Genetic predisposition to adverse drug events in dogs

Margo Karriker, PharmD

WALTHAM UCVMC-SD Clinical Nutrition Program, University of California Veterinary Medical Center, San Diego, USA

Dr. Karriker graduated with a doctorate from the School of Pharmacy at the University of North Carolina, Chapel Hill in 2003. She completed a two-year Veterinary Clinical Pharmacy residency at the University of California, Davis Veterinary Teaching Hospital in 2005 and began her position as the WALTHAM Specialist in Clinical Pharmacy for the WALTHAM UCVMC-SD Clinical Nutrition Program in San Diego the same year. While continuing to serve in this position, Dr. Karriker is also clinical faculty at the UC San Diego Skaggs School of Pharmacy and pharmacist for a federal Veterinary Medical Assistance Team.

KEY POINTS

- There is growing evidence that clear genetic links exist between the phenotypic characteristics of dogs and adverse drug reactions
- The fields of pharmacogenetics and pharmacogenomics are rapidly expanding the promise of the clinical application of genetic data to help prevent adverse events, predict drug behavior, and discover new drug targets for study
- Among the most widely studied genetic variances observed in dogs is the multi-drug resistance (MDR) gene deletion mutation resulting in altered p-glycoprotein expression and altered drug behavior in Collies and related breeds
- The future of pharmacogenetics and its clinical applicability will have a lasting impact on the clinical decisions we make in the future

Introduction

The goal of drug treatment is to maximize the therapeutic effect while minimizing adverse drug events and drug interactions. Seldom is drug therapy this simplistic. Complex patients, variable disease processes, and intricate medication regimens complicate most clinical scenarios. There are times when our best effort at predicting, avoiding, and managing adverse drug events is not enough.

While we have long noted trends in medication events seemingly related to breed, age, environmental influence and other factors, we have never been able to map these events to one genetic location or associate them unequivocally with one site in the genome.

In recent years, there has been much focus on the genetic basis of the pharmacokinetic (absorption, distribution, metabolism, excretion) and pharmacodynamic (drug interactions with targets such as receptors and transporters) interactions drugs have in the body. This field of study has been termed pharmacogenetics and is designed to provide an understanding of the genetic variation in populations that predict an individual's response to drug therapy. This field of study promises us the ability to effectively and efficiently predict a sound outcome

in almost every patient as it relates to identified targets of drug therapy.

With the completion of the human genome project and continued focus on mapping the genetic profile of other species, pharmacogenetic data and, in turn, clinical predictors of drug response, are likely to become more commonplace in veterinary practice. As our clinical picture progresses toward continued treatment of complex disease states, management of chronic conditions, and diagnosis of less common disease processes, the ability to predict a patient's interaction with their medication regimen, and perhaps a clinical success probability, should prove to be an invaluable part of comprehensive therapy.

Currently, we have a small amount of clinical and anecdotal evidence that allows us to have some insight into breed or population characteristics that appear to predispose animals to adverse drug events. Until recently, these insights were based primarily on the phenotypic, or visually expressed, traits such as hair coat and eye color. A pharmacogenetic approach to adverse drug events stands to provide us with drug therapy that is unique to each patient and allows for an individualized medication regimen (1).

As we currently stand, a population of seemingly similar animals may experience a variety of clinical responses to one common drug treatment. The variable clinical response we see may be genetically based in some cases and the insight pharmacogenetics gives us will change our practice on a real-time level. Using focused screening tests, we may be able to predict which animals express the altered protein or gene sequence responsible for the adverse event associated with a specific drug or class of drugs. With the tools that pharmacogenetics offers, we will likely be able to avoid or alter this event by modifying our drug choices.

Table 1.
Examples of inherited and acquired variations in enzymes, receptors and drug transporters found to be clinically relevant in human medicine (2)

Enzyme	Variant phenotypes	Drug affected	Response modification
Plasma pseudocholinesterase	Slow, ester hydrolysis	Succinylcholine	Prolonged apnea
Thiopurine methyltransferase (TPMT)	Poor TPMT methylators	6-Mercaptopurine 6-Thioguanine Azathioprine	Bone marrow toxicity, liver damage
Aldehyde dehydrogenase	Fast, slow metabolizers	Ethanol	Slow: Facial flushing Fast: Protection from liver cirrhosis
Catechol-O-methyltransferase	High, low methylators	Levodopa Methyldopa	Increased or decreased response
CYP 2D6	Ultra rapid Extensive Poor metabolizers	Debrisoquine Nortryptilline Dextromethoraphan	Ultra rapid: Drug resistance Extensive: Lung cancer Poor: Increased toxicity
CYP 2C9	Poor metabolizers	Tolbutamide, S-warfarin, phenytoin, Non-steroidal anti-inflammatory agents	Increase response or toxicity
CYP 2C19	Poor, extensive hydroxylators	Omeprazole	Poor: Increased toxicity, reduced efficacy
Transporters			
Multiple drug resistance transporter (MDR-1)	Overexpression	Many - See *Table 2*	Drug resistance
Receptors			
B_2 Adrenoreceptor	Receptor downregulation	Albuterol	Poor asthma control
5-HT_{2A} seroteronergic receptor	Multiple polymorphisms	Clozapine	Variable drug efficacy
HER_2	Overexpression (breast cancer)	Trastuzumab	Variable drug efficacy

Current evolution of pharmacogenetics

Many years ago, the idea of organisms possessing the capacity to adapt to influences placed on them by environmental or therapeutic factors was thought to be based in inheritance. In the early 1900's the studies of several investigators provided proof that there was indeed a connection between the biochemical processes that dictate drug metabolism and genetics (2). One of the first reports of an inherited difference in response to a foreign chemical was published by Snyder in 1932. Based on previous work by Fox, Blakeslee and Salmon, "taste blindness" to phenylthiocarbamide (PTC) was described and linked to an autosomal recessive trait in humans (3). Later, during World War II, Archibald Garrod noticed a link between the development of a primiquine hemolysis reaction and African-American soldiers. Further study showed that this hemolysis was the product of a genetic deficiency of glucose-6-phosphate dehydrogenase (G-6-PD) (4).

As time progressed, observations of succinylcholine, isonizid, and debrisoquine helped relate individual patients' responses to a genetic pattern. These early reports and case studies laid the ground work for modern pharmacogenetics.

Since those first advances, contemporary technology has allowed the study of pharmacogenetics to move forward at a heightened pace. Today, there is a focus on both pharmacogenetics (a collaboration of biochemistry and pharmacology to correlate phenotypic markers to specific genetic links) and on pharmacogenomics. Pharmacogenomics differs from pharmacogenetics in its more global focus. Using advanced technology such as high-throughput DNA sequencing, gene mapping and bioinformatics, studies can focus on interpatient variables to predict differences in drug behavior and response. The genomic approach will allow us to study the basis of observed drug response, and predict responses, identify new drug targets, and individualize therapy in a way that reduces both drug cost and prevents unwanted effects. Some of the inherited or acquired variations in drug enzymes, transporters and receptors seen to be clinically relevant in human medicine are outlined in ***Table 1***.

Several recent pharmacogenetic discoveries have proven to be clinically relevant to veterinary patients. The canine population is one of the more ideal settings to explore the genetics of a population. With traceable breed divergence, intensive inbreeding programs, and short time periods between generations, the process of finding the genetic link to a given drug event is quite realistic. Several papers have already reviewed pharmacogenetics in both human and veterinary medicine (2,5). This article will serve to summarize the genetic predictors of adverse drug events of clinical significance that have been identified in dogs.

Multi-drug resistance (MDR-1) mutation and p-glycoprotein

Multiple sources and clinical case reports have now reported the linkage between certain herding breeds and adverse drug events related to antiparasitics and other drugs. Avermectins are a widely used class of drugs used in veterinary medicine to treat internal and external parasites. One specific compound in this class is ivermectin, which works to paralyze invertebrate organisms by activating the GABA (gamma amino butyric) or glutamate gated chloride channels of the peripheral nervous system. Mammals generally have GABA expression within the central nervous system protected by the blood-brain barrier.

Clinically, it has been observed that Collies ***(Figure 1)*** and other related breeds are seen to be more sensitive to the CNS effects of ivermectins revealing clinical signs such as tremors, hypersalivation, coma, depression and ataxia. First described in the 1980's, very small doses (1/100-1/200th of standard) were shown to elicit these profound and acute adverse events in some, but not all, Collies and related breeds. While exploring several possibilities, such as altered protein binding, it was seen that Collies demonstrating adverse events had a higher brain concentration of ivermectin relative to non-sensitive Collies.

The basis of this observation has been shown to be linked to a deletion mutation in the MDR-1 (Multi Drug Resistance 1) gene sequence that results in a series of premature stop codons to be expressed halting formation of almost 90% of the resulting p-glycoprotein amino acid sequence (6). P-glycoprotein, first identified in the mid-1970's, is a 170kDa glycosylated membrane protein that functions to infer intrinsic resistance to a wide variety of drug substances by exporting these

substances out of the body. P-glycoprotein expression can be found in several tissues including the brain, where it works to maintain an integral portion of the blood-brain barrier; the gut, where its location on the brush border of enterocytes, limits the absorption and bioavailability of substrates; on the surface of tumor cells, where it infers multi-drug resistance; and the renal proximal tubules, where its presence accelerates the secretion of substrates into the urine (7).

P-glycoprotein's role in the blood-brain barrier was first shown in MDR-1 knockout mice. Investigations with ivermectin showed that the knockout population was 50-100 times more sensitive to the neurologic effects relative to the wild-type mice. By showing that ivermectin is a substrate for p-glycoprotein, the link between the absence of this protein and the reported adverse events was clear (8). The consequence of alterations in the expression of this protein stands to have great clinical significance due to its wide tissue expression and relative lack of substrate specificity.

The distribution of the MDR-1 mutation among the canine population has been described. Overall, it has been reported that almost 75% of Collies in the US, France and Australia have one mutant allele for the expression of altered p-glycoprotein. Affected breeds are suspected to have similar lineage and include other herding breeds such as Old English Sheepdogs, Australian Shepherds, Shelties, English Shepherds, Border Collies, German Shepherds, Long-haired Whippets, and Silken Windhounds. Case reports in other non-related breeds are less common at this time.

Figure 1. Standard American Collie.

Many drugs, of veterinary clinical significance, have been shown to be substrates of p-glycoprotein ***(Table 2)***. During drug absorption, p-glycoprotein may significantly reduce the oral bioavailability of its substrates. As shown in MDR-1 knockout mice, p-glycoprotein substrate bioavailability is considerably greater than wild-type mice. Investigations to take advantage of this process showed that the oral bioavailability of docetaxel, a substrate, was increased almost 20-fold when administered with a p-glycoprotein inhibitor. As drugs are distributed in the body, p-glycoprotein again plays a role. For those substrates that may cause adverse effects if given entry into the central nervous system, testes, or placenta, breeds suffering from the MDR-1 deletion mutation will be more likely to experience adverse events even at low clinical doses. Animals that are heterozygous for the deletion may not experience adverse events initially or with one dose, but at high or chronic doses, toxicity may still occur.

During drug excretion, p-glycoprotein expression on the renal tubules has altered the clearance of some substrates, particularly chemotherapeutic medications. The concurrent administration of a p-glycoprotein inhibitor to rats decreases the billiary and renal clearance of doxorubicin.

Clearly the implications of the MDR-1 deletion mutation and subsequent expression of p-glycoprotein will stand to gain even more clinical relevance as we are able to understand and test for those animals that have altered expression. Currently, at least one commercial laboratory will analyze samples for canines to provide genotyping information (Washington State University, www.vetmed.wsu.edu/vcpl).

Cytochrome P450 enzymes

Drug metabolism is mediated by several complex systems. While better understood in humans, the CYP450 (cytochrome P450) enzyme system is becoming better delineated in canines. This class of enzymes is responsible for the metabolism of a wide variety of drugs and can be expressed in several locations within the body. It has been shown that these enzymes may be induced or inhibited by certain drugs; they may be over or underexpressed in certain populations; and they may vary as to their expression in certain individuals within a population.

CYP1A2 has been shown to be deficient in 10% of one small population of Beagles (9). While few drugs used clinically in veterinary medicine have been identified to be substrate of this enzyme, future directions may provide greater clinical relevance.

CYP2B11 has up to a 14-fold variation in its activity in dogs of mixed breed pedigree, Greyhounds were shown to have a particularly low activity within the larger canine population. Several drugs including propofol are substrates of CYP2B11 and some evidence that this enzyme may be expressed differently in male and female dogs (10).

There is also some evidence that CYP2D15 may exhibit polymorphism in dogs. In Beagles, approximately half of the population appears to metabolize well celecoxib (a substrate of CYP2D15), while the other half appears to be poor metabolizers. This effect has been shown to increase the half-life of elimination of this drug up to 5-fold (11). While the extrapolation of this finding to other non-steroidals of similar structure, such as deracoxib, has not been shown, further clinical evidence and study may produce more information. Other drugs are shown to be CYP2D15 substrates in humans, although these are used less frequently in veterinary medicine.

As the exploration of the metabolic process of drug metabolism in dogs continues to grow, we may have evidence to show that there are more variants in the metabolic enzymes. As we know more about the source of these mutations, we will likely find that other breeds may be affected and expanded drug classes are involved. Further evidence will likely help us avoid dangerous drug-drug, drug-food, and drug-breed interactions.

Thiopurine S-methyl transferase (TPMT)

Thiopurine S-methyl transferase has been studied in humans and is shown to catalyze the methylation of drugs such as 6-mercaptopurine and azathioprine. Recently, a genetic polymorphism has been identified in dogs that leads to some considerable variation in the expression of this enzyme. Salavaggione, *et al.* (12) found that TPMT levels in the average canine red blood cell (RBC) were similar to those found in human studies. Furthermore, it was found that this drug-metabolizing enzyme has greatly varying levels, even in seemingly similar breed populations, which is also similar to the situation in humans. Their study included 56 different dog breeds and mixed breed subjects. The study first observed the TPMT levels occurring in 145 samples and found the level of activity varied over a 9-fold range. Using information on the sequence and structure of the TPMT gene, the researchers resequenced all of the exons of the canine TPMT gene using DNA from 39 dogs selected based on different levels of RBC TPMT activity. Subsequently, nine polymorphisms were observed. Six of the nine polymorphisms were associated with 67% of the

Table 2.
Substrates of p-glycoprotein (7)

Cytotoxic drugs
Doxorubicin
Vincrisitine
Vinblastine
Cardiac drugs
Digoxin
Immunosuppressives
Cyclosporin
Antiemetic drugs
Ondansetron
Antidiarrheal agents
Loperamide
Antibiotics
Erythromycin
Steroids
Dexamethasone
Hydrocortisone
H_2-blockers
Cimetidine
Ranitidine
Others
Ivermectin
Selamectin
Moxidectin
Milbemycin
Morphine
Phenytoin
Rifampin
Amitriptyline

variation in level of the RBC TPMT activity in the 39 samples. When those 6 single-nucleotide polymorphisms were assayed using DNA from all 145 dogs, 40% of the phenotypic variance could be explained by these polymorphisms.

The clinical application of these observed variations can not be fully elucidated at this time. We know that those patients with low TPMT activity are at much higher risk of developing potentially lethal toxicity when administered thiopurine drugs at standard doses. Likewise, those patients with high enzyme activity are likely to see clinical failure due to under-treatment. It has been observed that Giant Schnauzers have lower TPMT activity, while Alaskan Malamutes had high TPMT activity (13). While the clinical application of this information may not be readily available until standardized testing methods have been established, it is nonetheless important to consider that variations do occur that may predispose some breeds to greater risk of adverse events such as bone marrow suppression, and should be considered when observing the clinical picture.

Sighthounds and anesthesia

The sighthound class has long been a highly domesticated, strictly bred, group of dogs. Predisposing these animals to a myriad of physiological and anatomical anomalies, the physical makeup of these dogs has seemingly caused idiosyncratic reactions to certain classes of drugs. As described above, some of the reactions we see related to sensitivity to anesthetic agents, such as propofol, may likely be related to a cytochrome P450 variance relative to other non-related breeds. We do see other reactions that are more likely the consequence of breeding patterns, exercise pressures and performance demands placed on these animals by humans.

Sighthounds are typically thought to include the Greyhound, Whippet, Borzoi, Irish Wolfhound, Basenji, Saluki and Rhodesian Ridgeback and were bred to hunt based on sight, as opposed to scent. They have been characteristically stream-lined to show similar characteristics of lean body mass, prominent musculature, long limbs and a deep thorax. These breeds tend to have highly stressed demeanors and can be more likely to have stress-related complications when placed in the clinical environment. They have much less body fat than other breeds lending them a greater risk for adverse events from those more lipophilic drugs such as barbiturates that are cleared from the brain to muscle and fat with subsequent elimination by the liver. While anecdotal evidence has shown that not all sighthounds have the same reaction to barbiturates and propofol, these reactions are likely contributed to a combination of factors, including polymorphic gene expression, variable enzyme expression and environmental influences.

Idiosyncratic sulfonamide toxicity

There is a growing body of evidence that supports the theories that pharmacogenetic differences can also potentiate adverse drug reactions that are not related to a drug concentration and are not predictable. These idiosyncratic reactions have been characterized in humans for some time. The sulfonamide antibiotics (sulfamethoxazole, sulfadiazine, sulfadimethoxine) have been shown to cause numerous dose-dependant reactions in dogs such as hematuria, non-regenerative anemia, and an interference with thyroid hormone synthesis. Other adverse reactions have also been seen at therapeutic doses that tend to be more generalized and more typical of delayed immunologic reactions and can even manifest once a short treatment (10 days or less) has been completed. These reactions tend to include signs such as hepatotoxicity, skin eruptions, fever, hemolytic anemia, uveitis, polyarthritis, proteinuria and facial swelling. Work is currently underway in the United States to characterize dogs with these reactions using ELISA for anti-drug antibodies, *in vitro* cytotoxicity assays and other methodologies (14).

Conclusions

We have clear data that helps to verify, on a genetic basis, some of the adverse drug effects we have seen in certain breeds of dogs. The previous approaches of identifying a genetic predisposition to adverse drug events based on phenotype and then searching for the genetic link will soon give way to identifying predisposing genetic factors and modifying drug therapy in anticipation of the events. Several genetic factors that have clinical implication with regard to drug therapy have already been described. Pharmacogenetics provides us with the opportunity to practice, on a clinical level, the best possible drug therapy. We will be able to screen for those animals who may be at risk; test to verify their genotypic

characteristics; and customize drug therapy for the individual rather than the population. With the advances that clear genetic answers provide us, we will be able to identify new and more precise drug targets to allow for drug therapy that minimizes adverse events and maximizes therapeutic benefit. Academic and clinical support for the research efforts in pharmacogenetics and pharmacogenomics stand to benefit the whole of veterinary medicine for years to come.

REFERENCES

1. Roses AD. Pharmacogenetics. *Human molecular genetics* 2001; 10(20), 2261-2267.
2. Mancinelli L, Cronin M, Sadée W. Pharmacogenomics: the promise of personalized medicine. *AAPS PharmSci* 2000; 2(1).
3. Snyder LH. Studies in human inheritance. *Ohio J Sci* 1932; 32: 436-468.
4. Carsen PE, Flanagan CL, Iokes CE, *et al.* Enzymatic deficiency in primaquine-sensitive erythrocytes. *Science* 1956; 124: 484-485.
5. Mealey KL. Pharmacogenetics. The Veterinary clinics of North America. *Small animal practice* 2006; 36(5): 961-973.
6. Neff MW, Robertson KR, Wong AK, *et al.* Breed distribution and history of canine mdr1-1Delta, a pharmacogenetic mutation that marks the emergence of breeds from the collie lineage. *Proceedings of the National Academy of Sciences of the United States of America* 2004; 101(32): 11725-11730.
7. Sakaeda T, Nakamura T, Okumura K. MDR1 genotype-related pharmacokinetics and pharmacodynamics. Biological & pharmaceutical bulletin 2002; 25(11): 1391-1400. Plumb, D. Veterinary Drug Handbook. 5th Edition. Iowa State Press 2005.
8. Lankas GR, Cartwright ME, Umbenhaur D. P-glycoprotein deficiency in a subpopulation of CF-1 mice enhances avermectin-induced neurotoxicity. *Toxicol Appl Pharm* 1997; 143: 357-365.
9. Tenmizu D, Endo Y, Naguchi K, *et al.* Identification of the novel canine CYP1A2 1117 SNP causing protein deletion. *Xenobiotica* 2004; 34: 835-846.
10. Hay Krauss BL, Greenblatt DJ, Venkatakrishnan K, *et al.* Evidence for propofol hydroxylation by cytochrome P4502B11 in canine liver microsomes: breed and gender differences. *Xenobiotica* 2000; 30(6): 575-588.
11. Paulson SK, Reitz B, Engel L, et al. Evidence for polymorphism in the canine metabolism of the cyclooxygenase 2 inhibitor celecoxib. *Drug Metab Dispos* 1999; 27(10): 1293-1299.
12. Salavaggione OE, Kidd L, Prondzinski JL, *et al.* Canine red blood cell thiopurine S-methyltransferase: companion animal pharmacogenetics. *Pharmacogenetics* 2002; 12(9): 713-724.
13. Kidd LB, Salavaggione OE, Szumlanski, *et al.* Thiopurine methyltransferase activity in the red blood cells of dogs. *J Vet Int Med* 2004; 18(2): 214-218.
14. Trepanier LA. Idiosyncratic toxicity associated with potentiated sulfonamides in the dog. *J Vet Pharmacol Therap* 2004; (27): 129-138.