RESEARCH PROGRESS REPORT SUMMARY

Grant 01601: Identifying the Genetic Cause of Fatal Neonatal Liver Disease

Principal Investigator: Dr. Peter A.J. Leegwater, PhD

Research Institution: University of Utrecht

Grant Amount: $66,042.00

Start Date: 1/1/2012 **End Date:** 6/30/2015

Progress Report: Mid-Year 4 (FINAL)

Report Due: 6/30/2015 **Report Received:** 5/8/2015

Recommended for Approval: Approved

(Content of this report is not confidential. A grant sponsor's CHF Health Liaison may request the confidential scientific report submitted by the investigator by contacting the CHF office. The below Report to Grant Sponsors from Investigator can be used in communications with your club members.)

Original Project Description:

Intrahepatic portosystemic shunt (IHPSS) is a liver disease caused by patency of the embryonal ductus venosus. The ductus venosus ensures blood to flow from the placenta to vital organs like the lungs and heart without traversing the liver. This vessel should close within a few days after birth so that the liver is no longer bypassed by the vital portal blood flow. IHPSS leads to underdevelopment of the liver and severe (finally lethal) liver dysfunction. The condition is common in many large dog breeds and occurs rarely in man. Based on studies in Irish wolfhounds IHPSS is hereditary. Pedigree analysis in Irish Wolfhounds suggests that two genes act in concert to cause IHPSS. A large series of DNA samples from phenothypially characterized cases and controls are available which permits genetic analysis with the required statistical power. By comparing DNA variants between affected and healthy Irish wolfhounds we will be able to select genomic regions which harbor the genes responsible for this severe liver disorder. The responsible mutations will then be identified by large scale DNA sequence analysis of these regions. Once gene mutations have been proven to cause IHPSS a DNA test can be developed to assist breeders in reducing the incidence the disorder. In addition, with the available DNA of many large breed dogs diagnosed with IHPSS we can immediately extrapolate findings to other large dog breeds.

Grant Objectives:

The objective of the project is to elucidate the gene mutations that cause the phenotype of IHPSS in Irish wolfhounds. Based on previous research we hypothesize that two genes act in concert causing IHPSS. By combining high-throughput genotyping with sophisticated bioinformatic analysis tools we will test this hypothesis. The associated genome regions will be analyzed by large scale DNA sequencing to identify the underlying causative genes. This will enable the development of a test which will aid in breeding strategies in order to prevent the disease.

Publications:

- Van Steenbeek FG, van den Bossche L, Leegwater PA, Rothuizen J.; Inherited liver shunts in dogs elucidate pathways regulating embryonic development and clinical disorders of the portal vein.; Mamm Genome. 2012 Feb;23(1-2):76-84.

- Van Steenbeek FG, Spee B, Penning LC, Kummeling A, Van Gils HM, Grinwis GCM, Van Leenen D, Holstege FCP, Vos-Loohuis M, Rothuizen J, Leegwater PAJ (2013) Altered subcellular localization of Heat Shock Protein 90 is associated with impaired expression of the Aryl Hydrocarbon Receptor pathway in dogs. ; Plos One, Accepted for publication.

- Van Steenbeek FG, Van den Bossche L, Grinwis GCM, Kummeling A, Van Gils IHM, Groot Koerkamp MJA, Van Leenen D, Holstege FCP, Penning LC, Rothuizen J, Leegwater PAJ, Spee B (2013) Aberrant gene expression in dogs with portosystemic shunts. ; Plos One, Accepted for publication.

Report to Grant Sponsor from Investigator:

The DNA samples of 39 Irish wolfhounds with a shunt and 71 controls that had been collected were used for DNA typing. The results from 170,000 positions on the DNA have been obtained and we ran several analyses on these data. Two regions displayed association with the occurrence of a shunt. We selected these two chromosomal regions for large scale DNA sequencing in cases and controls. This resulted in a large amount of interesting DNA variants of which we selected 14 for follow up in Irish wolfhounds (37 cases, 86 controls), Hovawarts (11 cases, 14 controls) and Bernese mountain dogs (14 cases, 14 controls). Four of these variants were significantly associated with IHPSS in Irish wolfhounds. We continuously receive samples of Irish wolfhounds from across Europe. We will use these for additional validation of the results. In the near future we will determine the relevance of these findings by introducing the mutations in cultured cells that mimic the properties of veins.