RESEARCH PROGRESS REPORT SUMMARY

Grant 01771: Defining the Unique Genetic Markers in Dogs That Define Immune Function, Disease Resistance and Tissue Transplantation

Principal Investigator: Dr. Aravind Ramakrishnan, MD

Research Institution: Fred Hutchinson Cancer Research Center

Grant Amount: $178,200.00

Start Date: 1/1/2013 **End Date:** 12/31/2014

Progress Report: Mid-Year 2

Report Due: 6/30/2014 **Report Received:** 6/24/2014

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:

The Major Histocompatibility Complex (MHC) is a region of the genome that contains genes that code for a group of cell surface proteins known as Dog leukocyte antigens (DLA). DLA play important roles in the immune system including the recognition of self as well as recognition of foreign pathogens such as bacteria and viruses. The MHC genes are highly polymorphic and each gene has many different forms or alleles. Matching of MHC alleles between the donor and recipient is important for the success of stem cell and tissue transplants. Specific assortments of MHC alleles or haplotypes have been associated with an increased risk for the development of diabetes and auto immune diseases in humans. Knowledge of these associations has been valuable in understanding disease mechanisms. Recently we have developed improved methods for identifying the different forms of the DLA genes, in a large number of dogs of diverse breeds. In this application, we propose to characterize haplotypes, in over 1200 dogs from at least 50 breeds using a high throughput sequencing strategy. The distribution and frequency of different forms of each of these genes and their specific clustering among different breeds will greatly enhance our knowledge of the genetic diversity among breeds. The methodology and data gained from this study will enhance the power of association studies between MHC types and canine diseases. Such a database will also enable tissue transplantation from unrelated but matched donors as a treatment for advanced malignancies and other diseases, among dogs of most breeds.

Grant Objectives:

The goal of the project is to construct haplotypes of DLA alleles from about 1200 dogs of about 50 AKC pure breeds. The methodology and data gained from this study will identify the level of diversity of DLA alleles in different breeds and enhance the power of association studies between DLA haplotypes and canine diseases. Such a database will also enable tissue transplantation from unrelated but matched donors as a treatment for advanced malignancies and other diseases, among dogs of most breeds.

Publications:

- Venkataraman GM, Geraghty D, Fox J, Graves SS, Zellmer E, Storer BE, Torok-Storb BJ, Storb R. Canine DLA-79 gene: an improved typing method, identification of new alleles and its role in graft rejection and graft-versus-host disease. Tissue Antigens. 2013 Apr; 81(4):204-11. PubMed PMID: 23510416; PubMed Central PMCID: PMC3605710.

- Tsai KL, Starr-Moss AN, Venkataraman GM, Robinson C, Kennedy LJ, Steiner JM, Clark LA. Alleles of the major histocompatibility complex play a role in the pathogenesis of pancreatic acinar atrophy in dogs. Immunogenetics. 2013 Jul; 65(7):501-9. Epub 2013 Apr 21. PubMed PMID: 23604463.

Report to Grant Sponsor from Investigator:

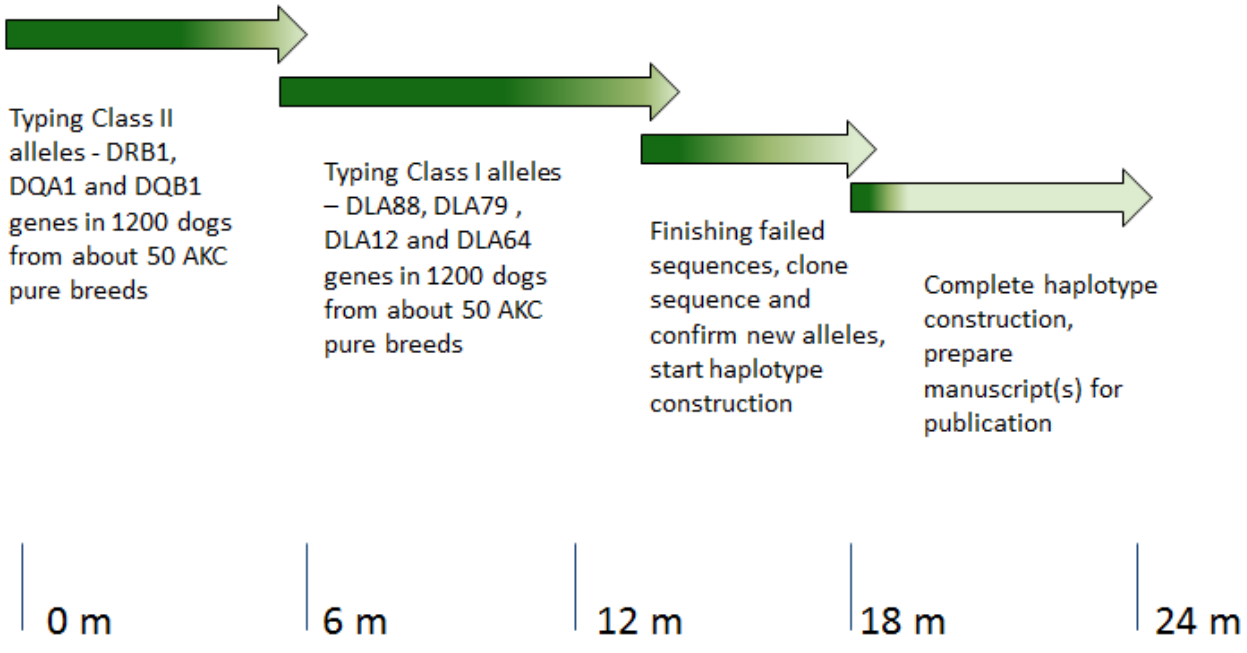
The Major Histocompatibility Complex (MHC) is a region of the genome in dogs that contains genes that encode a group of cell surface proteins known as Dog leukocyte antigens (DLA). DLA play important roles in the immune system including the recognition of self as well as recognition of foreign pathogens such as bacteria and viruses. The MHC genes are highly polymorphic i.e., each gene has many different forms or alleles. Matching of these MHC alleles between the donor and recipient is important for the success of treatments such as stem cell and organ transplants. Specific assortments of MHC alleles, comparable to 7 beads of different colors on a string, constitute a haplotype. Recently we have developed improved methods for identifying variants of the DLA genes, in a large number of dogs of diverse breeds. In this project, we proposed to characterize haplotypes, in over 1200 dogs from 50 pure AKC breeds using DNA sequencing.

The goal of the project is to construct haplotypes of DLA alleles from about 1200 dogs of about 50 AKC pure breeds. This can be divided into 4 major tasks.

1. Typing the 4 polymorphic class I genes
2. Typing the 3 polymorphic class II genes
3. Cloning and/or confirming new and ambiguous alleles for all classI and II genes.
4. Analyzing and deriving haplotype compositions.

Aim 1 is about 90 % complete and Aim 2 is also about 90 % complete. Work on Aim 3 is in progress. Work on Aim 4 will commence shortly. Allele identities have been established for all the three class II genes, viz., DRB-1, DQA-1 and DQB-1 in over 900 dogs. Many new alleles have been found for all three genes. Allele identities have been established for two of the 4 class I genes, viz., DLA88, and DLA79 in over 900 dogs. Many new alleles have been found for both of these genes. Typing DLA64 and DLA12 genes is nearing completion. Haplotyping analysis will commence shortly.

Specific haplotypes have been associated with an increased risk for the development of diabetes and auto immune diseases in humans. Knowledge of these associations has been valuable in understanding disease mechanisms. The distribution and frequency of different forms of each of these genes and their groupings among different breeds will greatly enhance our knowledge of the genetic diversity among breeds. The methodology and data gained from this study will enhance the power of association studies between MHC types and canine diseases. For example, in collaboration with Dr. Leigh Anne Clark's group at the Clemson University in South Carolina we have identified an association of a specific DLA class I allele with pancreatic acinar atrophy in the German Shepherd Dog. Such a database will also immediately enable tissue transplantation from unrelated but matched donors as a treatment for advanced malignancies and other diseases, among the dogs of most breeds.



We have achieved about 90 percent of the stated goals for this time, though we have changed our strategy. Work is being done in batch mode for logistics and cost efficiencies. Work is progressing very well and we do not anticipate any issues that will affect the successful completion of this project in time.