et al. 1998; Paulson et al. 1999). One mRNA variant (CYP2D15δ) contained a large in-frame deletion corresponding to 51 amino acids in exon 3, and another (CYP2D15V2) contained this deletion variant as well as three amino acid substitutions (Roussel et al. 1998; Paulson et al. 1999). The enzymatic function did not appear to be significantly decreased from wild type for those variants with amino acid changes with the exception of CYP2D15WT2, which showed a substrate-dependent reduction in activity (Paulson et al. 1999). Both CYP2D15 exon 3 deletion variants were inactive (Roussel et al. 1998; Paulson et al. 1999).

CYP2D15 genetic variation was proposed to explain polymorphic in vivo celecoxib clearance and in vitro celecoxib hydroxylation in Beagle dogs (Paulson et al. 1999). However, the investigators did not go on to establish an association between the presence of any CYP2D15 variant and slow or fast celecoxib clearance or liver metabolism. The CYP2D15 variant allele frequencies have not been reported for Beagles or any other dog breed. One barrier to progress in this area is the lack of a publicly available complete and accurate canine CYP2D15 gene sequence.

CYP3A enzyme subfamily

The human CYP3A isoforms, CYP3A4 and CYP3A5, are arguably the most important for drug metabolism in people, constituting 24% and 4%, respectively, of total CYP liver content and collectively participating in the metabolism of over 30% of clinically used drugs (Zanger and Schwab 2013; Achour et al. 2014). Furthermore, CYP3A4 is the primary CYP expressed in intestinal enterocytes and contributes considerably to the first-pass metabolism of orally administered drugs (Ding and Kaminsky 2003; Von Richter et al. 2004). CYP3A4 and 3A5 are both inducible by PXR and CAR ligands (Burk et al. 2004) and have wide overlapping substrate specificity including immunosuppressants, macrolide antibiotics, anticancer drugs, statins, antidepressants, and opioids (Zanger and Schwab 2013). Although interindividual variability in hepatic CYP3A4 expression is very high, over 100-fold (Zanger and Schwab 2013), none of the 43 variant alleles reported for CYP3A4 explain this variability (Pharmacogene Variation Consortium 2018). CYP3A4*22 was identified as a CYP3A4 null allele. However, the low CYP3A4*22 allele frequency in people (2.1% globally) indicates that it does not significantly contribute to overall CYP3A4 interindividual variability (Zanger and Schwab 2013). Of the 23 variant alleles recognized for CYP3A5 (Pharmacogene Variation Consortium 2018), the CYP3A5*3 intron variant causing aberrant mRNA splicing is the most important. Population differences in CYP3A5*3 allele frequency largely explain why only 5–10% of Caucasians but up to 60% of African or African-Americans have hepatic CYP3A5 expression. However, the impact of CYP3A5*3 on drug pharmacokinetics, efficacy, and toxicity appears to be somewhat limited, since most drugs metabolized by CYP3A5 are also metabolized by CYP3A4. The CYP3A5*3 allele was associated with decreased tacrolimus clearance and drug dose requirement in kidney transplant patients (Staatz et al. 2010a, b; Wang et al. 2010b; Birdwell et al. 2012).

Two CYP3A isoforms have been identified and characterized in dogs, CYP3A12 and 3A26 (Fraser et al. 1997). Canine CYP3A12 (the canine orthologue of human CYP3A4 also referred to as canine CYP3A4) is highly abundant in liver and intestinal mucosa, constituting 28% and over 50% of quantified CYP protein in those tissues, respectively (Heikkinen et al. 2015). CYP3A26 is also found in liver but at much lower concentrations (0.8% of CYP protein), and could not be quantified in the intestinal mucosa (Heikkinen et al. 2015). Like human CYP3A enzymes, both CYP3A12 and CYP3A26 are inducible by similar PXR and CAR ligands, although compound-specific differences have been noted (Chen et al. 2009). Known canine CYP3A substrates include diazepam, vinblastine, and testosterone (Shou et al. 2003; Achanta and Maxwell 2016). It is not known whether

the canine CYP3A enzymes also metabolize a diverse group of substrates, such as human CYP3A. CYP3A26 appears to have a far lower capacity to metabolize CYP3A substrates compared with CYP3A12 possibly because of amino acid differences in the substrate-binding site (Fraser et al. 1997; Shou et al. 2003; Locuson et al. 2009). To date, only one CYP3A12 variant, called CYP3A12*2, has been reported. This variant was discovered by sequencing cDNA clones from Beagle liver mRNA and differed at five nucleotides from the reference sequence resulting in five amino acid changes (Paulson et al. 1999). Evaluation of testosterone 6β-hydroxylation by recombinant wild type and variant CYP3A12*2 showed no significant differences in kinetic parameters (Paulson et al. 1999). No other CYP3A12 substrates were examined, and therefore, substrate-specific differences cannot be excluded. The CYP3A12 variant allele frequency among the general dog population is unknown, as is the clinical relevance of this variant warranting further study. No CYP3A26 variants have been reported.

NADPH cytochrome P450 oxidoreductase

NADPH cytochrome P450 oxidoreductase (POR) is the obligate electron donor to all microsomal CYP enzymes and also donates electrons to cytochrome b_5, heme oxygenase, squalene monooxygenase, and 7-dehydrocholesterol reductase (Pandey and Sproll 2014). POR also catalyzes the one-electron reduction of several anticancer prodrugs, such as mitomycin C and tirapazamine (Zanger and Schwab 2013). POR is inducible by PXR and CAR (Zanger and Schwab 2013). POR knockout mice are embryonic lethal and liver-specific POR knockout mice have a marked decrease in drug metabolism and lipid accumulation (Pandey and Sproll 2014). In humans, POR is highly polymorphic with 40 variant alleles currently recognized (Pharmacogene Variation Consortium 2018). Individuals with rare variants causing substantially reduced enzyme function are diagnosed with P450 oxidoreductase deficiency, a form of congenital adrenal hyperplasia (Pandey and Sproll 2014). More common variants with less severe functional impact have been described that influence CYP function in an isoform-dependent fashion (Pandey and Sproll 2014). The most common and best characterized POR polymorphism is the A530 V variant (POR*28) that has an allele frequency ranging from 25 to 45% across ethnicities (Pandey and Sproll 2014). The results of multiple studies suggest that individuals with the POR*28 allele have higher in vivo CYP3A activity (Gong et al. 2017). In addition, in vitro studies of the POR*28 variant suggest that the enzyme may have reduced mitomycin C-metabolizing capabilities and cause reduced CYP2D6 activity, but results have not been confirmed in vivo (Gong et al. 2017).

Two nonsynonymous variants have been discovered in the dog POR gene that appear to influence recombinant CYP2B11 oxidase activity (preliminary data reported in Martinez et al. 2018). These variants showed the highest allele frequency in Scottish Deerhounds (36%) and Greyhounds (35%) compared with other dog breeds and so may contribute to anesthetic drug sensitivity caused by lower CYP2B11 metabolism in Greyhounds. These findings need to be confirmed by in vivo study of drug metabolism in genotyped dogs.

Thiopurine *S*-methyltransferase

Thiopurine *S*-methyltransferase (TPMT) is an enzyme of human pharmacogenetic significance (Wang et al. 2010a). TPMT is responsible for *S*-methylation and inactivation of a number of clinically used thiopurine drugs that include thioguanine, 6-mercaptopurine, and azathioprine. Azathioprine is used in both humans and dogs to treat immune-related diseases affecting the gastrointestinal tract, skin, and joints (Rodriguez et al. 2004). A small percentage of human and canine patients can experience severe life-threatening myelosuppression during treatment with standard doses of azathioprine (Rodriguez et al. 2004; Wang et al. 2010a). In humans, this has been largely attributed to low or absent TPMT activity that results in increased exposure to active thiopurine metabolites. Over 90% of low-activity phenotypes can be explained by 3 SNPs that affect the TPMT protein sequence and result in enhanced TPMT protein degradation (Weinshilboum 2003; Evans 2004). TPMT genotyping and/or measurement of red blood cell TPMT activity is recommended by the United States FDA prior to prescribing azathioprine in people to guide dosage adjustments and minimize toxicity (Clinical Pharmacogenetics Implementation Consortium 2018).

Three different studies in dogs ($n = 145$–300 dogs) showed red blood cell TPMT activities that were similar on average to activities reported for humans, but varied over ninefold between individual dogs (Salavaggione et al. 2002; Kidd et al. 2004; Rodriguez et al. 2004). Furthermore, the results of one of these studies suggested that TPMT activity varied by dog breed with some breeds (such as Giant Schnauzer) having low activity, while other breeds (such as Alaskan malamute) having high activity (Salavaggione et al. 2002). Treatment of 10 healthy, TMPT-phenotyped dogs with azathioprine for 30 days showed reduced blood neutrophil counts in dogs with low/intermediate TPMT activity but no change in dogs with high TPMT activity (Rodriguez et al. 2004). This latter result indicates that reduced TPMT activity may also predispose dogs to myelosuppression.

Sequencing of the TPMT gene in 39 dogs with high and low TPMT activity identified multiple genetic variants, although only one (Arg97Gln; allele frequency 21%) was

found to affect the TPMT protein sequence (Kidd et al. 2004). However, subsequent recombinant expression of the variant allozyme showed no effect on enzyme function and there was only a modest association of this genetic variant with in vivo activity (17% of variability explained), suggesting minimal functional impact of this variant. In contrast to humans, where 0.3% of the European population is deficient in TPMT activity resulting from homozygosity of inactivating polymorphisms, none of the dogs phenotyped to date showed absent TPMT activity, suggesting that a null allele for this trait may be rare in dogs or perhaps isolated to certain dog breeds (Salavaggione et al. 2002; Kidd et al. 2004; Rodriguez et al. 2004). A study that recruited 6 dogs that had experienced severe myelotoxicity with standard dose azathioprine treatment for clinical disease showed normal TPMT activity in all dogs (Rodriguez et al. 2004). This latter result suggests that other genetic (and nongenetic) factors may contribute to azathioprine toxicity in dogs.

Albumin

Albumin has an extraordinary capability to bind small molecules in the circulatory system and interstitium, playing a remarkable role in drug disposition and pharmacological effects of numerous drug molecules (Benet and Hoener 2002; Baker and Parton 2007; Bohnert and Gan 2013). The chemical structure of albumin is comparable in all mammals. In dogs and humans, serum albumin has a sequence homology of 79.8% (Yamada et al. 2016). Despite the homology of the primary structure of serum albumins between species, drug-binding properties differ considerably (Kosa et al. 2007; Pistolozzi and Bertucci 2008). Topologically, albumin consists of three domains (I–III), each of which is divided into two subdomains (A and B) (Zsila et al. 2011; Yamada et al. 2016). Human albumin has at least two drug-binding sites known as site I and site II, according to the classification of Sudlow et al. (Sudlow et al. 1975). Binding sites I and II are located in subdomains IIA and IIIA, respectively (Zsila et al. 2011). These binding sites differ with respect to shape, polarity, and binding specificities (Ghuman et al. 2005). A drug-binding study using warfarin and phenylbutazone suggests that binding site I of canine and human serum albumin differs with respect to drug-binding capabilities (Kosa et al. 1997). In contrast, the same study showed that binding site II of canine and human serum albumin seems to be comparable (Kosa et al. 1997). This is, at least in part, due to a relatively high homology (91.7%) of the amino acid sequence composing this binding site (Cys476-Pro499) (Kaneko et al. 2008). Beagles, among other breeds, can express three electrophoretic albumin variants, F, S, and FS (Mgheni et al. 1979), with autosomal codominant inheritance AlbF and Albs (Day et al. 1971). The role of these variants in binding small molecules is unknown. Notably, sequencing analysis of albumin cDNA from Beagles revealed two single-nucleotide polymorphisms, G1075T and A1422T, leading to amino acid changes Ala335Ser and Glu450Asp, respectively. A1422T (Glu450) can affect drug binding to albumin site II (Ghuman et al. 2005). G1075T and A1422T albumin polymorphisms have been associated with differences in the extent of plasma protein binding and disposition of an investigational drug in Beagles (Ito et al. 2009). It remains unknown if Ala335Ser and Glu450Asp alter the extent and affinity of binding other drugs. The human albumin gene has been reported to be polymorphic (Minchiotti et al. 2008), but few mutations within subdomains IIA and IIIA have been identified (He and Carter 1992; Sugio et al. 1999). Except for mutations c.269T4C (p.Leu90Pro) (Sunthornthepvarakul et al. 1994), c.725G4A p.Arg242His (Petersen et al. 1994, 1995), and c.725G4C (p.Arg242Pro) (Petersen et al. 1994; Wada et al. 1997), which form strong binding sites for triiodothyronine or thyroxine, mutations in subdomains IIA and IIIA do not appear to alter drug binding to a clinically significant degree (Minchiotti et al. 2008). Whether or not comparable mutations exist in dogs is unknown.

Malignant hyperthermia

Malignant hyperthermia (MH) is a rare heritable drug hypersensitivity disorder that occurs in humans and dogs, as well as other species. Classical cases in human patients occur following administration of a triggering agent, including depolarizing muscle relaxants (especially succinylcholine) and potent inhalant anesthetics (including halothane, isoflurane, and sevoflurane). Clinical signs can include muscle rigidity, increased body temperature, tachycardia, acidosis, hyperkalemia, rhabdomyolysis, and disseminated intravascular coagulation. Death can ensue if treatment is not instituted rapidly. Incidence estimates for human MH range from 1/10,000 to 1/250,000 anesthetic procedures. However, the prevalence of susceptibility in the population is likely higher (estimated up to 1/400 people), since only a proportion of susceptible individuals are likely to be exposed to triggering agents (Rosenberg et al. 2015; Alvarellos et al. 2016).

A recent study (the largest to date) indicates that about 76% of MH cases in people can be explained by mutations in the ryanodine receptor (*RYR1*) gene (Miller et al. 2018). All but one of the mutations were found in the heterozygous state suggesting a dominant "gain-of-function" effect. The *RYR1* gene product (RyR) is a homotetrameric calcium channel located in the sarcoplasmic reticulum membrane that facilitates muscle contraction by releasing calcium stores into the myoplasm. MH mutations typically result in enhanced sensitivity of the channel to opening by anesthetic drugs. Mutation "hotspots" are found in *RYR1* exons 15, 44, and 46, likely reflecting the importance of these protein

regions in channel function. The same study also showed that about 1–2% of human MH cases are explained by mutations in the calcium voltage-gated channel subunit alpha1 S (*CACNA1S*) gene. The *CACNA1S* gene product (called $Ca_v1.1$) is the voltage sensing subunit of the pentameric dihydropyridine receptor (DHPR), an L-type calcium channel located in the T-tubule membrane adjacent to the RyR receptors. Physical interaction of DHPR with RyR forms the basis for skeletal muscle excitation–contraction coupling. Finally, a rare mutation in the SH3 and cysteine-rich domain-containing protein 3 (STAC3) gene (p.Trp284Ser) was recently associated with MH in 10 homozygous patients based on clinical signs after inhalant anesthetic administration and response to dantrolene treatment (Zaharieva et al. 2018). The STAC3 gene product is involved in excitation–contraction couple and associates with $Ca_v1.1$. The remaining ~ 30% of MH cases remain unexplained.

Sporadic cases of anesthesia-induced malignant hyperthermia in dogs have been reported over the past 45 years (Short and Paddleford 1973; Bagshaw et al. 1978; Cohen 1978; Sawyer 1981; Leary et al. 1983; O'Brien et al. 1983; Kirmayer et al. 1984; Otto 1992; Chohan and Greene 2011; Adami et al. 2012). Of these 10 reported cases, four (40%) were Greyhounds suggesting a breed predisposition (Bagshaw et al. 1978; Cohen 1978; Leary et al. 1983; Kirmayer et al. 1984). The remaining dogs were a variety of different breeds. The diagnosis of malignant hyperthermia in each of these cases was based on clinical presentation without specific confirmatory diagnostics. However, one research group (O'Brien et al. 1983) established a colony of MH dogs. Subsequent studies of these dogs and their descendants have verified similarity to human MH by in vivo halothane–succinylcholine challenge testing including a positive response to dantrolene therapy, and by in vitro caffeine–halothane contracture testing of muscle biopsies (Nelson 1991; Roberts et al. 2001). Furthermore, pedigree analysis indicated that the MH phenotype was transmitted as an autosomal dominant trait, while genetic marker linkage analysis isolated a genomic region containing the canine *RYR1* gene (Roberts et al. 2001). Gene sequencing identified a nonsynonymous variant in the *RYR1* gene c.1640T > C p.Ala547Val that completely cosegregated with the drug-sensitivity phenotype. The amino acid at this position is highly conserved across species and is near pathogenic mutations in the human gene.

It is unclear whether canine RYR1 p.Ala547Val mutation is causative in other cases of canine MH. One case report of a suspected MH reaction to anesthesia (Adami et al. 2012) indicated that a "genetic test (PCR assay)" had been performed, but was negative for "a mutation of the RYR1 gene" with a reference to the canine p.Ala547Val mutation. However, no other information was provided regarding the assay, especially whether it was specific for p.Ala547Val or scanned for other RYR1 (or CACNA1S) mutations. As for human MH, it is likely that multiple rare mutations may account for canine MH.

It should also be mentioned that (unexpected) hyperthermia has been reported in dogs following other (non-pharmacologic) triggers including moderate exercise and stress (Rand and O'Brien 1987; O'Brien et al. 1990; Dickinson and Sullivan 1994; Thrift et al. 2017). This has been termed canine stress syndrome by analogy to porcine stress syndrome, a model of human malignant hyperthermia that occurs in pigs. It has been proposed that this may represent a milder form of MH. Several dogs (a Border collie and an English Springer Spaniel) were systemically evaluated and showed in vitro caffeine–halothane contracture test results similar to dogs susceptible to MH (Rand and O'Brien 1987; O'Brien et al. 1990). Consequently, there may be overlap in the pathophysiology of hyperthermia caused by different triggers. The genetic basis for canine stress syndrome has not been reported to date.

Beta$_1$ adrenergic receptor

There are two polymorphisms (p.Ser48Gly and p.Arg389Gly) of the human β_1 adrenoreceptor that are thought to be clinically relevant. Both polymorphisms are present in > 10% of Caucasians, African-Americans, Asians, and Latino-Hispanics (Parry et al. 2013). Both are gain-of-function polymorphisms that augment β_1 receptor activity compared to wild-type individuals. Affected individuals are more sensitive to the effects of β_1 adrenoreceptor antagonists, such as atenolol, carvedilol, and others. Like their human counterparts, dogs with certain cardiac diseases require treatment with β_1 adrenoreceptor antagonists. To identify potential reasons for variable efficacy of β1 adrenoreceptor antagonists in dogs, the canine β_1 adrenoreceptor gene was sequenced in five dog breeds with a high incidence of cardiac disease (Cavalier King Charles Spaniel, Boxer, Newfoundland, Great Dane, and Doberman pinscher). Two polymorphic sites were detected, both within the cytoplasmic tail of the β_1 adrenoreceptor (Maran et al. 2013). The first locus harbors either a six or nine base-pair deletion at base pair 1260, while the second locus harbors a 24 base-pair deletion starting at base pair 1315. In a subsequent study, dogs with deletions at both loci for were evaluated for phenotypic differences in response to treatment with atenolol compared to control dogs (Meurs et al. 2015). Dogs with the double deletion had a blunted response to atenolol (i.e., reduction in heart rate was significantly less) compared to control dogs. Because this polymorphism appears to be present in a substantial portion of dog breeds that are at risk for developing cardiac disease and, therefore, might require treatment with β_1 adrenoreceptor antagonists, a pharmacogenetic approach to treatment might be advantageous.

Precision medicine for cancer treatment

Oncology-focused precision medicine employs a different approach than the pharmacogenetic discoveries previously discussed. Rather than identifying patient genotypes that predict adverse drug reactions, the intent of cancer precision medicine is to identify mutations in DNA from tumor cells rather than DNA from non-neoplastic host (patient) cells. Identified tumor cell mutations can then theoretically be used to select targeted therapies (drugs or immunotherapies) designed specifically for those tumor cell mutations. For example, afatinib is a kinase inhibitor indicated for human patients with metastatic non-small cell lung cancer, whose tumor has one of the two very specific mutations of the gene encoding epidermal growth factor receptor (EGFR). The FDA lists over 200 drugs with pharmacogenomics biomarkers included as a component of the drug label (U.S. Food & Drug Administration 2018). Nearly, half of those are anticancer drugs. Many of these drugs received FDA approval with attendant-labeling requirements that stipulate the use of that drug for a specific type of cancer that has a specific mutation as detected by an FDA-approved genetic test. There are no direct correlates in veterinary medicine—that is, there are currently no FDA-approved drugs specifically indicated for dogs with defined mutations.

The closest example of precision medicine in dogs involves a receptor tyrosine kinase inhibitor (toceranib) that is FDA approved for treating grade II or III cutaneous mast cell tumors, a common neoplasm in dogs. Dogs with mast cell tumors that have a specific c-*kit* gene mutation, an internal tandem duplication with altered KIT expression, are reported to be more likely to respond to toceranib than dogs, whose mast cell tumors do not have c-*kit* mutations (60% versus 31%, respectively) (London 2009). However, unlike the FDA-approved human kinase inhibitors, the label does not stipulate that toceranib use is indicated only for mast cell tumors with specific c-kit mutations. In addition, since dog genetic testing does not currently require FDA oversight, there is not an FDA-approved genetic test for c-*kit* available for dogs. Therefore, while toceranib comes the closest to being a true, evidence-based precision medication for treating canine cancer patients, it lacks many important characteristics of human-targeted therapeutic agents.

Ongoing research has identified potential "actionable" genotypes in several canine neoplasms including hemangiosarcoma (Wang et al. 2017), melanoma (Hernandez et al. 2018), osteosarcoma (Monks et al. 2013), and others. Several somatic mutations in these canine tumors are thought to correspond to well-characterized oncogenic mutations in human cancers, including PIK3CA, TP53, PTEN, PLCG1, NRAS, BRAF, and others. However, clinical trials are necessary to confirm if identified mutations actually respond to the targeted inhibitors (i.e., binimetinib for specific BRAF mutations). Until such time, offering direct-to-consumer cancer genome sequencing with the implication that it might lead to precision medicine is potentially misleading, as there is currently no evidence to support those claims.

Valuable information could be generated from prospective studies using canine tumor genome information paired with patient survival times after treatment with targeted therapies. This could, over the long term, provide evidence with which to select appropriate chemotherapeutic drugs for canine cancer patients. Clinical trials along those lines are desperately needed to move the field of canine precision medicine forward.

Optimistically, precision medicine for canine cancer patients is several years away. The authors urge companies that are considering or currently offering this service for veterinary patients to provide evidence of its efficacy before seeking payment from pet owners.

Canine pharmacogenetic testing considerations

Quality control is as relevant for pharmacogenetic testing, as it is for genetic testing for disease traits. This article will not duplicate information that has been adequately discussed in other articles of this special issue. However, there are features of veterinary pharmacogenetic diagnostic testing services that are unique and warrant discussion. In particular, predicting a safe and effective drug-dosing regimen based on genetics in the context of treating a specific veterinary patient requires the expertise of a veterinary clinical pharmacologist with training in genomics. This is necessary, because most veterinarians simply have not been trained to interpret pharmacogenetic testing results in the context of a particular patient with a specific disease that is being treated with multiple drugs. Pharmacogenetics is not currently a component of veterinary curricula for U.S. Colleges of Veterinary Medicine, so it is inappropriate for entities that market pharmacogenetic tests to expect veterinarians to interpret results. Similarly, individuals trained in human genetic counseling lack the expertise to provide pharmacogenetic counseling for veterinary patients because of the differences among drugs used in humans versus dogs, but also because of dramatically different drug dispositions between the two species (Dalgaard 2015).

Even highly competent geneticists that research veterinary species have demonstrated a critical lack of understanding of veterinary pharmacogenetics. For example, in a recent opinion piece authored by a vertebrate geneticist and two

veterinarians without expertise in veterinary pharmacology and genomics entitled, "Pet genomics medicine runs wild," published in *Nature*, the authors stated that three different ABCB1 mutations have been associated with "the phenotype" (Moses et al. 2018). The authors go on to state their concern that genetic testing companies currently test for only one ABCB1 mutation. Their statement regarding multiple polymorphisms resulting in drug-sensitivity phenotypes is simply wrong and was based solely on a website that carries a disclaimer regarding the accuracy of its information. Therefore, while their intentions were good, the end result may be harmful for dogs by discouraging dog owners and veterinarians from testing dogs for the ABCB1-1Δ mutation prior to treatment with drugs that are well documented to cause life-threatening adverse drug reactions in dogs with the ABCB1-1Δ genotype.

In conclusion, companies that market genetic testing for veterinary patients should have the expertise to help owners and veterinarians interpret not only the genetic results, but how to incorporate those results with other diagnostic information to optimize the patient's therapeutic outcome. It would be beneficial to all involved if veterinary genetic testing companies could form partnerships or collaborate with the veterinary researchers, such as cardiologists, neurologists, ophthalmologists, pharmacologists, etc., that have studied the genetic mutation in the context of dogs and cats with the disease.

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Compliance with ethical standards

Conflict of interest KLM receives royalties from Washington State University for MDR1 (ABCB1) genotyping patents. All the other authors declare that they have no conflict of interest.

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