RESEARCH PROGRESS REPORT SUMMARY

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

Principal Investigator: Beverly Torok-Storb, PhD; Michael Harkey, PhD

Research Institution: Fred Hutchinson Cancer Research Center

Grant Amount: $178,196.82

Start Date: 1/1/2013 **End Date:** 6/30/2018

Progress Report: FINAL

Report Due: 6/30/2018 **Report Received:** 7/10/2018

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Original Project Description:

The Major Histocompatibility Complex (MHC) genes encode proteins that are critical for a wide range of biological functions, from immune protection against infectious disease to the predisposition of an individual to develop diabetes and auto immune diseases. The MHC genes in the dog are incompletely characterized, thereby severely limiting our ability to full define the cause of many canine diseases. Dr. Ramakrishnan has developed improved methods for identifying the different forms of canine MHC genes in a large number of dogs of diverse breeds. In this study he will characterize the patterns of MHC genetic variation 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 (stem cell transplants) and other diseases (tissue transplantation). Fully defining the canine MHC will have broad impact across canine health, including oncology, immunology and infectious disease.

Publications:

Three related manuscripts[1,2,3] have been published recently. A manuscript entitled “Gastric Dilatation-Volvulus is Strongly Associated with Specific Alleles of Three Canine Immune-System Genes: DLA-88, DLA-DRB1 and TLR5” deals with Bloat disease in Great Danes and identifies potential candidate DLA antigens in its etiology. Incidentally, 3 new DLA-88 alleles were identified as part of this work. This

paper is published, at the American Journal of Veterinary Research. A companion paper entitled, "The Canine Gut Microbiome is Associated with Higher Risk of Gastric Dilatation-Volvulus and High Risk Genetic Variants of the Immune System" is focused on changes in the gut microbiome that may influence bloat. This paper is published in PLoS One. A third manuscript entitled "Thirteen novel canine DLA-88 alleles identified by sequence-based typing" primarily deals with characterizing new DLA-88 alleles from a subset of dogs from our study and reports 13 new alleles. This paper is published at the Journal, HLA. We anticipate publishing a fourth paper within the next 3-4 months that describes the new DLA88 typing method and the new DLA88 alleles, identified in the 288 dogs. Two papers related to this project have been published earlier[3, 4], during the Grant period, as previously reported. One deals with developing a new method for typing DLA-79 alleles, identification of new alleles and the role of DLA-79 alleles in transplantation. The second paper, as collaboration with Dr. Leigh Anne Clark's group at the Clemson University in South Carolina, deals with association of DLA class I and II alleles with pancreatic acinar atrophy in the German Shepherd Dog. A presentation by our collaborator that relates to canine endocrine insufficiency and DLA alleles in German Shepherd Dogs and Pembroke Welsh Corgi was presented at the Canine/Feline International Genomics conference at Cambridge, MA Sept. 2013.

1. Gopalakrishnan M. Venkataraman, Lorna J. Kennedy, Marie-Térèse E. Little, Scott S. Graves, Beverly J. Torok-Storb, and Rainer Storb. Thirteen novel canine DLA-88 alleles identified by sequence-based typing. HLA HLA. 2017 Sep;90(3):165-170)

2. Michael A. Harkey , Alexandra M. Villagran, Gopalakrishnan M. Venkataraman, Wendy M. Leisenring, Meredith A. Hullar, Beverly J. Torok-Storb. Gastric Dilatation-Volvulus is Strongly Associated with Specific Alleles of Three Canine Immune-System Genes: DLA-88, DLA-DRB1 and TLR5. Am J Vet Res. 2017 Aug;78(8):934-945.

3. Meredith A. J. Hullar, Johanna W. Lampe, Beverly J. Torok-Storb, Michael A. Harkey. The Canine Gut Microbiome is Associated with Higher Risk of Gastric Dilatation-Volvulus and High Risk Genetic Variants of the Immune System. PLoS One. 2018 Jun 11;13(6).

4: 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.

5: 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.

Presentations:

Investigation of the role of the major histocompatibility complex in canine exocrine pancreatic insufficiency.

Tsai KL, Evans JM, Starr-Moss AN, Venkataraman GM, Kennedy LJ, Steiner JM and Clark LA. 7th International Conference on Advances in Canine and Feline Genomics and Inherited Diseases. Cambridge, MA, September 23 -- 27, 2013.

Report to Grant Sponsor from Investigator:

The stated goal of the project is to construct haplotypes of DLA alleles from about 1200 dogs representing approximately 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 we can achieve in our 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.

As of 6/29/17, our goals shifted abruptly, since the primary researcher in this study, Gopalakrishnan Venkataraman (GK), left the lab. GK had generated an enormous data set, and had discovered a large number of new DLA88 alleles. But about 300 dogs, recruited for the study, had not yet been examined. Dr. Michael Harkey, who had worked with GK, and runs a Core that specializes in DLA typing, had agreed to take over this study over the last 6 months. He has been working with Dr. Dan Geraghty, an expert in HLA typing in humans, to develop a rapid, low-cost method of DLA typing.

During the tenure of this study a new sequencing technology has developed and matured to such a level that it now more cost effective, more accurate, and orders of magnitude faster than the methods we have been using. This is the so-called “Next Gen Sequencing” (NGS), which involves simultaneous sequencing of up to 1.5 million templates and automated computer analysis of the results in an over-night run. Since this method is clonal (single molecule based), it reads each allele separately from each gene, and eliminates the ambiguity of conventional sequence reads that average two different alleles. Dr. Geraghty has developed an HLA typing service based on this technology (Nelson et al., 2015. “An integrated genotyping approach for HLA and other complex genetic systems”. Human Immunology, 12:928-938.

We proposed that the best use of the remaining $25,000 in the grant would be to develop and test a similar high throughput method for DLA88 in dogs, to apply the method to type the remaining 288 dogs, and to identify any new DLA88 alleles from this effort. A six-month no-cost extension was granted to complete this effort.

Specific Aims:

Our goals for this part of the study were to:

1. Develop a Next Gen Sequencing approach for DLA88 typing. 100% complete
2. Identify novel alleles of DLA88. 100% complete
3. Validate the new method with established methods. 90% complete

We have completed the Next Gen Sequence analysis of 288 dogs, and identified 26 novel alleles that met our initial selection criteria. We are now in the process of validating the data by conventional Sanger Sequencing. So far, 17 candidate novel alleles have been confirmed. So we believe that we have developed a faster, cheaper approach to DLA typing, and that most of the 26 candidate novel alleles of DLA88 will prove to be real. The new DLA88 alleles will be a significant contribution to the current canine MHC database.

More importantly, the proof-of principle work on NGS-based DLA typing sets the stage for rapid, inexpensive DLA typing. In the context of the growing understanding of the many roles MHC genes play in health and disease, this approach can facilitate large-scale studies of the impact of MHC genotypes on diseases. In addition, these are the genes that must be matched to facilitate a tissue or organ transplant. A fast, accurate, inexpensive method of DLA typing will accelerate the research, and provide more affordable clinical data for transplantation. It is our hope that further investment will be made in developing a complete NGS-based DLA genotyping capability in the near future.

We are now in the process of final verification of the last samples, and have begun preparing a manuscript for publication in HLA. No further funding is required to complete this plan.