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CANINE HEALTH FOUNDATION
PREVENT TREAT & CURE

RESEARCH PROGRESS REPORT SUMMARY

Grant 02242: Understanding the Genetics of Adverse Drug Reactions in Sighthounds

Principal Investigator: Michael Court, BVSc, PhD

Research Institution: Washington State University

Grant Amount: $149,944.15

Start Date: 2/1/2016 **End Date:** 7/31/2018

Progress Report: FINAL

Report Due: 7/31/2018 **Report Received:** 8/8/2018

(The content of this report is not confidential and may be used in communications with your organization.)

Original Project Description:

Life-threatening unanticipated reactions to drugs with a narrow margin of safety (such as those used for anesthesia and to treat cancer) are a common concern for dog owners and veterinarians. However, research conducted at Washington State University has enabled development of a simple cheek swab test (the MDR1 gene test) that is now being used by veterinarians to identify dogs that should either avoid or have reduced doses of certain drugs used to treat cancer and parasite infections. Using a similar strategy the investigators have been conducting research to identify the cause of extremely slow recovery from anesthesia (up to several days) in a high proportion of greyhounds, and also in other sighthound breed dogs (such as Scottish deerhound, Borzoi, Whippets, etc.). The investigators have recently discovered a mutation in a gene that is known to be essential for metabolism (breaking down) many commonly used anesthetic drugs (such as propofol), as well as many other drugs used in dogs. Interestingly in addition to sighthound breeds, this gene mutation is also found in some other breeds such as Border Collies. The purpose of this research project is to prove that this mutation can cause decreased drug metabolism, while also determining which drugs and which dog breeds are likely to be most impacted. The ultimate goal of this study is to develop a genetic test that could be used by veterinarians to guide the safe use of these drugs in dogs with the gene mutation.

Publications:

Court MH, Martinez SE. (2018) Drug metabolism polymorphism in Greyhounds: Identification and functional characterization of cytochrome P450 2B11 gene variants. Manuscript draft prepared for submission to PLoS One.

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Martinez SE, Pandey AV, Court MH. (2018). Drug metabolism polymorphism in Greyhounds: Identification and characterization of P450 oxidoreductase variants and their effects on CYP2B11 and CYP2D15 function. Manuscript draft prepared for submission to PLoS One.

Presentations:

Court, MH. "Sighthound Pharmacogenetics Research at WSU". Invited presentation to the Annual National Meeting of the Borzoi Club of America. Richland, WA, May 23, 2018.

Martinez SE, Pandey AV, Court MH. "Implications of canine cytochrome P450 oxidoreductase genetic polymorphisms in drug metabolism". American Academy of Veterinary Pharmacology and Therapeutics 20th Biennial Symposium, Potomac, MD, USA. May 21-24, 2017.

Court, MH. "Drug metabolism polymorphisms in dogs and cats". American Academy of Veterinary Pharmacology and Therapeutics 20th Biennial Symposium Potomac, MD, USA. May 21-24, 2017.

Court, MH. "Good Dog, Bad Genes: Pharmacogenomics of breed-related adverse drug reactions". Invited presentation to the Genetics Program, School of Veterinary Medicine, University of California, Davis, CA, USA. May 15, 2017.

Report to Grant Sponsor from Investigator:

Idiosyncratic drug reactions, including extremely slow recovery from anesthesia, have been found with significant frequency in Greyhounds as well as other sighthound breeds. Our laboratory discovered two mutations in a gene that encodes for an enzyme, called P450 oxidoreductase (POR), which is essential for the metabolism (breaking down) of many drugs used in dogs, including anesthetics. These two mutations in the gene lead to three different mutant POR enzymes being made, called 2, 3 and 4 (1 is the normal enzyme). POR partners with other enzymes responsible for drug metabolism, called P450 enzymes, and this partnership is required for the P450 enzymes to work. We found that the POR mutant 3 was quite common in Greyhounds, Scottish Deerhounds and other sighthound breeds, as well as in some other breeds including Border Collies. POR mutant 2 was found primarily in Rottweilers, Doberman Pinschers and Chow Chows. POR mutant 4 was the rarest of the mutations and so far we have only found it in Greyhounds and Scottish Deerhounds.

We predicted that these mutant POR enzymes would affect some but not all of the drug metabolizing P450 enzymes it partners with based on preliminary research in our laboratory as well as previously published scientific articles. To determine what effect, if any, POR mutations had on the enzyme's function as well as the effects the mutations may have when partnered with two drug metabolizing P450 enzymes, we used an insect cell system and programed the cells to express these dog proteins.

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Our first set of studies examined the POR enzyme function on its own. Results showed that the mutations did not significantly affect the enzyme's basic functions compared to the normal enzyme. This indicated to us that the mutations did not cause a total loss of enzyme function. Our second set of studies partnered the enzyme with a P450 drug metabolizing enzyme responsible for the metabolism of many anesthetic drugs. We looked at how efficiently the enzyme partnership was able to break down three different drugs, including the anesthetic, propofol. We found that the POR mutant 3 and mutant 4 caused the three drugs to be metabolized 37% and 69% slower, respectively compared to the normal POR enzyme. In the third set of studies, we partnered the POR enzyme with another P450 drug metabolizing enzyme that is responsible for the metabolism of many commonly used veterinary drugs, including tramadol, and we again looked at how efficiently the enzyme partnership was able to break down two different drugs. We did not find any difference between the mutant POR enzymes and the normal enzyme with this P450 partnership in the speed of drug break down.

We concluded that the POR mutant 3 and mutant 4 enzyme effects were dependent on which drug metabolizing P450 enzyme they were partnered with. We only partnered the POR mutants with two P450 enzymes but there are many more P450 enzymes involved in drug metabolism that POR partners with. It is important to determine if the POR mutants affect the drug metabolizing activities of these other P450 enzymes too. While the POR mutant 2 did not seem to affect the drug metabolizing activities of the two P450 enzyme partners we examined, we cannot conclude that the mutation has not effect on all of the partner enzymes and this mutation should continue to be studied.

We saw a significant decrease in drug metabolism activity in the partnership of the POR mutant 3 and 4 with the P450 drug metabolizing enzyme involved in many anesthetic drugs. Given the frequency of the mutant 3 in sighthound breeds, it is possible that this mutation, along with the less common mutant 4, may be the causes of slow recovery from anesthesia along with other idiosyncratic drug reactions. However; prior to recommending genetic testing for these mutations to veterinarians and owners, it is essential that we be able to prove that these mutations can cause decreased drug metabolism in dogs. Thanks to the generous support of the AKC-CHF, Borzoi Club of America, Chow Chow Club and Saluki Health Research, we look forward to being able to do just that with Grant 02529: Understanding the Genetics of Adverse Drug Reactions in Sighthounds: Phase II.