Research Support History for Pug Dog Club of America, Inc.

October 2, 2023

Agency	Grant and Research Program Area	Fund	Commitment Date	Support Amount	Progress/Publications
AKC CHF	*02791-A: Neurofilament light chain concentrations in dogs with meningoencephalitis* Dr. Christopher L Mariani, DVM, PhD; North Carolina State University Program Area: Neurology Project Dates: 2/1/2020 to 7/31/2022; Grant Amount: $9,473.00	DAF	12/19/20	$3000.00	Meningoencephalitis of unknown etiology (MUE) is a common and devastating disorder that is most prevalent in small and toy breed dogs such as Pugs, Maltese and Chihuahuas. Although dogs frequently respond to anti-inflammatory or immunosuppressive therapy, many dogs suffer relapses or worsen in the face of such therapy, and this condition is ultimately fatal in most cases. Currently available diagnostic tests including magnetic resonance imaging (MRI) and spinal fluid (CSF) analysis are necessary to make a diagnosis of MUE but are not helpful in predicting the course of disease or likelihood of survival. In addition, these tests are expensive and their role in monitoring the response to therapy is uncertain. There is a critical need for novel biomarkers that will help predict responses to therapy and to monitor ongoing therapy, ideally using a blood sample. Neurofilament light chain (NF-L) is a protein found in neurons and released into the CSF and blood after injury to the central nervous system. NF-L has emerged as a promising biomarker of brain inflammation in humans, largely due to the development of a sensitive assay that can detect very small concentrations of this protein. This study will measure NF-L within the CSF and serum of dogs with MUE and compare these concentrations with control samples. The investigators will evaluate the utility of NF-L to predict patient response to therapy and prognosis. Neurofilament Light Chain Concentrations in Dogs with Meningoencephalomyelitis 2022 ACVIM Hybrid Forum **Christopher L. Mariani**[1]; et al., [1]NC State University; NF-L concentrations were significantly higher in meningoencephalomyelitis dogs than in healthy controls for both serum and CSF. However, matched serum and CSF concentrations in meningoencephalomyelitis dogs were not well correlated. We found no differences in CSF or serum concentrations between dogs that did or did not survive to hospital discharge or between those surviving greater than or less than 3 months. Finally, we found no differences in CSF or serum concentrations between dogs that did or did not have persistent neurological deficits after treatment. Conclusion: Dogs with meningoencephalomyelitis have markedly elevated CSF and blood concentrations of NF-L compared to healthy controls. NF-L was not associated with outcomes, although further study is required to adequately answer these questions.

Agency	Grant and Research Program Area	Fund	Commitment Date	Support Amount	Progress/Publications
AKC CHF	*03077-A: Cooling strategies during exertional hyperthermia* Dr. Cynthia M. Otto, DVM, PhD; Trustees of the University of Pennsylvania Program Area: General Canine Health Project Dates: 10/1/2022 to 3/31/2024; Grant Amount: $15,836.04	Cash	1/24/23	$ 500.00	One of the greatest risks for working and sporting dogs is heat injury. Exposure to high temperature environments or generation of heat through exercise can lead to a progression from hyperthermia (increased core temperature), to heat stress (initial physiologic response to increased core temperature), to heat injury (changes in physiologic function, mild-moderate organ damage) and ultimately to heat stroke (heat injury with neurologic signs and organ damage). To eliminate heat, dogs are unable to sweat, except through their paw pads. A dog's core temperature can reach over 105°F during normal exercise. Although this body temperature does not result in heat injury in most conditioned dogs, it does put dogs at risk of heat injury/heat stroke if activity continues or heat dissipation is compromised by the dog's own physiology or the environmental conditions. Dog owners/handlers are taught to recognize the signs of heat stress and respond by stopping activity and cooling the dog. One of the classic cooling methods recommended in veterinary literature is to apply isopropyl alcohol to the paw pads to enhance evaporative cooling. Alternatively, submersion of the dog in cool water has been suggested. No study has ever evaluated the comparative cooling efficacy of these two methods. This cross-over study will compare the core temperature response to each active cooling method to no active cooling in 10 conditioned dogs with exertional heat stress following sprint intervals for 10 min or peak temperature of 105°F. Results will provide important guidance for the health of exercising dogs.
AKC CHF	*02772: Identifying early stage ultra-rare mutations as predictive biomarkers of lymphoma in high-risk versus low risk breeds* Dr. Daniel E L Promislow, PhD; University of Washington Program Area: Oncology - Lymphoma Project Dates: 3/1/2020 to 6/30/2022; Grant Amount: $75,600.00	DAF	12/14/21	$1000.00	The most common type of cancer in dogs is lymphoma, with ~80,000 cases diagnosed annually in the United States. Breeds vary in their risk of lymphoma, but it is unclear why there is variation despite considerable effort to identify the genetics of cancer risk and progression in dogs. Cancer typically arises from the accumulation of non-inherited (i.e. somatic) mutations. However, variation among breeds in cancer risk could be due to breed-specific variation in the types of mutations, the rate of accumulation of mutations, or the downstream effects of mutations in healthy dogs. This study will use novel sequencing technology to test the hypothesis that breed-specific lymphoma risk is due to variation in the frequency and type of rare precancerous mutations. Normally, measuring these low-frequency mutations has been beyond the range of standard sequencing technology, which is limited to detecting mutations present in >1% of cells. The new technology applied here represents a >10,000-fold improvement in accuracy, enabling the investigators to accurately detect a precancerous mutation present at a single site at a frequency of just one out of every 10 million DNA base

Agency	Grant and Research Program Area	Fund	Commitment Date	Support Amount	Progress/Publications
					pairs. By determining if mutation frequency in blood of healthy high-risk and low-risk dogs can predict lymphoma risk, this work could lead to the development of novel tests for the early diagnosis and prognosis of canine lymphoma. This work has the potential to shed light on the mechanisms that underlie breed-specific variation in lymphoma risk, and in the long term, could lead to the development of novel tests for the early diagnosis and prognosis of canine lymphoma.
AKC CHF	*02966: Corneal cross-linking - using light to save sight.* Dr. Simon Anton Pot, DVM; University of Zurich Program Area: Ophthalmology Project Dates: 2/1/2022 to 7/31/2024; Grant Amount: $30,000.00	Cash	12/14/21 1/24/23	$2,000.00 $2,000.00	Brachycephalic dog breeds are particularly susceptible to the development of corneal ulcers but all dogs can be affected. Corneal ulcer treatment aims to eliminate bacteria, stop the melting process, and allow normal healing to resume. First-line treatment involves medical therapy by frequent application of antibiotic and enzyme inhibitor eyedrops, as well as pain relief. Treatment success varies, and antibiotic resistance remains a concerning issue. If intensive medical therapy does not achieve this goal, surgery is often indicated. Corneal cross-linking (CXL) was introduced in human medicine to increase tissue strength in weakened areas of the cornea and is used as corneal ulcer treatment to resist enzymatic digestion. CXL also effectively kills both antibiotic-resistant and sensitive bacteria. The 15-minute CXL procedure involves the application of Riboflavin (Vitamin B2) drops onto the cornea and illumination with ultra-violet (UV) light. Despite evidence suggesting that CXL helps heal patients with corneal ulcers, it is not clear that CXL works better than or equally well as existing medical therapy in dogs. In this study, 10 animal hospitals have joined efforts to launch a clinical trial to determine whether CXL will allow canine patients to heal more quickly and with a lower risk of deterioration compared to state-of-the-art medical therapy The "Corneal cross-linking- using light to save sight" study is progressing. So far, 41 dogs have been enrolled into this multicenter prospective clinical trial. As soon as 90 dogs have been recruited, the collected data will be unmasked, analyzed, and reviewed by a panel of independent specialists, who will offer recommendations regarding study continuation. We organize regular online discussion sessions for the cooperating veterinary ophthalmology clinics to facilitate information flow, patient enrollment, and adherence to the study protocol. Kowalska M, Hafezi F, Pot SA, Hartnack S. Medical management versus PACK-CXL in dogs with infectious keratitis: a randomized controlled trial protocol. Animals MDPI. 2022. 12(20), 2862. doi.org/10.3390/ani12202862 .

Agency	Grant and Research Program Area	Fund	Commitment Date	Support Amount	Progress/Publications
AKC CHF	*02502: Precision Medicine for Canine Lymphoma* Dr. Nicola J Mason, BVetMed, PhD; Trustees of the University of Pennsylvania Program Area: Oncology - Lymphoma Project Dates: 3/1/2018 to 2/28/2022; Grant Amount: $72,077.64	Cash Cash DAF	12/14/21 12/19/20 12/19/20	$1,000.00 $1,000.00 $1,000.00	Wang, G., Wu, M., Durham, A. C., Mason, N. J., & Roth, D. B. (2021). Canine Oncopanel: A capture-based, NGS platform for evaluating the mutational landscape and detecting putative driver mutations in canine cancers. *Veterinary and Comparative Oncology*. https://doi.org/10.1111/vco.12746
AKC CHF	*02589: Genetic Basis of Canine Spinal Abnormalities* Dr. Kari J. Ekenstedt, DVM, PhD; Purdue University Program Area: Neurology Project Dates: 4/1/2019 to 3/31/2021; Grant Amount: $112,993.00	Cash	4/2/2019 12/19/20 12/14/2021	$5,000.00 $2,500.00 $2,000.00	This study will identify potential genes and risk alleles to better understand the genetic basis of canine spinal abnormalities using comparisons between affected and unaffected dogs. The identification of these genes and risk alleles will advance knowledge with an ultimate goal to develop genetic tests and/or a genetic risk model to help predict healthy spines for good health in breeds with tightly curled tails such as Pugs, French Bulldogs, English Bulldogs, Boston Terriers and Basenjis. Publications: Paper in preparation (note: this is parallel work, not technically supported by this AKC OAK, but used in the preliminary data of the grant proposal): Dreger DL, Cook S, Lim CK, Friedenberg S, McCue ME, Conzemius MG, and Ekenstedt KJ. Genetic causes of "short spine" in dogs mirror human vertebral dysplasias. Submission planned to Nature. Submission anticipated early winter 2022/2023. **Presentations:** Bhowmik N and Ekenstedt KJ. "Investigating Selective Sweeps Associated with Brachycephaly and Screw Tail in Brachycephalic Dogs: A Proof-of-Concept Study with Runs of Homozygosity Islands". 2022 Purdue Veterinary Medicine Research Day, Purdue University, West Lafayette, IN. • We plan to submit an updated version of this work to the 11th International Conference on Canine and Feline Genetics and Genomics, which has very recently been pushed back again, to fall (October 2022), because of the pandemic. We may also submit an abstract to the 2022 ICEOS meeting (International Congress on Early Onset Scoliosis), which will be held in November 2022. Bhowmik N and Ekenstedt KJ. "Investigating Selective Sweeps Associated with Brachycephaly and Screw Tail in Brachycephalic Dogs: A Proof-of-Concept Study with Runs of Homozygosity Islands". 2022 Purdue Veterinary Medicine Research Day, Purdue University, West Lafayette, IN. **Report to Grant Sponsor from Investigator:** In total, to date we have recruited 206 samples for the first aim of this grant, and to access data from more dogs of other studies, brought our sample total to 664 dogs. All dogs are purebred dogs of any age/any health status from Pugs, Boston Terriers, French Bulldogs, Bulldogs, and Basenjis. We always take samples from any of these breeds. We have now generated SNP array data on these dogs (~200 from Purdue), and combined it with existing SNP data via shared pooled data with our collaborators. This is an excellent way to increase the

Agency	Grant and Research Program Area	Fund	Commitment Date	Support Amount	Progress/Publications
					amount of data without increasing cost. We included an additional breed with existing data to act as a control (the Boxer). We have subjected all of this data to a selective sweep analysis – a lengthy process that is now complete. We have identified small sweep regions shared by these breeds, and several sweep regions that are exclusive to specific breeds. We will investigate these further now by looking at existing whole-genome sequence, and generating new whole-genome sequence from dogs submitted for this work under this grant. In addition, we have built a new, larger database of dogs who have been seen at the Purdue Vet Teaching Hospital and have radiographs of various sections of their spine. Dr. Caroline Fulkerson, willl provide phenotypes by reading all radiographs (normal spine, or spine not normal and classified via type of abnormality). We have identified 943 dogs, representing 9 breeds (157 are Pugs) and we will recruit DNA samples from these dogs right away for a case/control genome-wide association study). We are now regularly receiving samples from Pugs affected with a spinal myelopathy. Three Pugs with PM (Pug Myelopathy) are included in the samples currently being whole genome sequenced; these dogs can serve both as cases for PM, but also for their tail phenotype in the sweep analysis. We will gladly accept all samples from affected Pugs for this planned case/control genetic comparison. We would ideally like to tease out the genetic differences between Pugs with and without thoracic hemivertebrae and Pugs who do or do not progress to a spinal myelopathy. We do not know yet whether these conditions (hemivertebrae and Pug myelopathy) are related; currently we suspect they are not, but future work should help us address this. Progress Reports filed 9/27/19, 5/7/20, 10/19/21, 4/6/22/, 11/10/22.
AKC CHF	*02275: Disease Risks Associated with Spay and Neuter: A Breed-Specific, Gender-Specific Perspective* Dr. Benjamin L Hart, DVM, PhD; University of California, Davis Program Area: Musculoskeletal Conditions and Disease Project Dates: 9/1/2016 to 2/28/2018; Grant Amount: $61,783.58	DAF	6/5/2018	$2,500.00	Golden Retriever http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0055937 Labrador Retriever and Golden Retriever http://dx.doi.org/10.1371/journal.pone.0102241.g001 German Shepherd Dog onlinelibrary.wiley.com/doi/10.1002/vms3.34/full Hart, B. L., et al. (2020). Assisting Decision-Making on Age of Neutering for 35 Breeds of Dogs: Associated Joint Disorders, Cancers, and Urinary Incontinence. Frontiers in Veterinary Science, 7. https://doi.org/10.3389/fvets.2020.00388
AKC CHF	*02452-A: Targeting the T helper cell inflammatory pathway in meningoencephalomyelitis of unknown etiology* Dr. Renee Barber, DVM, PhD; University of Georgia Program Area: Neurology Project Dates: 1/1/2018 to 6/30/2019; Grant Amount:	Cash	6/5/2018	$2,500.00	Meningoencephalomyelitis of unknown origin (MUO) is a common neurological disorder of dogs that results in inflammation of the brain and/or spinal cord causing depression, seizures, blindness, difficulty walking, and death. All dogs can be affected but young to middle aged small and toy breed

Agency	Grant and Research Program Area	Fund	Commitment Date	Support Amount	Progress/Publications
	$8,845.00				dogs (such as the Chihuahua, Maltese, Pug, and Yorkshire Terrier) are often affected. Currently, brain biopsy is the only means of definitive diagnosis prior to death and the ideal treatment is not known. There is a critical need to improve diagnosis and treatment of MUO. The investigators will identify changes in the immune system associated with inflammation that occurs in the brains and spinal cords of affected dogs, looking for specific products of the immune response, such as interferon-gamma and interleukin 17, in blood and cerebrospinal fluid. Identification of these products could lead to development of new diagnostic tests, strategies for more effective treatment, and improved prognosis prediction. AKCCHF-A Lay Summary MUO grant.pdf Barber, R., & Barber, J. (2022). Differential T-cell responses in dogs with meningoencephalomyelitis of unknown origin compared to healthy controls. *Frontiers in Veterinary Science*, *9*. https://www.frontiersin.org/articles/10.3389/fvets.2022.925770
AKC CHF	*10284: Oncology-Lymphoma Research Program Area Support* Program Area: Oncology – Lymphoma	DAF	1/26/2017	$2,000.00	http://www.pugdogclubofamerica.com/uploads/7/4/8/2/74828213/akc_chf_2309_my2_summary.pdf See Program Area Publications HERE
AKC CHF	*10284: Oncology-Lymphoma Research Program Area Support* Program Area: Oncology – Lymphoma	Cash	1/26/2017	$3,000.00	http://www.pugdogclubofamerica.com/uploads/7/4/8/2/74828213/akc_chf_2309_my2_summary.pdf
AKC CHF	*02165-MOU: Biomarker Development in Canine Degenerative Myelopathy for Diagnosis and Longitudinal Monitoring of a Therapeutic Approach* Dr. Joan R. Coates, DVM, MS; University of Missouri, Columbia Program Area: Neurology Project Dates: 1/1/2015 to 6/30/2020; Grant Amount: $154,077.00	DAF	10/13/2015	$2,500.00	Toedebusch, C. M., et al. (2017). Cerebrospinal Fluid Levels of Phosphorylated Neurofilament Heavy as a Diagnostic Marker of Canine Degenerative Myelopathy. *Journal of Veterinary Internal Medicine*, *31*(2), 513–520. https://doi.org/10.1111/jvim.14659 Toedebusch, Christine M.et al. (2019). Lumbar spinal cord microglia exhibited increased activation in aging dogs compared with young adult dogs. *GeroScience*. https://doi.org/10.1007/s11357-019-00133-8 Toedebusch, Christine M., et al. (2018). Arginase-1 expressing microglia in close proximity to motor neurons were increased early in disease progression in canine degenerative

Agency	Grant and Research Program Area	Fund	Commitment Date	Support Amount	Progress/Publications
					myelopathy, a model of amyotrophic lateral sclerosis. *Molecular and Cellular Neuroscience*, *88*, 148–157. https://doi.org/10.1016/j.mcn.2018.01.009
AKC CHF	*02111-A: The skin mycobiome (fungal microbiome) of healthy and allergic dogs* Dr. Jan S Suchodolski, DVM, PhD; Texas A&M AgriLife Research Program Area: Dermatology and Allergic Disease Project Dates: 6/1/2014 to 5/31/2015; Grant Amount: $12,959.86	DAF	7/10/2014	$1,000.00	Meason-Smith, C., et al. (2015). What is living on your dog's skin? Characterization of the canine cutaneous mycobiota and fungal dysbiosis in canine allergic dermatitis. *FEMS Microbiology Ecology*, *91*(12), fiv139. https://doi.org/10.1093/femsec/fiv139
AKC CHF	*02116-A: The cutaneous resistome of dogs: the effect of antimicrobial selective pressure on the canine microbiome.* Dr. Shelley C Rankin, PhD; University of Pennsylvania Program Area: Dermatology and Allergic Disease Project Dates: 6/1/2014 to 7/31/2016; Grant Amount: $12,312.00	DAF	7/10/2014	$1,000.00	Related Grants 00857-A: Treatment of Canine Atopic Dermatitis with a Novel Immune Modulating Vaccine 00572-A: Heritability of Atopic Dermatitis in West Highland White Terriers
AKC CHF	*02118-A: Identification of a Lipid Receptor in the Canine Endometrium to Support Non-Invasive Therapy in Pyometra* Dr. Cordula Bartel, PhD; University of Veterinary Medicine of Vienna Program Area: Reproductive Conditions and Disease Project Dates: 7/1/2014 to 11/30/2015; Grant Amount: $10,368.00	DAF	7/10/2014	$1,000.00	Gabriel, C., et al. (2016). The physiological expression of scavenger receptor SR-B1 in canine endometrial and placental epithelial cells and its potential involvement in pathogenesis of pyometra. *Theriogenology*, *85*(9), 1599-1609.e2. https://doi.org/10.1016/j.theriogenology.2016.01.021 Mair, G., et al. (2017). Combining RPL27 with OAZ1 or RPL8 as Universal Normalizers of Gene Expression in Canine Endometrial Studies. *International Journal of Veterinary Science and Technology*, *1*(1), 23–24.
AKC CHF	*01828: Mapping of Genetic Risk Factors for Canine Hip Dysplasia* Dr. Antti Iivanainen, DVM, PhD; University of Helsinki and the Folkhälsan Institute of Genetics Program Area: Musculoskeletal Conditions and Disease Project Dates: 1/1/2014 to 12/31/2016; Grant Amount: $79,207.71	DAF	11/7/2013	$1,000.00	Mikkola, L., et al. (2019). Genetic dissection of canine hip dysplasia phenotypes and osteoarthritis reveals three novel loci. *BMC Genomics*, *20*(1027), 1–13. https://doi.org/10.1186/s12864-019-6422-6 Mikkola, L. I., et al. (2019). Novel protective and risk loci in hip dysplasia in German Shepherds. *PLOS Genetics*, *15*(7), e1008197. https://doi.org/10.1371/journal.pgen.1008197
AKC CHF	*01731: Potential association between altered gut microbiota and development of meningoencephalomyelitis of unknown etiology (MUE) in dogs* Dr. Nick D Jeffery, BVSc, PhD; Iowa State University	Cash	11/12/2012	$2,500.00	Jeffery, N. D., et al. (2017). The Association of Specific Constituents of the Fecal Microbiota with Immune-Mediated Brain Disease in Dogs. *PLOS ONE*, 12(1), e0170589. Retrieved from

Agency	Grant and Research Program Area	Fund	Commitment Date	Support Amount	Progress/Publications
	Program Area: Neurology Project Dates: 1/1/2013 to 6/30/2015; Grant Amount: $31,076.60				http://dx.doi.org/10.1371%2Fjournal.pone.0170589
AKC CHF	*01827: High-throughput (metagenomic) sequencing for identification of bacteria associated with canine periodontitis and oral health.* Dr. Marcello Pasquale Riggio, PhD; University of Glasgow Program Area: General Canine Health Project Dates: 1/1/2013 to 12/31/2014; Grant Amount: $31,000.00	Cash	11/12/2012	$2,500.00	
AKC CHF	*01455: Mapping Genes Associated with Necrotizing Meningoencephalitis in Dogs* Dr. Simon R. Platt, BVMS; University of Georgia Program Area: Neurology Project Dates: 1/1/2011 to 12/31/2014; Grant Amount: $74,809.46	DAF	1/17/2012	$5,000.00	Barber, Renee M., et al. (2011). Identification of Risk Loci for Necrotizing Meningoencephalitis in Pug Dogs. *Journal of Heredity*, *102*(Suppl_1), S40–S46. https://doi.org/10.1093/jhered/esr048 Barber, R.M., et al. (2012). Broadly Reactive Polymerase Chain Reaction for Pathogen Detection in Canine Granulomatous Meningoencephalomyelitis and Necrotizing Meningoencephalitis. *Journal of Veterinary Internal Medicine*, *26*(4), 962–968. https://doi.org/10.1111/j.1939-1676.2012.00954.x Schrauwen, I., Barber, R. M., et al. (2014). Identification of Novel Genetic Risk Loci in Maltese Dogs with Necrotizing Meningoencephalitis and Evidence of a Shared Genetic Risk across Toy Dog Breeds. *PLoS ONE*, *9*(11), e112755. https://doi.org/10.1371/journal.pone.0112755
AKC CHF	*01455: Mapping Genes Associated with Necrotizing Meningoencephalitis in Dogs* Dr. Simon R. Platt, BVMS; University of Georgia Program Area: Neurology Project Dates: 1/1/2011 to 12/31/2014; Grant Amount: $74,809.46	Cash	12/21/2010	$10,000.00	
AKC CHF	*01455: Mapping Genes Associated with Necrotizing Meningoencephalitis in Dogs* Dr. Simon R. Platt, BVMS; University of Georgia Program Area: Neurology Project Dates: 1/1/2011 to 12/31/2014; Grant Amount: $74,809.46	DAF	12/21/2010	$2,000.00	
AKC CHF	*01312: Association mapping study of Legg-Calve-PerthesDisease in the West Highland White Terrier, Yorkshire Terrier, and Cairn Terriers* Dr. Alison Starr-Moss, PhD; Clemson University Program Area: Musculoskeletal Conditions and Disease Project Dates: 1/1/2010 to 6/30/2013; Grant Amount: $78,688.00	Cash	3/30/2010	$2,500.00	Starr-Moss, A. N., Nowend, K. L., Alling, K. M., Zepp, E. J., & Murphy, K. E. (2012). Exclusion of COL2A1 in canine Legg-Calve´- Perthes disease. *Animal Genetics*, *43*(1), 112. https://doi.org/10.1111/j.1365-2052.2011.02215.x
AKC CHF	*01248: Whole Genome Association Analyses for Cryptorchidism in Dogs*	Cash	3/30/2010	$2,500.00	Zhao, X., Onteru, S., Saatchi, M., Garrick, D., & Rothschild, M. (2014). A genome-wide association study for

Agency	Grant and Research Program Area	Fund	Commitment Date	Support Amount	Progress/Publications
	Dr. Max F. Rothschild, PhD; Iowa State University Program Area: Reproductive Conditions and Disease Project Dates: 1/1/2010 to 12/31/2010; Grant Amount: $74,036.11				canine cryptorchidism in Siberian Huskies. *Journal of Animal Breeding and Genetics*, 131(3), 202–209. https://doi.org/10.1111/jbg.12064
AKC CHF	*01160-A: Development of Contract-Enhanced Magnetic Resonance Angiography for the Diagnosis of Congenital Vascular Liver Disease in Dogs* Dr. Wilfried Mai, DVM PhD; University of Pennsylvania Program Area: Hepatic Disease Project Dates: 5/1/2008 to 4/30/2010; Grant Amount: $12,679.00	DAF	9/9/2009	$1,000.00	Mai, W., & Weisse, C. (2011). Contrast-enhanced Portal Magnetic Resonance Angiography in Dogs with Suspected Congenital Portal Vascular Anomalies: Portal MRA. *Veterinary Radiology & Ultrasound*, 52(3), 284–288. https://doi.org/10.1111/j.1740-8261.2010.01771.x
AKC CHF	*01188-A: Capacity for Respiratory-Based Thermoregulation in Brachycephalic Breeds* Dr. Michael Scott Davis, DVM, PhD; Oklahoma State University Program Area: Lung and Respiratory Disease Project Dates: 1/1/2009 to 12/31/2011; Grant Amount: $11,517.57	DAF	9/9/2009	$1,000.00	Davis, M. S., et al. (2017). Effect of brachycephaly and body condition score on respiratory thermoregulation of healthy dogs. *Journal of the American Veterinary Medical Association*, 251(10), 1160–1165. https://doi.org/10.2460/javma.251.10.1160
AKC CHF	*00779: Characterization of the Canine Y Chromosome: Identifying Genes that Cause Male Infertility* Dr. William J. Murphy, PhD; Texas A&M University Program Area: Reproductive Conditions and Disease Project Dates: 7/1/2007 to 12/31/2009; Grant Amount: $203,344.00	DAF	11/9/2006	$1,000.00	Li, G., Davis, B. W., Raudsepp, T., Pearks Wilkerson, A. J., Mason, V. C., Ferguson-Smith, M., O'Brien, P. C., Waters, P. D., & Murphy, W. J. (2013). Comparative analysis of mammalian Y chromosomes illuminates ancestral structure and lineage-specific evolution. *Genome Research*, *23*(9), 1486–1495. https://doi.org/10.1101/gr.154286.112
AKC CHF	*01241-A: Detection of DNA damage in response to cooling storage in canine spermatozoa using single-cell gel electrophoresis (comet assay)* Dr. Cristina Gobello, DVM; National University of La Plata Program Area: Reproductive Conditions and Disease Project Dates: 8/1/2009 to 3/31/2011; Grant Amount: $8,402.00	DAF	9/9/2009	$500.00	
AKC CHF	*01105: Understanding the Dynamics of Canine Influenza Virus Transmission in Dog Populations and Intervention Strategies for Reducing Transmission* Dr. Cynda Crawford, DVM PhD; University of Florida Program Area: Immunology and Infectious Disease Project Dates: 1/1/2009 to 12/31/2011; Grant Amount:	Cash	9/9/2009	$2,500.00	Anderson, T. C., et al. (2013). Prevalence of and exposure factors for seropositivity to H3N8 canine influenza virus in dogs with influenza like illness in the United States. *Journal of the American Veterinary Medical Association*, 242(2), 209–216. https://doi.org/10.2460/javma.242.2.209

Agency	Grant and Research Program Area	Fund	Commitment Date	Support Amount	Progress/Publications
	$104,220.00				Anderson, T. C. (2011). Diagnosis and Epidemiological Investigation of the H3N8 Canine Influenza Virus (Doctoral Dissertation, University of Florida)
AKC CHF	*01099: Degenerate PCR for Detection of Viral, Bacterial, and Rickettsial Genera in Pugs and Maltese Dogs with Necrotizing and Granulomatous Meningoencephalitis* Dr. Scott J Schatzberg, DVM PhD; University of Georgia Program Area: Treatment Project Dates: 1/1/2009 to 12/31/2010; Grant Amount: $67,612.50	DAF	7/7/2009	$2,500.00	Barber, R. M., et al. (2010). Evaluation of Brain Tissue or Cerebrospinal Fluid with Broadly Reactive Polymerase Chain Reaction for *Ehrlichia*, *Anaplasma* , Spotted Fever Group *Rickettsia*, *Bartonella*, and *Borrelia* Species in Canine Neurological Diseases (109 Cases). *Journal of Veterinary Internal Medicine*, *24*(2), 372–378. https://doi.org/10.1111/j.1939-1676.2009.0466.x Nghiem, P. P., & Schatzberg, S. J. (2010). Conventional and molecular diagnostic testing for the acute neurologic patient. *Journal of Veterinary Emergency and Critical Care*, *20*(1), 46–61. https://doi.org/doi:10.1111/j.1476-4431.2009.00495.x Schatzberg, S. J., et al. (2009). Broadly Reactive Pan-Paramyxovirus Reverse Transcription Polymerase Chain Reaction and Sequence Analysis for the Detection of *Canine Distemper Virus* in a Case of Canine Meningoencephalitis of Unknown Etiology. *Journal of Veterinary Diagnostic Investigation*, *21*(6), 844–849. https://doi.org/10.1177/104063870902100613 Talarico, L. R., & Schatzberg, S. J. (2010). Idiopathic granulomatous and necrotising inflammatory disorders of the canine central nervous system: A review and future perspectives. *Journal of Small Animal Practice*, *51*(3), 138–149. https://doi.org/10.1111/j.1748-5827.2009.00823.x
AKC CHF	*01099: Degenerate PCR for Detection of Viral, Bacterial, and Rickettsial Genera in Pugs and Maltese Dogs with Necrotizing and Granulomatous Meningoencephalitis* Dr. Scott J Schatzberg, DVM PhD; University of Georgia Program Area: Treatment Project Dates: 1/1/2009 to 12/31/2010; Grant Amount: $67,612.50	DAF	6/4/2009	$2,500.00	
AKC CHF	*01244-A: DRB, DQA, and DQB Gene Sequencing and Allele Determination in the Pug Dog* Dr. Kimberly A Greer, PhD; Indiana University East Program Area: Prevention Project Dates: 5/1/2009 to 10/31/2010; Grant Amount: $12,960.00	DAF	3/23/2009	$6,480.00	Greer, Ka, et al. (2010) Necrotizing meningoencephalitis of Pug Dogs associates with dog leukocyte antigen class II and resembles acute variant forms of multiple sclerosis. *Tissue Antigens*. 76, 110-8. http://dx.doi.org/10.1111/j.1399-0039.2010.01484.x Pedersen, N., et al. (2011). Dog Leukocyte Antigen Class II–Associated Genetic Risk Testing for Immune Disorders of Dogs: Simplified Approaches Using Pug DogNecrotizing Meningoencephalitis as a Model.

Agency	Grant and Research Program Area	Fund	Commitment Date	Support Amount	Progress/Publications
					Journal of Veterinary Diagnostic Investigation, 23(1), 68–76. https://doi.org/10.1177/104063871102300110
AKC CHF	*01056-A: Determine the Effect of Stenotic Nares on the Development of the Brachycephalic Syndrome in Brachycephalic Dogs* Dr. Joe G. Hauptman, DMV; Michigan State University Program Area: Treatment Project Dates: 1/1/2008 to 12/31/2009; Grant Amount: $2,838.78	DAF	6/2/2008	$1,000.00	
AKC CHF	*00985-A: In vivo Effects of Tetracycline on Canine Refractory Ulcers* Dr. Heather Chandler, PhD; The Ohio State University Program Area: Treatment Project Dates: 4/1/2007 to 9/30/2008; Grant Amount: $12,707.00	DAF	6/2/2008	$1,000.00	Chandler, H. L., et al. (2010). In vivo effects of adjunctive tetracycline treatment on refractory corneal ulcers in dogs. *Journal of the American Veterinary Medical Association*, 237(4), 378–386. https://doi.org/10.2460/javma.237.4.378
AKC CHF	*00963: Genotyping Small Breed Dogs with Portosystemic Vascular Anomalies and Microvascular Dysplasia* Dr. Sharon A. Center, DVM; Cornell University Program Area: Prevention Project Dates: 6/1/2008 to 12/31/2011; Grant Amount: $189,489.00	DAF	6/2/2008	$7,000.00	From internet accessed 7.25.20 https://pubmed.ncbi.nlm.nih.gov/17144823/
AKC CHF	*00759: Investigation of Antigenic Causes of Vaccine- Associated Allergic Reactions in Dogs* Dr. George E. Moore, DVM, PhD; Purdue University Program Area: Immunology and Infectious Disease Project Dates: 1/1/2007 to 12/31/2008; Grant Amount: $31,631.12	DAF	11/9/2006	$1,000.00	Moore, G. E., & HogenEsch, H. (2010). Adverse Vaccinal Events in Dogs and Cats. *Veterinary Clinics of North America: Small Animal Practice*, 40(3), 393–407. https://doi.org/10.1016/j.cvsm.2010.02.002
AKC CHF	*00779: Characterization of the Canine Y Chromosome:Identifying Genes that Cause Male Infertility* Dr. William J. Murphy, PhD; Texas A&M University Program Area: Reproductive Conditions and Disease Project Dates: 7/1/2007 to 12/31/2009; Grant Amount: $203,344.00	DAF	11/9/2006	$1,000.00	Li, G., et al. (2013). Comparative analysis of mammalian Y chromosomes illuminates ancestral structure and lineage-specific evolution. *Genome Research*, 23(9), 1486–1495. https://doi.org/10.1101/gr.154286.112
AKC CHF	*00640: Linkage Disequilibrium Analysis of Markers Associated with Pug Dog Encephalitis* Dr. Kimberly A Greer, PhD; Texas A&M University Program Area: Prevention	Cash	7/18/2006	$15,457	Greer, K. A., Wong, A. K., Liu, H., Famula, T. R., Pedersen, N. C., Ruhe, A., Wallace, M., & Neff, M. W. (2010). Necrotizing meningoencephalitis of Pug Dogs associates with dog leukocyte antigen class II and resembles acute variant

Agency	Grant and Research Program Area	Fund	Commitment Date	Support Amount	Progress/Publications
	Project Dates: 12/1/2006 to 11/30/2008; Grant Amount: $49,464.00				forms of multiple sclerosis. *Tissue Antigens*. https://doi.org/10.1111/j.1399-0039.2010.01484.x
AKC CHF	*00640: Linkage Disequilibrium Analysis of Markers Associated with Pug Dog Encephalitis* Dr. Kimberly A Greer, PhD; Texas A&M University Program Area: Prevention Project Dates: 12/1/2006 to 11/30/2008; Grant Amount: $49,464.00	Cash	7/18/2006	$15,457	Greer, Kimberly A., Schatzberg, S. J., Porter, B. F., Jones, K. A., Famula, T. R., & Murphy, K. E. (2009). Heritability and transmission analysis of necrotizing meningoencephalitis in the Pug. *Research in Veterinary Science*, *86*(3), 438–442. https://doi.org/10.1016/j.rvsc.2008.10.002 Levine, J. M., Fosgate, G. T., Porter, B., Schatzberg, S. J., & Greer, K. (2008). Epidemiology of Necrotizing Meningoencephalitis in Pug Dogs. *Journal of Veterinary Internal Medicine*, *22*(4), 961–968. https://doi.org/10.1111/j.1939-1676.2008.0137.x Young, B. D., Levine, J. M., Fosgate, G. T., de Lahunta, A., Flegel, T., Matiasek, K., Miller, A., Silver, G., Sharp, N., Greer, K., & Schatzberg, S. J. (2009). Magnetic Resonance Imaging Characteristics of Necrotizing Meningoencephalitis in Pug Dogs. *Journal of Veterinary Internal Medicine*, *23*(3), 527–535. https://doi.org/10.1111/j.1939-1676.2009.0306.x
AKC CHF	*0002501: Transmission Analysis of Breed Specific Necrotizing Encephalitis in the Pug Dog: Pedigree Collection Phase* Dr. Kimberly A Greer, PhD; Texas A&M University Program Area: Prevention Project Dates: 12/31/2002 to 12/31/2003; Grant Amount: $3,500.00	Cash	8/27/2002	$1,750.00	
AKC CHF	*0002012: Development of PCR Multiplexed Canine MarkerPanels for the Purposes of Genome Screening and Linkage Analysis* Dr. Marcia Eggleston, PhD; University of California, Davis Program Area: General Canine Health Project Dates: 7/19/2000 to 9/30/2002; Grant Amount: $130,000.00	Cash	8/31/2000	$5,000.00	Eggleston, M. L., et al. (2002). PCR multiplexed microsatellite panels to expedite canine genetic disease linkage analysis. *Animal Biotechnology*, 13(2), 223–235. https://doi.org/10.1081/ABIO-120016191
AKC CHF	*0002631: Polymerase Chain Reaction for Herpesviruses From Paraffinized Brains and Fresh Tissues in Cases of Pug Dog Encephalitis* Dr. Stephen C. Barr, PhD; Cornell University Program Area: Treatment Project Dates: 4/1/2004 to 3/31/2005; Grant Amount: $34,451.00	DAF	2004	$18,530.5	Schatzberg, S. J., Haley, N. J., Barr, S. C., de Lahunta, A., & Sharp, N. J. H. (2005). Polymerase chain reaction screening for DNA viruses in paraffin-embedded brains from dogs with necrotizing meningoencephalitis, necrotizing leukoencephalitis, and granulomatous meningoencephalitis. *Journal of Veterinary Internal Medicine*, 19(4), 553–559.

Agency	Grant and Research Program Area	Fund	Commitment Date	Support Amount	Progress/Publications
AKC CHF	*0002650: Transmission Analysis of Breed Specific Necrotizing Encephalitis in the Pug Dog* Dr. Keith E. Murphy, PhD; Texas A&M University Program Area: Prevention Project Dates: 7/1/2003 to 6/30/2004; Grant Amount: $25,400.00	DAF	2003	$12,700	
MAF	D06CA-066 *Mapping Genes Associated with Canine Mast Cell Tumors* Dr. Maja L. Arendt; Uppsala University, University of Cambridge Program Area: Cancer Project Dates: Grant Amount:	Cash	4/9/2007	$3000	Mast cell tumor (MCT) is the most common skin tumor in dogs. A particularly high incidence of this disease has been reported in certain breeds, such as golden retrievers, shar-peis, Labrador retrievers and boxers. Scientists at MIT's, Broad Institute and the Ohio State University mapped genes associated with MCTs. They compared the genomes of 88 golden retrievers with MCT with those of over 103 healthy golden retrievers serving as control subjects. The team identified and localized five candidate loci as genetic risk factors for mast cell tumors. They reported that the locus on chromosome 17 has the strongest association, suggesting that it is a major risk factor for the disease. Although they did not identify the specific gene(s), they have narrowed down the search. Determining these genetic mutations will in turn allow for the development of genetic tests to identify dogs at risk for MCT cancer, leading to better treatment strategies and breeding management. https://journals.plos.org/plosgenetics/article?id=10.1371/journal.pgen.1005647 2015
MAF	D06CA-066 *Mapping Genes Associated with Canine Mast Cell Tumors* Dr. Maja L. Arendt; Uppsala University, University of Cambridge Program Area: Cancer, Project Dates: Grant Amount:	Cash	6/11/2008	$3000	https://www.nature.com/articles/ng.525 2010 The unique canine breed structure makes dogs an excellent model for studying genetic diseases. Within a dog breed, linkage disequilibrium is extensive[1,2], enabling genome-wide association (GWA) with only around 15,000 SNPs and fewer individuals than in human studies[1,3]. Incidences of specific diseases are elevated in different breeds, indicating that a few genetic risk factors might have accumulated through drift or selective breeding. In this study, a GWA study with 81 affected dogs (cases) and 57 controls from the Nova Scotia duck tolling retriever breed identified five loci associated with a canine systemic lupus erythematosus (SLE)–related disease complex that includes both antinuclear antibody (ANA)–positive immune-mediated rheumatic disease (IMRD) and steroid-responsive meningitis-arteritis (SRMA). Fine mapping with twice as many dogs validated these loci. Our results indicate that the homogeneity of strong genetic risk factors within dog breeds allows multigenic disorders to be mapped with fewer than 100 cases and 100 controls, making dogs an excel-

Agency	Grant and Research Program Area	Fund	Commitment Date	Support Amount	Progress/Publications
					lent model in which to identify pathways involve in human complex cases.
MAF	D08CA-002 *Altered expression of antimicrobial peptide genes in the skin of dogs with atopic dermatitis and other inflammatory skin conditions* Dr. Mark Rutherford, University of Minnesota Program Area: Dermatology, Project Dates: Grant Amount:	Cash	4/5/2010	$3000	Results: Altered Gene Expression May Worsen Dogs' Skin Allergies Atopic dermatitis, skin inflammation caused by environmental allergens, affects many dogs, many of whom develop secondary skin infections that aggravate suffering and require antibiotic treatment. The skin's immune system contains small antimicrobial peptides (proteins) that defend the skin against infections. Humans with atopic dermatitis and secondary bacterial infections are deficient in certain antimicrobial peptides, so scientists are now investigating whether allergic dogs also lack these peptides. Researchers extracted RNA from samples and then applied standardized assays to measure and compare defensin gene expression. Defensins are small blood and tissue peptides that help prevent and clear infections. They also measured gene expression from various skin regions and discovered significant variation in expression depending on the body location of these skin samples. Researchers learned that atopic dogs showed altered expression of several antimicrobial genes, though in unpredictable ways. The data are encouraging in that the pattern of gene expression may predict dogs at risk for secondary skin infections during atopic dermatitis; however, further study is needed to control for breed, gender and age effects on expression of these genes. https://pubmed.ncbi.nlm.nih.gov/23701024/
MAF	D07CA-055 *A Canine Clinical Trial: Analgesic Efficacy of Liposomal Hydromorphone for Forelimb Amputation* Dr. Lesley Smith, University of Wisconsin-Madison Program Area: Anesthesiology, Project Dates: Grant Amount:	Cash	4/5/2010	$3000	Results: Study Finds More Effective Pain Management Approach After Surgery Pain management is critical to an animal's recovery and quality of life after surgery. With major surgeries, such as limb amputation, effective pain relief is required for several days after surgery. Researchers at the University of Wisconsin used Morris Animal Foundation funding to determine the optimal dose of a novel formulation of the drug hydromorphone that lasts up to four days after a single injection. They also discovered that the new hydromorphone formulation outperforms the most commonly used analgesic, and few dogs treated with hydromorphone required additional medications for pain control. In addition to relieving pain more immediately and for longer periods of time, this pain management formulation may allow hospitalized patients to be discharged to their owners more quickly after major surgery, which may also help limit costs.

Agency	Grant and Research Program Area	Fund	Commitment Date	Support Amount	Progress/Publications
MAF	D09CA-019 *Developing a Diagnostic Test to Determine an Animal's Spay Status* Dr. Ned Place, Cornell Program Area, Project Dates: Grant Amount:	Cash	12/20/2010	$3000	https://pubmed.ncbi.nlm.nih.gov/21908283/ Anti-Müllerian hormone (AMH), or Müllerian inhibitory substance, is a hormone that is best known for its production by fetal testes in mammals and as the inhibitor of Müllerian (paramesonephric) duct development in males. However, following the development of the Müllerian ducts into the oviduct, uterus, and upper vagina in female mammals, the ovaries produce AMH, which can be found in measurable amounts within the peripheral circulation, especially in adults. The ovaries appear to be the sole source of AMH in the circulation; therefore, it may be a useful marker in clinically relevant situations when an assessment of the presence or absence of ovaries or ovarian remnants in dogs and cats is important. Overall, a single measurement of serum AMH concentration was highly effective at distinguishing ovariohysterectomized from intact adult animals. In addition, the assay also accurately identified several cases of ovarian remnant syndrome.
MAF	D10-406 *Mapping Genes Associated with Inflammatory Brain Disorder in Dogs* Dr. Renee Barber, University of Georgia Program Area: Neurology, Project Dates: Grant Amount:	Cash	12/20/10 01/23/12	$3000 $3000	https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0112755 Identification of Novel Genetic Risk Loci in Maltese Dogs with Necrotizing Meningoencephalitis and Evidence of a Shared Genetic Risk across Toy Dog Breeds Necrotizing meningoencephalitis (NME) affects toy and small breed dogs causing progressive, often fatal, inflammation and necrosis in the brain. Genetic risk loci for NME previously were identified in pug dogs, particularly associated with the dog leukocyte antigen (DLA) class II complex on chromosome 12, but have not been investigated in other susceptible breeds. We sought to evaluate Maltese and Chihuahua dogs, in addition to pug dogs, to identify novel or shared genetic risk factors for NME development.). This suggests a shared genetic background exists between all breeds and confers susceptibility to NME, but effect sizes might be different among breeds. In conclusion, we identified the first genetic risk factors for NME development in the Maltese, chromosome 4 and chromosome 15, and provide evidence for a shared genetic risk between breeds associated with chromosome 15 and DLA II. Last, DLA II and IL7R both have been implicated in human inflammatory diseases of the central nervous system such as multiple sclerosis, suggesting that similar pharmacotherapeutic targets across species should be investigated. https://academic.oup.com/jhered/article/102/Suppl_1/S40/897030 Identification of Risk Loci for Necrotizing Meningoencephalitis in Pug Dogs Necrotizing meningoencephalitis (NME), a fatal inflammato-

Agency	Grant and Research Program Area	Fund	Commitment Date	Support Amount	Progress/Publications
					ry disorder of the brain and its surrounding membranes, primarily affects small dog breeds including Pugs, Maltese and Chihuahuas. The cause of NME is unknown; however, it is known to be inherited within families of Pugs, which indicates that genetic factors play a role in its development. Dr. Renee Barber, funded by a Morris Animal Foundation Fellowship award, investigated the significance of two previously identified broad genetic regions of interest that may be associated with Pugs developing NME. Dr. Barber and the research team confirmed that these regions (on two specific chromosomes) predispose some Pugs to this condition.
MAF	D12CA-803 *Reprogramming Canine Stem Cells to Develop New Therapies* Dr. Deborah Guest, Animal Health Trust Program Area:, Project Dates: Grant Amount:	Cash	1/23/2012	$3000	Results: Researchers Successfully Develop Canine Stem Cells Stem cell therapy offers promise for treating many orthopedic, neurologic and cardiovascular injuries and diseases. Induced pluripotent stem cells (iPSCs) are a type of stem cell developed from adult cells that have been reprogrammed back to an early stage. When they are in such an early stage of development the cells have the potential to develop into any cell type that could be needed for a specific treatment. One advantage of treatment with iPSCs over traditional stem cells is the reduced risk of rejection by the patient's immune system. Although techniques to produce iPSCs have been developed for humans, mice, monkeys, pigs and horses, they have yet to be created for dogs. With Morris Animal Foundation funding, researchers from the Animal Health Trust successfully determined the conditions required to generate canine iPSCs from clinically normal canine adult cells in the lab. By establishing the methods required to generate canine iPSCs, this research has formed the basis for future work to assess the therapeutic potential of canine iPSCs. These data stand to improve the health and welfare of dogs suffering from a wide range of conditions and injuries. Important next steps will include work to turn iPSCs into specific cell types with therapeutic relevance to dogs. Researchers also hope to generate iPSCs from dogs with inherited diseases, leading to a greater understanding of these diseases and their treatment. FINAL Report 7.27.20
MAF	D13CA-031 *Studying How Mast Cell Tumors Spread* Dr. Cheryl London, Ohio State University Program Area: Cancer, Project Dates: Grant Amount:	Cash	11/16/2012	$3000	**Final Report: Researchers identify key factors involved in aggressive mast cell disease in dogs.** Mast cell tumors are one of the most common skin tumors in dogs. Mast cells are white blood cells that play important roles in allergic response, wound healing and inflammation. MCTs vary widely from localized masses that can be cured with surgery, to aggressive masses that rapidly spread to other organs. Dogs suffering from aggressive MCTs respond poorly to conventional therapies, including surgery, chemotherapy

Agency	Grant and Research Program Area	Fund	Commitment Date	Support Amount	Progress/Publications
					and radiation. New strategies desperately are needed to improve survival rates in these patients. In previously funded studies, researchers from The OSUdiscovered increased amounts of a small piece of RNA in aggressive canine MCTs (a microRNA termed miR-9). It's known miR-9 contributes to tumor spread in human cancers. Based on these findings, our researchers suspected increased miR-9 expression would induce changes in normal mast cells to make them act in an aggressive manner as found in MCTs. To explore this theory, Morris Animal Foundation-funded researchers developed a mouse model that produces increased miR-9 in mast cells to study how this genetic material affects disease behavior in dogs. Using this unique model, the team found evidence of miR-9 playing a critical role in promoting mast cell invasion. This study furthered our understanding of the factors leading to aggressive canine MCTs. This new information may help in the development of new diagnostic tools for specific therapeutic targets involving miR-9s. The positive outcomes of this research also resulted in an NIH training grant award to a promising young investigator who will continue to explore the role of miR-9 in mast cell disease. (D13CA-031)
MAF	D16CA-048 *Controlling itch and inflammation in dogs with atopic dermatitis*, Dr. Wolfgang Beaumer, North Carolina State University Program Area: Dermaology, Project Dates: Grant Amount:	Cash	02/23/2016	$3000	Update: Morris Animal Foundation-funded researchers from North Carolina State University are investigating the role of an inflammation-promoting protein, cytokine thymic stromal lymphopoietin (TSLP), in atopic dermatitis, a chronic relapsing allergic skin disease. Recent studies show that TSLP plays a central role in the initiation and maintenance of atopic dermatitis in several species. However, little is known about TSLP and its itch-inducing role in dogs. So far, the researchers have confirmed the increased expression of TSLP in canine skin cells in response to house dust mite antigen under laboratory conditions. Because there is a large overlap between itch and pain signaling, the team currently is trying to produce enough recombinant canine TSLP to investigate the role of TSLP in skin tissue samples and sensory neurons from atopic dogs. Once these steps are complete, the team will be able to investigate how current treatments and anti-TSLP antibodies interact to control TSLP expression and secretion in skin cells, and if these treatments impair TSLP function. About 10 percent of all dogs are affected by atopic dermatitis. While current treatments provide relief, some atopic dogs suffer from lifelong itching and inflammation which can lead to self-trauma. Many of the currently available therapies for atopic dermatitis are associated with significant side effects, such as liver problems and diabetes mellitus linked to long-term steroid use. Because little is known about the biological

Agency	Grant and Research Program Area	Fund	Commitment Date	Support Amount	Progress/Publications
					mechanisms of itch in dogs, developing new therapies has been difficult. This study is providing valuable, new information on the mechanisms behind the itch associated with atopic dermatitis, and may help identify TSLP as a new therapy target to control symptoms of itch and inflammation in chronic atopic dogs. (D16CA-048) https://onlinelibrary.wiley.com/doi/pdf/10.1002/brb3.1428
MAF	D17CA-042 *Whole Exome Sequencing Analysis in Canine Mast Cell Tumors to Identify Potential Therapeutic Targets*, Dr. Matthew Breen, North Carolina State University Program Area: Cancer, Project Dates: Grant Amount:	Cash	01/09/2017	$10,000	FINAL REPORT: Researchers identify promising new therapeutic target for aggressive mast cell tumors. Morris Animal Foundation-funded researchers at North Carolina State University identified a new genetic mutation in high-grade mast cell tumors, a common type of skin tumor in dogs. The team hopes this new finding will help progress treatment discovery for dogs with this devastating disease. Scientists already knew some high-grade (aggressive) mast cell tumors have a mutation in a gene called *KIT*. Drug therapy aimed at inhibiting this gene has been successfully used in the treatment of some tumors. Unfortunately, not all high-grade mast cell tumors have this mutation, so the team conducted a study to see if more mutations linked to the cancer could be found. Researchers performed an in-depth, genetic analysis of high-grade mast cell tumor cases without the *KIT* mutation, looking for new genetic variants. The study focused on mast cell tumors in dogs of any breed so results could be applicable across the general dog population. However, findings may be particularly beneficial to high-risk breeds for this cancer, including pugs and boxers. Data analysis helped identify a gene frequently mutated or deleted in canine mast cell tumors not associated with *KIT* gene mutation. Certain therapies aimed at targeting the deficiency of this same gene have shown favorable response in human cancer patients. The team hopes that similar therapies could be beneficial in dogs as well. New therapeutic targets are critically needed for high-grade mast cell tumors that do not harbor KIT mutations, to help improve the clinical management of this cancer. Findings from this study may enhance diagnostic, prognostic and treatment options for this cancer in dogs. The next steps include the screening of a larger number of cases for the presence of the new mutations. This information will help researchers learn more about the effect of these mutations on tumor cells and assist with the screening and testing of new treatments for high-grade mast cell tumors.

Agency	Grant and Research Program Area	Fund	Commitment Date	Support Amount	Progress/Publications
MAF	D22CA-028 *Identifying Genes Associated with Meningoencephalitis.* Dr. Renee Barber, University of Georgia Program Area: Neurology, Project Dates: Grant Amount:	Cash	12/14/21 01/24/23	$2,000.00 $2,000.00	Morris Animal Foundation-funded researchers at the University of Georgia are working to identify immune system genes associated meningoencephalitis of unknown origins (MUO), a common autoimmune disorder affecting the nervous system of dogs. Researchers are using state-of-the-art sequencing of blood cells reflecting different states of the disease, including dogs in remission, to help identify immune system genes that contribute to disease. In the first arm of the study, the team collected and sequenced samples from 13 dogs with MUO and will compare these data against 26 age-and breed-matched control dogs. The team currently needs to recruit 13 more control dogs to have enough samples for robust final data analysis. In the second arm of the study, the team is comparing RNA from dogs with MUO at diagnosis and after treatment to identify genes that contribute to illness. The team has enrolled the target number of 39 dogs with MUO and is working on obtaining follow-up samples from these cases. So far, the team has obtained samples from 13 dogs in this cohort that have responded to treatment. The team is working to obtain additional follow-up samples from dogs that relapsed or did not respond to treatment. Despite aggressive therapy, only one-third of dogs with MUO have successful long-term outcomes. Learning more about genetic factors associated with the development of the disease could point the way toward more effective treatments, better diagnostic tests and improved long-term outcomes for affected dogs. This work is primarily focused on toy and small breed dogs, including pugs, Maltese, Chihuahua, dachshund and shih tzu breeds, which are more commonly impacted by MUO than larger breeds. R. Barber 3/8/22

Agency	Grant and Research Program Area	Fund	Commitment Date	Support Amount	Progress/Publications
MAF	D23CA-513 *Targeting Frailty to Improve Health span* Principal Investigator: Natasha Olby, DVM, North Carolina State University Start Date: TBA Projected Duration: 3 Years Study Cost: $249,062.00	Cash	01/24/2023	$1,000	Description: Frailty is a physical state in which health reserves are limited, decreasing response to stress, and increasing death rate. In people, frailty is defined by weight loss, weakness, exhaustion, slow walking and low activity. While frailty is important in the assessment of elderly people and is treated to improve health, the idea of frailty is rarely used in veterinary medicine. Researchers developed a frailty tool for veterinarians that uses owner questions to assess frailty areas, and assessment of body and muscle condition. The tool accurately predicts six-month death rate; data shows dogs who are frail in greater than or equal in two areas are three times more likely than non-frail dogs to die within six months. The team will conduct a clinical trial to assess how frail dogs, as defined by their frailty tool, respond to exercise. Dogs will be allocated to either a standard care group or a rehabilitation group that includes added daily in-home exercise and three months of weekly in-clinic exercise targeting endurance, strength and balance. Findings will fill knowledge gaps of frailty in dogs, how frailty changes over time and how dogs respond to exercise to improve canine patient health and quality of life.

Agency	Grant and Research Program Area	Fund	Commitment Date	Support Amount	Progress/Publications
MAF	D21CA-837 *Understanding How Coinfections Impact Urinary Tract Infections* Luke Borst, DVM, PhD, DACVP, North Carolina State University,	Cash	1/19/2021	$3,000	**RESULTS: Bacterial Coinfections Associated with Worse Outcomes in Dogs with Urinary Tract Infections.** Morris Animal Foundation-funded researchers at North Carolina State University studied the genetic and clinical significance of coinfections of *E. coli* and *Enterococcus* bacteria in dogs with urinary tract infections. UTIs are very common in dogs and frequently caused by these two species of bacteria. In earlier studies, the team found these bacteria work together to survive in harsh environments similar to the urinary system. This led the team to investigate how coinfections with *E. coli* and *Enterococcus* bacteria influences prognosis and treatment of sick dogs. This study had two arms. In the first arm, the team looked at cultures of both bacteria in the laboratory. They found that the presence of *Enterococcus sp.* enhanced growth of *E. coli* on media that mimicked conditions in the urinary tract. The team also identified genes present in certain *E. coli* strains that helped promote infection. In the second arm of the study, the team reviewed the records of dogs with urinary tract infections. Data showed coinfections with both bacteria were associated with worse outcomes in dogs with UTIs. Disease recurrence, number of hospital visits, cost of therapy and antibiotic-drug resistance, as well as other factors, increased when both these bacteria were present in the urine of dogs with UTIs compared to when only one of the bacteria was present. This study provided valuable information on how coinfections with two common bacteria augment infection in dogs with UTIs. Findings will help improve treatment of urinary tract infections and inform the development of new nonantimicrobial-based therapies that are urgently needed given the growing problem of drug-resistant UTI infections in both people and dogs. **PUBLICATIONS PENDING** *Escherichia coli* and *Enterococcus spp*. in Dogs with Polymicrobial Urinary Tract Infections: A 5-Year Retrospective Study, *Journal of Veterinary Internal Medicine* (*accepted*) Canine uropathogenic and avian pathogenic *Escherichia coli* harboring conjugative plasmids exhibit augmented growth and exopolysaccharide production in response to *Enterococcus faecalis*. (*in preparation)*
ARCF UGA	*Pug Dog Encephalitis* Dr. Scott J. Schatzberg, University of Georgia	Cash	2009	$12,500	Dr. Schatzberg contributed to numerous publications at UGA but there is no record of what exact study this grant supported.

Agency	Grant and Research Program Area	Fund	Commitment Date	Support Amount	Progress/Publications
Mich State	*Investigation of Ataxia and Hind Limb Weakness in Pug Dogs* Jon S. Patterson, DVM, PhD, Dipl ACVP; Dept. of Pathobiology and Diagnostic Investigation, Veterinary Diagnostic Laboratory, Michigan State University College of Veterinary Medicine (MSU CVM) retired	Cash	2015 1/17/2017	$15,000 $ 4,000	Progress report for 2019 received. Data is being analyzed for publication. The work at Michigan State is completed. The next phase is the genetics work at Purdue funded by AKC CHF on pug spinal disease. Ian J. Wachowiak[1], Jon S. Patterson[2], Kathryn M. Winger[3], Kathleen L. Smiler[4], Robert Cole[5], Rachel Moon[5], Michael Kluz[6], Lisa R. Bartner[1], (2022) *Thoracolumbar Myelopathies in Pug Dog*s, [Manuscript submitted for publication.] [1]Colorado State University; Department of Small Animal Clinical Sciences; [2]Michigan State University; Department of Pathobiology and Diagnostic Investigation; [3]Michigan State University; Department of Small Animal Clinical Sciences; Neurology; [4]Michigan State University College of Veterinary Medicine Affiliate; ACLAM, [5]Auburn University Radiology; Department of Small Animal Clinical Sciences; [6]VCA Jackson, Michigan Animal Hospital

Agency	Grant and Research Program Area	Fund	Commitment Date	Support Amount	Progress/Publications
OFA	*Support to Develop OFA Implementation of BOAS RFG testing in the USA* Orthopedic Foundation for Animals 2300 E Nifong Blvd Columbia, MO 65201-3806 Email: ofa@offa.org	Cash	12/14/21	$1,000.00	With encouragement from the Bulldog Club of America the French Bulldog Club of America, and the Pug Dog Club of America, the OFA is joining this international effort and licensing the RFGS for implementation here in the US. To achieve success, the program require participation from multiple stakeholders. The OFA is organizing the effort, will establish the database for exam results and the resulting certifications for dogs in the normal range, and will forward the results to The Kennel Club in the UK for inclusion in the international statistics. Veterinarians with a specialized interest in the brachycephalic breeds and respiratory health will undergo training an approval as RFGS assessors. Breed clubs will organize and sponsor screening events to make the program available regionally. And responsible breeders can screen their breeding stock and include the results in their selection criteria. The RFGS is currently in a pilot phase here in the US. Pending successful beta testing, systems development and assessor training, a full program roll out is anticipated in late 2022 or early 2023. Additional information, as it becomes available, will be released through the OFA media channels as well as the Parent Clubs.

Agency	Grant and Research Program Area	Fund	Commitment Date	Support Amount	Progress/Publications
ETHOS	*Ethos Discovery research to help find a cure for PDE:* Dr. Rebecca Windsor, ETHOS Discovery, Westborough, MA Phase 1 – Pilot study completed in 2020. Demonstrated altered cytokines in dogs at high risk for PDE. Phase 2 – Symptoms of early encephalitis in Pugs (SEEP) study – completed summer 2021 Identified progressive neurological exam abnormalities suggesting symptoms in the brain and spinal cord in 8/36 dogs, all at medium/high genetic risk. MRI/spinal fluid analysis performed and abnormalities noted in 6/8 Pugs that could be consistent with meningitis and very early necrosis. Phase 3 – Therapeutic trials to prevent PDE – 2021 Pugs with abnormal MRIs will be treated with conventional therapy (immunosuppressive approach) or stem cell therapy (immunomodulatory approach). See if MRI/spinal fluid changes have improved.	Cash	12/14/21 01/24/23	$1,000.00 $1000.00	Leukocyte and cytokine variables in asymptomatic Pugs at genetic risk of necrotizing meningoencephalitis. **Windsor R**, Stewart SD, Talboom J, Lewis C, Naymik M, Piras IS, Keller S, Borjesson DL, Clark G, Khanna C, Huentelman M. J Vet Intern Med. 2021 Nov;35(6):2846-2852. doi: 10.1111/jvim.16293. Epub 2021 Oct 23. PMID: 34687084 Free PMC article. A potential early clinical phenotype of necrotizing meningoencephalitis in genetically at-risk pug dogs. **Windsor R**, Stewart S, Schmidt J, Mosqueda M, Piras I, Keller SM, Steinmetz B, Borjesson DL, Huentelman M, Khanna C. J Vet Intern Med. 2022 Jul;36(4):1382-1389. doi: 10.1111/jvim.16444. Epub 2022 May 27. PMID: 35621070 Free PMC article.

Respectfully Submitted,
Kathleen L. Smiler, DVM, DACLAM
PDCA Health Committee
September 30, 2023