COHA Translational Fellowship Opportunity for Residency-Trained Veterinary Specialists

Fellowship at the Referral Center for Animal Models of Human Genetic Disease (RCAM; P40 10939)

Area of Research: The RCAM goals are:

1. To identify hereditary metabolic diseases within the dog and cat population,
2. To develop precise diagnostic tests for carriers and affected animals,
3. To recommend informed breeding of carrier and normal animals to preserve gene pools,
4. To maintain colonies of hereditary disorders in dogs and cats to better understand disease pathogenesis and to develop therapies, and
5. To provide tissues samples and large animal models of human genetic disease to investigators in order to better understand disease pathogenesis and as preclinical models for potential human clinical trials.

University/Department: Department of Clinical Sciences and Advanced Medicine, School of Veterinary Medicine, University of Pennsylvania

Primary Mentor: Charles H Vite, DVM, PhD, Dipl ACVIM (Neurology), Professor of Neurology, vite@vet.upenn.edu; Director of RCAM (https://www.vet.upenn.edu/research/core-resources-facilities/referral-center-for-animal-models)

Mentor Team: John H. Wolfe, VMD, PhD, Professor of Pathology and Medical Genetics
Margret Casal, Dr. Med Vet, PhD, Professor of Medical Genetics
Paula Henthorn, PhD, Professor of Medical Genetics

Description of Potential Research Project(s): In the past five years, in collaboration with the office of Therapeutics for Rare and Neglected Diseases at NIH, the Niemann Pick disease type C1 (NPC1) cat model accelerated the translation of a promising experimental therapy in the NPC1 mouse model to children by providing critical information on route of delivery, scaling of dose, adverse events, and surrogate biomarkers that would not have been feasible using the mouse model. Notably, this work led to the development of a phase 1/2a trial of 2-hydroxypropyl-beta-cyclodextrin that has yielded promising efficacy data and supported the initiation of a multinational phase 2b/3 clinical efficacy trial that has been approved by both the FDA and EMA. In collaboration with Edimer Pharmaceuticals, neonatal dogs with X-linked ectodermal dysplasia (XLHED) received the recombinant protein EDA200, which improved the development of sweat glands, teeth, tear production and pulmonary function that is normally compromised in these dogs. The results of these experiments were submitted to

the FDA and supported the first clinical trials of children with XLHED. In a U01 collaborative project with the NIH Magnuson Clinical Center, the feline alpha-mannosidosis model was used to evaluate CSF directed delivery of adeno-associated viral vectors to treat the brain to support development of an IND for a first-in-human gene therapy trial using a CSF delivery route for AAV in alpha-mannosidosis. Similar preclinical trials in large animal models of the mucopolysaccharidoses, globoid cell leukodystrophy, hemophilia, cystinuria, and epilepsy have resulted in planned or ongoing clinical trials in patients. The fellow will focus on a disease model within their specialty (neurology, internal medicine, cardiology, clinical pathology, dermatology)

Additional Training Opportunities: The University of Pennsylvania is well equipped to support translational research. The majority of the project will be conducted at the School of Veterinary Medicine, however, resources available at the Perelman School of Medicine will also be utilized. The expertise and resources for translational research are unparalleled, with numerous centers including the Smilow Center for Translational Research, Institute for Translational Medicine and Therapeutics, and the Clinical and Translational Research Center. Research on rare diseases is heightened by the innovative Center for Orphan Disease Research and Therapy, which has research facilities dedicated to discovering and translating therapies. Collaborations between the school of veterinary medicine and school of medicine are encouraged and supported through regular conferences and symposia.

The fellow will be expected to attend weekly seminars at the Institute for Translational Medicine and Therapeutics, the Mahoney Institute of Neurological Sciences, and the Center for Neurodegenerative Disease Research. In addition to the aforementioned University of Pennsylvania facilities, the Wistar Institute and Children's Hospital of Philadelphia (CHOP) are located on campus and work in close collaboration with the university.

Dr. Vite and Dr. Wolfe are skilled in grant writing. They will devote time monthly to training in grant writing and the fellow will also be expected to assist in the submission of two grants currently in preparation.

The fellow will be expected attend/present at weekly lab meetings, and to present data yearly at the ACVIM symposia and at the American Society of Gene and Cell Therapy.

Fellowships are for 2 years and provide stipend and employee benefits at the NIH post-doctoral pay scale. Fellows may supplement their stipend with up to 25% effort towards clinical

work, if such work is in alignment with the research and career development plan.

All fellowships will have a start date of fall 2022.

Biosketches of primary mentor and mentor team (NIH format; 4 pages maximum for each; attach here)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Vite, Charles Herman

eRA COMMONS USER NAME (credential, e.g., agency login): CHARLESVITE

POSITION TITLE: Professor of Neurology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Cornell University, Ithaca, NY	B.S.	May 1986	Biology
Purdue University, West Lafayette, IN	D.V.M.	May 1990	Veterinary Medicine
University of Pennsylvania, Philadelphia, PA		June 1994	Neurology Residency
University of Pennsylvania, Philadelphia, PA	Ph.D.	May 2003	Cell and Molecular Biology

A. Personal Statement

I am an American College of Veterinary Internal Medicine (ACVIM) board-certified veterinary neurologist with expertise in clinical neurology, neuroanatomy, neuropathology, neuroradiology, and electrodiagnostic techniques. I received my PhD in Cell and Molecular Biology on gene therapy of the central nervous system (CNS) in the feline model of alpha-mannosidosis. These studies were the first to evaluate gene therapy of the CNS in a large animal (canine or feline) model of human disease. I currently manage the National Referral Center for Animal Models of Human Genetic Disease (RCAM; NIH OD P40-10939; PI: Vite). The RCAM consists of breeding colonies of more than 40 models of human genetic diseases, including many with CNS diseases such as dogs with globoid cell leukodystrophy and mucopolysaccharidoses, and cats with Niemann-Pick disease type C1 and alpha-mannosidosis.

The focus of my laboratory is to improve the characterization and treatment of hereditary neurodegenerative diseases by studying naturally-occurring large animal models of human diseases. My laboratory develops and evaluates experimental therapies including gene therapy, cell-based therapy, and pharmacotherapy in canine and feline models, and develops and validates ante-mortem biochemical and magnetic resonance markers of disease severity and progression. References that support my expertise include:

1. **Vite CH**, McGowan JC, Niogi S, Passini MA, Drobatz KJ, Haskins ME, Wolfe JH. Effective gene therapy for an inherited diffuse CNS disease in a large animal model. Ann Neurol 57: 355 – 364, 2005.
2. Swain GP, Prociuk M, Bagel JH, O'Donnell P, Berger K, Drobatz K, Gurda BL, Haskins ME, Sands MS, **Vite CH**. Adeno-associated virus serotypes 9 and rh10 mediate strong neuronal transduction of the dog brain. Gene Therapy 21: 28-36, 2014. PMC 3881028
3. **Vite CH**, Bagel JH, Swain GP, Prociuk M, Sikora TU, Stein VM, O'Donnell P, Ruane T, Ward S, Crooks A, Li S, Mauldin E, Stellar S, De Meulder M, Kao ML, Ory DS, Davidson C, Vanier MT, Walkley SU. Intracisternal cyclodextrin prevents cerebellar dysfunction and Purkinje cell death in feline Niemann-Pick type C1 disease. Sci Transl Med 7:276ra26, 2015. PMC4415615
4. Gurda BL, Bradbury AM, **Vite CH**. Canine and feline models of human genetic diseases and their contributions to advancing clinical therapies. Yale J Biol Med 90: 417-431, 2017. PMC5612185

B. Positions and Honors

Positions and Employment

1990-1992 Emergency and Critical Care Veterinarian, Veterinary Emergency Service, Inc., Fresno, CA 1992

1992-1994 Neurology Residency, School of Veterinary Medicine, Univ. of PA, Philadelphia, PA
1994 Introductory Magnetic Resonance Visiting Fellowship, Univ. of PA, Philadelphia, PA
1996 Neuro Magnetic Resonance Visiting Fellowship, Univ. of PA, Philadelphia, PA
1994-1997 Lecturer in Veterinary Neurology, School of Veterinary Medicine, Univ. of PA
1997-1998 Post-Doctoral Fellow, School of Veterinary Medicine, Univ. of PA
1998-2003 Senior Research Investigator, School of Veterinary Medicine, Univ. of PA
2004-2012 Assistant Professor, Dept. Clinical Studies, School of Veterinary Medicine, University of PA
2012-2018 Associate Professor and Chief, Section of Neurology, Dept. Clinical Studies, School of Veterinary Medicine, University of PA.
2014-pres Director, National Referral Center of Animal Models of Human Genetic Disease (P40 OD-10939)
2017-pres Division Head, Division of Inherited Metabolic Diseases, School of Veterinary Medicine, Univ. of PA
2018-pres Professor, Section of Neurology and Neurosurgery, Dept. Clinical Sciences and Advanced Medicine, School of Veterinary Medicine, University of PA.

Other Experience and Professional Memberships

1997 – present Diplomate American College of Veterinary Internal Medicine (ACVIM) Neurology
2000 - 2003 ACVIM Neurology Examination Committee
2003 - 2006 ACVIM Neurology Credentials Committee
2004 - 2005 ACVIM Neurology Neurosurgery Task Force
2012 – 2014 ACVIM/European College of Veterinary Neurology Liason
2011 – 2017 ACVIM Neurology Exam Rating Committee
2014 – 2017 ACVIM Neurology President
2004-2007, 2012-2015 Section Chief, Neurology, University of Pennsylvania
2013 – 2018 Institutional Animal Care and Use Committee, University of Pennsylvania
2015 – 2016 National Niemann Pick Disease Foundation Scientific Advisory Board
2015 – 2018 Vtesse Scientific Advisory Board
2012 - present Neuroscience Graduate Group, University of Pennsylvania
2002 - present International Society for Mannosidosis and Related Diseases Scientific Advisory Board
2002 - present National Mucopolysaccharidoses Society Scientific Advisory Board

Honors

2001 - present Phi Zeta Society
2005 Veterinary Medical Student Organization Teaching Award (Class of 2006)
2009 Veterinary Medical Student Organization Teaching Award (Class of 2010)
2014 Zoetis Award for Veterinary Research Excellence
2016 RARE Champion of Hope, Collaborations in Science and Technology, Global Genes
2016 Guardian Angel Award, Dana's Angels Research Trust

C. Contributions to Science

My early studies characterized naturally occurring diseases and their therapies in client-owned animals and were therefore useful to the field of veterinary medicine. Since then, my work utilizes breeding colonies of naturally-occurring canine and feline models of human genetic nervous system diseases to better understand disease pathogenesis, develop therapies, and develop biomarkers of disease severity and therapeutic efficacy. The diseases I have focused on include lysosomal storage diseases with CNS manifestations including Niemann-Pick disease type C1, Krabbe disease, alpha-mannosidosis, fucosidosis, and the mucopolysaccharidoses. These studies are highly translational in nature with the goal of developing improved therapies for human disease.

1. Niemann-Pick disease type C1 (NPC) disease is a progressive neurological lysosomal storage disease caused by mutations in the NPC1 gene, leading to an increase in unesterified cholesterol and several sphingolipids. There are currently no FDA-approved therapies for NPC disease. I characterized the feline model of NPC1 and performed critical studies on the efficacy and safety of 2-hydroxypropyl-beta-cyclodextrin (HPßCD) administration directly into the CNS of NPC cats. Administration of HPßCD into the cerebellomedullary cistern of presymptomatic cats prevented the onset of cerebellar dysfunction and resulted in Purkinje cell survival and near normal concentrations of brain cholesterol and sphingolipids, and

extended lifespan significantly. The cat model studies provided critical data on HPßCD efficacy that were crucial in advancing HPßCD into Phase 3 human clinical trials.

a. Bagel JH, Sikora TU, Prociuk M, Pesayco JP, Mizisin AP, Shelton GD, **Vite CH**. Electrodiagnostic testing and histopathological changes confirm peripheral nervous system myelin abnormalities in the feline model of Niemann-Pick disease type C. J Neuropath Exp Neurol 72:256-62, 2013. PMC3640270
b. **Vite CH**, Bagel JH, Swain GP, Prociuk M, Sikora TU, Stein VM, O'Donnell P, Ruane T, Ward S, Crooks A, Li S, Mauldin E, Stellar S, De Meulder M, Kao ML, Ory DS, Davidson C, Vanier MT, Walkley SU. Intracisternal cyclodextrin prevents cerebellar dysfunction and Purkinje cell death in feline Niemann-Pick type C1 disease. Sci Transl Med 7:276ra26, 2015. PMC4415615
c. Ory DS, Ottinger EA, Farhat NY, King KA, Jiang X, Weissfeld L, Berry-Kravis E, Davidson CD, Bianconi S, Keener LA, Rao R, Soldatos A, Sidhu R, Walters KA, Xu X, Thurm A, Solomon B, Pavan WJ, Machielse BN, Kao K, Silber SA, McKew JC, Brewer CC, **Vite CH**, Walkley SU, Austin CP, Porter FD. Intrathecal 2-hydroxy-beta-cyclodextrin decreases neurological disease progression in Niemann-Pick disease type C1: an ad hoc analysis of a non-randomized, open-label phase 1/2trial. Lancet 390:1758-1768, 2017. PMC 6176479
d. Gurda BL, Bagel JH, Fisher SJ, Schultz ML, Lieberman AP, Hand P, **Vite CH**, Swain GP. LC3 immunostaining in the inferior olivary nuclei of cats with Niemann-Pick disease type C1 is associated with patterned Purkinje cell loss. J Neuropath Exp Neurol 77: 229-245, 2018. PMC5989620

2. Krabbe disease is a leukodystrophy caused by mutations in the gene for galactosylceramidase, leading to a failure to adequately degrade galactosylceramide and psychosine (galactosylsphingosine). Most human patients are affected in infancy but there is no cure for Krabbe disease, although bone marrow transplantation prolongs life and is the current standard of care in children. I maintain a breeding colony of Krabbe dogs and am currently studying bone marrow transplantation, AAV gene therapy, and quantitative magnetic resonance methods to measure demyelination and the effects of treatment.
   a. McGowan JC, Haskins ME, Wenger DA, **Vite CH**. Investigating demyelination in the brain in a canine model of globoid cell leukodystrophy (Krabbe Disease) using magnetization transfer contrast. J Comp Assist Tomog 24: 316-321, 2000.
   b. Swain GP, Prociuk M, Bagel JH, O'Donnell P, Berger K, Drobatz K, Gurda BL, Haskins ME, Sands MS, **Vite CH**. Adeno-associated virus serotypes 9 and rh10 mediate strong neuronal transduction of the dog brain. Gene Therapy 21: 28-36, 2014. PMC 3881028
   c. Bradbury AM, Bagel JH, Jiang X, Swain GP, Prociuk ML, Fitzgerald CA, O'Donnell PA, Braund KG, Ory DS, **Vite CH**. Clinical, electrophysiological, and biochemical markers of peripheral and central nervous system disease in canine globoid cell leukodystrophy (Krabbe's disease). J Neurosci Res 94:1007-17, 2016. PMC5027978
   d. Bradbury AM, Rafi MA, Bagel JH, Brisson BK, Marshall MS, Pesayco Salvador JP, Jiang X, Swain GP, Prociuk ML, ODonnell P, Fitzgerald C, Ory DS, Bongarzone ER, Shelton GD, Wenger DA, **Vite CH**. AAVrh10 gene therapy ameliorates central and peripheral nervous system disease in canine globoid cell leukodystrophy (Krabbe disease). Hum Gene Ther 29: 785-801, 2018. PMC6066194

3. I began studies of AAV gene transfer into the CNS in large animal models of lysosomal storage diseases through a K08 (mentor: John Wolfe) in the feline model of alpha-mannosidosis. Alpha-mannosidosis is a lysosomal storage disease caused by a deficiency of lysosomal α-mannosidase activity, and resulting in the accumulation of oligosaccharides within the CNS. Human alpha-mannosidosis is characterized by predominantly progressive neurological dysfunction and early death. Pre-clinical studies of AAV-mediated gene therapy in affected cats brain resulted in correction of storage, improved myelination, and ameliorated neurological dysfunction. The studies in the feline model of alpha-mannosidosis have continued in Dr. Wolfe's laboratory, with my laboratory having an active collaboration.
   a. **Vite CH**, Passini MA, Haskins ME, Wolfe JH. Adeno-associated virus vector-mediated transduction in the cat brain. Gene Therapy 10: 1874-1881, 2003.
   b. **Vite CH**, McGowan JC, Niogi S, Passini MA, Drobatz KJ, Haskins ME, Wolfe JH. Effective gene therapy for an inherited diffuse CNS disease in a large animal model. Ann Neurol 57: 355 – 364, 2005.
   c. Yoon SY, Gay-Antaki C, Ponde D, Poptani H, **Vite CH**, Wolfe JH. Quantitative, non-invasive, in vivo longitudinal monitoring of gene expression in the brain by co-AAV transduction with a PET reporter gene. Mol Ther Methods Clin Dev 1: 14016, 2014. PMC4362377

d. Yoon SY, Bagel JH, O'Donnell PA, **Vite CH**, Wolfe JH. Clinical improvement of alpha-mannosidosis cat following a single cisterna magna infusion of AAV1. Mol Ther. 24:26-33. 2016. PMC4754545

4. Surrogate markers of neurological disease are necessary to measure disease severity and therapeutic success. These biomarkers are critical for providing the FDA with information on therapeutic efficacy to support the initiation of clinical trials for patients. I have developed and validated biochemical and magnetic resonance markers of CNS disease progression and efficacy of experimental therapies in naturally occurring canine and feline animal models of human CNS disease. Notably, several markers are planned for use or have been used in clinical trials.
   a. Porter FD, Scherrer DE, Lanier MH, Langmade SJ, Molugu V, Gale SE, Olzeski D, Sidhu R, Dietzen DJ, Fu R, Wassif CA, Yanjanin NM, Marso SP, House J, **Vite C**, Schaffer JE, Ory DS. Cholesterol oxidation products are sensitive and specific blood-based biomarkers for Niemann-Pick C1 disease. Sci Transl Med. 2:56ra81, 2010. PMC3170139
   b. Tortelli B, Fujiwara H, Bagel JH, Zhang J, Sidhu R, Jiang X, Yanjanin NM, Shankar RK, Carillo-Carasco N, Heiss J, Ottinger E, Porter FD, Schaffer JE, **Vite CH**, Ory DS. Cholesterol homeostatic responses provide biomarkers for monitoring treatment for the neurodegenerative disease Niemann-Pick C1 (NPC1). Hum Molec Genet 23: 6011-23; 2014. PMC4204776
   c. Kumar M, Duda JT, Yoon SY, Bagel J, O'Donnell P, **Vite** C, Pickup S, Gee JC, Wolfe JH, Poptani H. Diffusion Tensor Imaging for Assessing Brain Gray and White Matter Abnormalities in a Feline Model of α-Mannosidosis. J Neuropathol Exp Neurol. 2016 75:35-43. PMCID in progress.
   d. Bradbury A, Bagel J, Sampson M, Farhat N, Ding W, Swain G, Prociuk M, O'Donnell P, Drobatz K, Gurda B, Wassif C, Remaley A, Porter F, **Vite C**. Cerebrospinal fluid calbindin D concentration as a biomarker of cerebellar disease progression in Niemann-Pick Type C1 disease. J Pharmacol Exp Ther. 358:254-61, 2016. PMC4959104

5. Naturally occurring canine epilepsy has a prevalence estimated between 0.5 and 5.7% which is similar to that described in human patients. Canine epilepsy is a good model for testing pharmacotherapies and implantable devices because it shares similar response and refractory rates to humans for antiepileptic drugs and vagus nerve stimulation. I maintain epileptic dogs in our animal colonies and evaluate pharmacotherapy in these dogs. Also, in collaboration with Dr. Brian Litt, I have published on the development and validation of an implantable brain electrode system and the development of an algorithm to predict seizure occurrence in epileptic dogs.
   a. Howbert JJ, Patterson EE, Stead SM, Brinkmann B, Vasoli V, Crepeau D, **Vite CH**, Sturges B, Ruedebusch V, Mavoori J, Leyde K, Sheffield WD, Litt B, Worrell GA. Forecasting seizures in dogs with naturally occurring epilepsy. PLoS One. 9:e81920, 2014. PMC 3885383
   b. Brinkmann BH, Patterson EE, **Vite** C, Vasoli VM, Crepeau D, Stead M, Howbert JJ, Cherkassky V, Wagenaar JB, **Litt** B, Worrell GA. Forecasting Seizures Using Intracranial EEG Measures and SVM in Naturally Occurring Canine Epilepsy. PLoS One 10(8):e0133900, 2015. PMC4524640
   c. Davis KA, Ung H, Wulsin D, Wagenaar J, Fox E, Patterson N, **Vite C**, Worrell G, Litt B. Mining continuous intracranial EEG in focal canine epilepsy: Relating interictal bursts to seizure onsets. Epilepsia. 57:89-98. 2016. PMC4770560
   d. Ung H, Davis KA, Wulsin D, Wagenaar J, Fox E, McDonnell JJ, Patterson N, **Vite** CH, Worrell G, Litt B. Temporal behavior of seizures and interictal bursts in prolonged intracranial recordings from epileptic canines. Epilepsia 57: 1949-1957, 2016. PMC5241889

NCBI bibliography:
https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/45441950/?sort=date&direction=ascending

D. Additional Information: Research support

Ongoing Research Support

NIH-P40-OD010939 (PI Vite) 1/18/2019 – 12/31/2023

"Referral Center - Animal Models of Human Genetic Disease"

To discover, understand, and treat naturally occurring hereditary disorders, and genetic predispositions to disease, in dogs and cats that are orthologous to those found in human patients.

NIH-R01-NS096087-03 (PI Vite) 6/1/2016 - 5/31/2021
"Combination Therapy, Biomarkers, and Imaging in Canine Krabbe Disease"
Development of hemopoietic stem cell and gene therapy for canine Krabbe disease

Biomarin Pharmaceutical Inc (PI Vite) 7/30/2015-12/8/2022
"Intrathecal Recombinant GALC Administration to Treat Canine Krabbe Disease"
Natural history study in the Krabbe dog and evaluation of the safety and efficacy of intrathecal enzyme replacement therapy.

Dana's Angels Research Trust (PI Vite) 12/17/2012–06/30/2020
Support of Accelerated Research for NPC disease
"Combination Therapy for Feline NPC Disease"
Development of cyclodextrin and other small molecule therapies for NPC1 disease

Ara Parseghian Medical Research FDN.40041056 (PI Vite) 7/1/2019-6/30/2020
"Improving Extracerebellar NPC1 Disease Using Intravenous 2-hydroxypropyl-bbeta-cyclodextrin"
Intravenous cyclodextrin to treat hepatic pathology in NPC1 disease

University Of Pittsburgh (PI Vite) 12/1/2015-9/30/2020
"Treatment of Krabbe Disease Using Intravenous Adeno-Associated Virus Gene Therapy"
Gene therapy study to evaluate GALC activity in both the central and peripheral nervous system.

NIH R01-DK063973-11 (PI Wolfe) 9/23/2016 - 8/31/2020
"Gene Transfer and NMR Studies in Alpha-Mannosidosis Brain"
Investigate systemic vector gene delivery to the brain with novel AAV vectors in the feline alpha-mannosidosis model and develop MRS and DTI to monitor therapy non-invasively.
Role: co-Investigator

<u>Pending</u>
NIH-R01 NS115869-01A1 (PI Vite) 12/20 – 11/25
"AAV-mediated gene therapy for CNS disease correction in feline NPC1 disease"
Investigates optimized techniques to develop a membrane-bound protein to the lysosome to treat NPC disease.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.

Follow this format for each person. DO NOT EXCEED FIVE PAGES.

NAME: WOLFE, JOHN H.

eRA COMMONS USER NAME (agency login): JHWOLFE

POSITION TITLE: Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Ripon College, Ripon, WI	A.B.	1969	Philosophy
Harvard University, Cambridge, MA	no degree	1973-74	post-bacc general sciences
University of Pennsylvania, Philadelphia, PA	no degree	1975-77	post-bacc general sciences
University of Pennsylvania, Philadelphia, PA	V.M.D.	1978-82	Veterinary Medicine
University of Pennsylvania, Philadelphia, PA	Ph.D.	1986	Immunology (virology & genetics)
Sloan-Kettering Institute, New York, NY	Postdoc	1987	Molecular biology

A. Personal Statement

Dr. Wolfe's research program investigates vector-mediated gene transfer and stem cell transplantation to the brain in animal models of neurodevelopmental genetic diseases of children. Studies are done in large animal models with the same disease as humans as translational intermediates to optimize procedures, methods, and the biological effectiveness of the treatment strategies for eventual human clinical application. His research program is currently directed towards increasing the distribution of a therapeutic protein, which is critical in neurogenetic diseases because lesions are typically distributed widely in the brain. The work includes alternative routes of viral vector gene delivery to achieve widespread gene and protein (secreted from gene corrected cells) within the brain to correct the storage lesions globally. Another important goal is to develop methods to monitor changes in gene transduction and changes in pathology by non-invasive imaging modalities, which will be critical for clinical usage. Better understanding of the basic mechanisms by which gene vectors and stem cells are transported within and into the CNS are expected to lead to improved engineering for therapeutic applications. Other goals are to optimize doses, evaluate adverse effects, evaluate clinical improvement, and perform quantitative brain imaging for treatment-induced improvements and its correspondence changes in histopathological parameters.

Role in P40: (5%, 0.6CM). Dr. Wolfe is a Professor in the Dept of Pathobiology in the School of Veterinary Medicine (SVM) and the Dept of Pediatrics in the Perlman School of Medicine. He is a Stokes Investigator in the Research Institute of Children's Hospital of Philadelphia (CHOP), Director of the W.F. Goodman Center for Comparative Medical Genetics in the SVM, and heads the Program in Comparative Animal Biology (PICAB) of the Penn/CHOP CTSA grant (renewed 2016-2021), which is designed to provide infrastructure support to enhance research on novel treatment approaches using domestic animal models of human genetic diseases. Dr. Wolfe's role on this P40 grant application is to coordinate the PICAB with the disease discovery process of the P40 grant, and to institute the formal fee-for-service program for investigators from other institutions to utilize our facilities and, more importantly, our expertise for conducting experimental translational trials in dogs and cats. These studies will be done on a cost plus recovery basis to continue to reduce NCRR expenses and expand the NIH investment in these research programs. His effort will be complimentary to the educational initiatives of the CTSA U11 grant, enhancing each other without duplicating services. He also will provide advice and assistance to investigators on the P40 interested in addressing therapy for the CNS, including choice and design of gene transfer vectors and use and transplantation of neural stem cells or other appropriate cell types into the CNS. He will serve as a contact between P40 members and counterpart faculty at CHOP since most of the genetic diseases in animal models are seen as pediatric diseases in humans.

Recent reviews:

Simonato, M., Bennett, J., Boulis, N.M., Castro, M.G., Fink, D.J., Gray, S.J., Lowenstein, P.R., Vandenberghe, L.H., Wilson, T.J., Wolfe, J.H. and Glorioso, J.C. (2013) Gene therapy for neurological disorders. Are we getting there? Nat. Rev. Neurol., 9: 277-291. PMC3908892

Castle, M.J., Turunen, H.T., Vandenberghe, L.H. and Wolfe, J.H. (2016) Controlling AAV tropism in the nervous system with natural and engineered capsids. In: Gene Therapy for Neurological Disorders, F.P. Manfredsson, ed., Meth. Mol. Biol., 1382:133-149. PMCID: PMC4993104.

Siddiqi, F. and Wolfe, J.H. (2016) Stem cell therapy for the central nervous system in lysosomal storage diseases. Hum Gene Ther, 27: 749-757. PMCID: PMC5035913.

Hunter, J.E., Ramos, L. and Wolfe, J.H. (2016) Viral Vectors in the CNS. Reference Module in Neuroscience and Biobehavioral Psychology, Elsevier online. Invited contribution, in press.

Ramos, L., Hunter, J.E., Wolfe, J.H. (2016) Viral vector gene delivery to the brain for treating neurogenetic diseases. *In:* Drug and Gene Delivery to the Central Nervous System for Neuroprotection; Nanotechnological Advances; H.S. Sharma, D.F. Muresanu, A. Sharma (eds), Springer, New York. Invited review, in press.

B. Positions and Honors

Positions and Employment

1978 - 1984	NIH Veterinary Medical Scientist Trainee, Univ Pennsylvania, Schools of Medicine and Veterinary Medicine, Philadelphia, PA
1985 - 1987	Exxon Fellow, Molecular Biology Program, Sloan-Kettering Institute, New York, NY
1987 - pres	Assist Professor to Professor (1997), Pathology and Medical Genetics, School of Veterinary Medicine, Univ Pennsylvania, Philadelphia, PA
1992 - 2011	Adjunct Assistant Professor to Professor (1997), The Wistar Institute, Philadelphia, PA
1993 - pres	Member, Intellectual and Developmental Disabilities Research Center, Univ Pennsylvania and Children's Hospital of Philadelphia, Philadelphia, PA
1993 - 2001	Director, Veterinary Medical Scientist Training Program, Univ Pennsylvania, Philadelphia, PA
1993 - 2013	Associate Director, Core Center for Gene Therapy, Univ Pennsylvania, Philadelphia, PA
1997 - pres	Member, Mahoney Institute for Neurological Sciences, Univ Pennsylvania, Philadelphia, PA
1998 - pres	Stokes Investigator, Children's Hospital of Philadelphia, Philadelphia, PA
1999 - pres	Professor, Department of Pediatrics, Perelman School of Medicine, Univ Pennsylvania, Philadelphia, PA
2000 - pres	Director, WF Goodman Center for Comparative Medical Genetics, Univ Pennsylvania, PA
2002 - 2014	Member, Penn Genome Frontiers Institute and Scientific Advisory Council, Univ Penna, Philadelphia, PA
2006 - pres	Member, Institute for Translational Medicine and Therapeutics, Univ Penna, Philadelphia, PA
2007 - pres	Member, Penn Institute for Regenerative Medicine, Univ Penna, PA
2013 - pres	Member, Penn Center for Orphan Disease Research and Therapy, Univ Penna, Phila, PA.

Other Experience and Professional Memberships

NIH Reviewing: Member, 1990-94, Ad hoc NIDDK, NIAID, NHLBI. 1992, Mam. Genet Study Sec. 1994-98, Member, Medical Biochemistry Study Section. 1994-95, Chair, ad hoc reviews, MEDB Study Section. 1997, Ad hoc, Board of Scientific Counselors, NINDS. 1998, Chair, NIDDK SEP; Chair, NIGMS SEP. 2000, Ad hoc, MEDB; Chair, Biochem Sci IRG SEP; MCDN IRG SEP; 2002 Ad hoc MEDB, SEP/NINDS; 2003, 2004; GTIE SEP, 6/03, 2/04, 3/04; DBD 3/05; NCI 6/05; DDK-B-B1 3/06; NCF 6/06; DBD 2/07; MDCN SEP 3/07; NIDDK ZRG-B, 9/07; CSR Neurotech SEP, 2/08; GGD study section 6/09; ad hoc GGG-F and BDCN-T 6/09; DDK-B ad hoc 10/09; NT study section 2/10; DDK-B study section, 10/10; DBD 1/11; ZRG1-GGG-C 3/11; ZDK1-GRB-C 7/11; ZRG1-GGG-R 10/11; NSD-B 10/12; GGD 2/13; ZNS1 SRB-J 4/13; ZHD1 DRG-D 4/13; ZNS1 SRB-J 10/13; ZHD1 DRG-D (90) 1/14; ZHD1 DRG-D (91) 2/14; ZRG1 CBW56 3/14; DBD 6/14; ZRG1-BBBP-Y 1/15; NSDB 2/15; ZRG1 BDCN-R 3/15; DBD

6/15; NSDB - 10/15; ZRG1 BDCN-R - 11/15; ZRG1 BBBP-Y 1/16; GGD 2/16; NSD-B 6/16; ZRG1-BBBP-Y 9/16; NSD-B 10/16; ZRG1 BBBP-Y (45) - 2/17; NSD-B - 10/17; GGD-D - 11/17.

Professional Memberships: ASGCT, ASHG, ISSCR, ASM, AAAS, SfN, ESGCT.

Honors

1982, Elected Phi Zeta. 1982, Auxillary to SCAVMA Prize. 1982, Phi Zeta Award. 1987-92, NIH Special Emphasis Research Career Award. 1991, SmithKline Beecham Research Award. 1994-pres, John Morgan Society. 1994-96, President, ß Chapter, Phi Zeta. 1994-2011, Edit Bd., Gene Ther. 1998-2010, Edit. Bd., Hum Gene Ther. 2000-pres, Sci. Advis. Bd, Natl. MPS Soc 2000-pres, Distinguished Lect. Child. Hosp. LA. 2002, Leslie Nicholas Lect., Coll Phys & Surg. 2004, Keynote Speaker, 4th D.B. Marks Memorial Res. Conf. 2005-pres, Edit Bd, Molecular Therapy. 2007-08 & 2014-16 Chair, Neural Disorders Committee, Amer. Soc. Gene & Cell Therapy. 2013-pres. Executive Committee, International Consortium of Gene Therapy. 2015-pres, Board of Trustees, Ripon College.

C. Contributions to Science

1. **Development of experimental gene therapy for the CNS in alpha-mannosidosis.**
   a. Sun, H., Wang, Y., Haskins, M.E., Patterson, D.F. and Wolfe, J.H. (1999) Retrovirus vector-mediated correction and cross-correction of lysosomal a-mannosidase deficiency in human and feline fibroblasts. Hum. Gene Ther. 10: 1311-1319.
   b. Vite, C.H., McGown, J.G., Braun, K.G., Drobatz, K.J., Glickson, J.D., Wolfe, J.H. and Haskins, M.E. (2001) Histopathology, electrodiagnostic testing and magnetic resonance imaging show significant peripheral and central nervous system myelin abnormalities in the cat model of alpha-mannosidosis. J. Neuropath. Exp. Neurol., 60: 817-828.
   c. Vite, C.H., Niogi, S.N., McGowan, J.C., Passini, M.A., Drobatz, K.J., Haskins, M.E. and Wolfe, J.H. (2005) Effective gene therapy for an inherited diffuse CNS disease in a large animal model. Ann. Neurol., 57: 355-364.
   d. Yoon, S.Y., Bagel, J.H., O'Donnell, P.A., Vite, C.H. and Wolfe, J.H. (2016) Clinical improvement of alpha-mannosidosis cat following a single cisterna magna infusion of AAV1. Mol. Ther. 24: 26-33. PMCID: PMC4754545.
2. **In vivo imaging to monitor gene activity and therapeutic effects of vectors in the alpha-mannosidosis cat.**
   a. Vite CH, Magnitsky S, Aleman D, O'Donnell P, Cullen K, Ding W, Pickup S, Wolfe JH, Poptani H. (2008) Apparent diffusion coefficient reveals gray and white matter disease, and T2 mapping detects white matter disease in the brain in feline alpha-mannosidosis. AJNR Am J Neuroradiol. 29: 308-13. PubMed PMID: 17974615.
   b. Magnitsky S, Vite CH, Delikatny EJ, Pickup S, Wehrli S, Wolfe JH, Poptani H. (2010) Magnetic resonance spectroscopy of the occipital cortex and the cerebellar vermis distinguishes individual cats affected with alpha-mannosidosis from normal cats. NMR Biomed. 23: 74-9. PMCID: PMC3045771.
   c. Yoon, S.Y., Gay-Antaki, C., Ponde, D.E., Poptani H., Vite, C.H. and Wolfe, J.H. (2014) Quantitative, Non-Invasive, In Vivo Longitudinal Monitoring of Gene Expression in the Brain by co-AAV Transduction with a PET Reporter Gene. Mol. Ther. Meth. Clin. Devel., 1: 14016. PMCID: PMC4362377.
   d. Kumar M, Duda JT, Yoon SY, Bagel J, Vite C, Pickup S, Gee J, Wolfe JH, Poptani H. Diffusion tensor imaging for assessing gray and white matter abnormalities in the brain of a feline model of alpha-mannosidosis. J Neuropath Exp Neurol. 75(1): 35-43. PMCID not yet assigned.
3. **Development of gene therapy approaches to lysosomal storage diseases, using the Sly disease (MPS VII) mouse and dog models as a paradigm.**
   a. Wolfe JH, Deshmane SL, Fraser NW. (1992) Herpesvirus vector gene transfer and expression of beta-glucuronidase in the central nervous system of MPS VII mice. Nat Genet. 1(5): 379-84. PubMed PMID: 1338772.
   b. Wolfe JH, Sands MS, Barker JE, Gwynn B, Rowe LB, Vogler CA, Birkenmeier EH. (1992) Reversal of pathology in murine mucopolysaccharidosis type VII by somatic cell gene transfer. Nature. 360: 749-53. PubMed PMID: 1465145.
   c. Wolfe JH, Sands MS, Harel N, Weil MA, Parente MK, Polesky AC, Reilly JJ, Hasson C, Weimelt S, Haskins ME. (2000) Gene transfer of low levels of beta-glucuronidase corrects hepatic lysosomal

storage in a large animal model of mucopolysaccharidosis VII. Mol Ther. 2: 552-61. PubMed PMID: 11124056.

d. Ponder KP, Melniczek JR, Xu L, Weil MA, O'Malley TM, O'Donnell PA, Knox VW, Aguirre GD, Mazrier H, Ellinwood NM, Sleeper M, Maguire AM, Volk SW, Mango RL, Zweigle J, Wolfe JH, Haskins ME. (2002) Therapeutic neonatal hepatic gene therapy in mucopolysaccharidosis VII dogs. Proc Natl Acad Sci USA. 99: 13102-7. PubMed PMCID: PMC130593.

4. Transport of therapeutic protein and specific AAV serotype vectors within axonal pathways of the CNS.

a. Passini MA, Lee EB, Heuer GG, Wolfe JH. (2002) Distribution of a lysosomal enzyme in the adult brain by axonal transport and by cells of the rostral migratory stream. J Neurosci. 22: 6437-46. PubMed PMID: 12151523.

b. Cearley CN, Wolfe JH. Transduction characteristics of adeno-associated virus vectors expressing cap serotypes 7, 8, 9, and Rh10 in the mouse brain. Mol Ther. 2006 Mar;13(3):528-37. PubMed PMID: 16413228.

c. Cearley CN, Wolfe JH. A single injection of an adeno-associated virus vector into nuclei with divergent connections results in widespread vector distribution in the brain and global correction of a neurogenetic disease. J Neurosci. 2007 Sep 12;27(37):9928-40. PubMed PMID: 17855607.

d. Castle MJ, Perlson E, Holzbaur EL, Wolfe JH. (2014) Long-distance axonal transport of AAV9 is driven by dynein and kinesin-2 and is trafficked in a highly motile Rab7-positive compartment. Mol Ther. 22: 554-66. PMCID: PMC3944332.

5. Cell-based treatments for disseminated CNS lesions in neurogenetic disease.

a. Snyder EY, Taylor RM, Wolfe JH. (1995) Neural progenitor cell engraftment corrects lysosomal storage throughout the MPS VII mouse brain. Nature. 374: 367-70. PMID: 7885477.

b. Taylor RM, Wolfe JH. (1997) Decreased lysosomal storage in the adult MPS VII mouse brain in the vicinity of grafts of retroviral vector-corrected fibroblasts secreting high levels of beta-glucuronidase. Nat Med. 3: 771-4. PMID: 9212105.

c. Flax JD, Aurora S, Yang C, Simonin C, Wills AM, Billinghurst LL, Jendoubi M, Sidman RL, Wolfe JH, Kim SU, Snyder EY. (1998) Engraftable human neural stem cells respond to developmental cues, replace neurons, and express foreign genes. Nat. Biotechnol. 16: 1033-9. PMID: 9831031.

d. Weerakkody, T.N., Patel, T.P., Yue, C., Takano, H., Anderson, H.C., Meaney, D.F., Coulter, D.A. and Wolfe, J.H. (2013) Engraftment of exogenous undifferentiated neural stem cells disrupts cortical network activity. Mol. Ther., 21: 2258-2267. PMCID: PMC3863790.

6. Primary neural stem cell-based transplantation.

a. Heuer GG, Skorupa AF, Prasad Alur RK, Jiang K, Wolfe JH. (2001) Accumulation of abnormal amounts of glycosaminoglycans in murine mucopolysaccharidosis type VII neural progenitor cells does not alter the growth rate or efficiency of differentiation into neurons. Mol Cell Neurosci. 17: 167-78. PMID: 11161477.

b. Walton RM, Magnitsky SG, Seiler GS, Poptani H, Wolfe JH. (2008) Transplantation and magnetic resonance imaging of canine neural progenitor cell grafts in the postnatal dog brain. J Neuropathol Exp Neurol. 67: 954-62. PMCID: PMC2856607.

c. Chaubey S, Wolfe JH. (2013) Transplantation of CD15-enriched murine neural stem cells increases total engraftment and shifts differentiation toward the oligodendrocyte lineage. Stem Cells Transl Med. 2: 444-54. PMCID: PMC3673756.

d. Griffin TA, Anderson HC, Wolfe JH. (2015) Ex vivo gene therapy using patient iPSC-derived NSCs reverses pathology in the brain of a homologous mouse model. Stem Cell Reports. 4: 835-46. PMCID: PMC4437470.

D. Other Support

U01-HD-79066-03 (MPIs: Wolfe, J.H., Kaler, S.G.) 12/24/14-11/30/18 (NCE) 3.0 cal mo
NIH-NICHD
Choroid Plexus-Directed Gene Therapy for Alpha-Mannosidosis
This U01 is an intramural/extramural collaborative project with the NIH clinical center to evaluate CSF directed delivery of AAV vectors to treat the brain in alpha-mannosidosis in mouse and cat models, and perform a natural history study of human patients (at the NIH Magnuson Clinical Center). The goal is to develop an IND for a first-in-human gene therapy trial using a CSF delivery route for AAV.

R01-NS088667-03 (PI: Wolfe, J.H.) 2/1/15-1/31/20 2.4 cal mo
NIH/NINDS
Disseminated gene delivery to the CNS by human iPSC-derived neural stem cells.
This project investigates cell culture and genetic modifications to increase the engraftment of neural stem cells in the post-natal brain.

R01-NS038690-14 (PI: Wolfe, J.H.) 9/1/11-8/31/18 (NCE) 2.4 cal mo
NIH/NINDS
Disseminated AAV Vector Transport in the Brain via Neuronal Pathways.
This grant studies the axonal transport of AAV vectors within the brain and their distribution in vivo within specific neurological pathways as strategies to reduce the dosage needed to correct global CNS lesions.

R01-DK063973-11 (PI: Wolfe, J.H.) 9/23/16-8/31/20 2.4 cal mo
NIH/NIDDK
Gene Transfer and NMR Studies in Alpha-Mannosidosis Brain
This project is to evaluate vascular delivery of gene vectors to treat CNS and other organs, and evaluate non-invasive imaging methods to monitor gene transfer and therapeutic effects in the brain.

UL1-TR001878-01 (G Fitzgerald, Prog Dir) 7/1/16-5/31/21 0.6 cal mo
NIH-NCATS
Institutional Clinical and Translational Science Award
Program in Comparative Animal Biology (PI: Wolfe, J.H.)
This small sub-program supports interactions between veterinary and medical faculty to advance translation by evaluating experimental therapeutics in domestic animal diseases that are similar to human diseases.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Henthorn, Paula S.

eRA COMMONS USER NAME (credential, e.g., agency login): HENTHORN

POSITION TITLE: Professor of Medical Genetics

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Illinois, Champaign, IL	B.S.	May 1978	Genetics & Devel. Biol.
University of Wisconsin, Madison	Ph.D.	Dec 1984	Genetics

A. Personal Statement

I am a Ph.D geneticist, trained. I trained in genetics at the University of Wisconsin, where I studied the molecular basis of several forms of human hemoglobinopathies, early in the days of DNA cloning and sequencing. In particular, I cloned and sequenced the DNA spanning and surrounding several very large deletions (of unknown length at the time, but later found to be on the order of 100 kilobase pairs) removing multiple genes in the beta hemoglobin gene locus. I then went on to two postdoctoral fellowships at the University of Pennsylvania. The first included cloning and sequencing for the first time, several human alkaline phosphatase cDNAs using protein-producing cloning vectors and antibody-based screening and followed by cloning and sequencing the genes encoding those cDNAs, and provided the basis for determining the molecular basis of hypophospatasia in humans (a bone disease caused by tissue non-specific alkaline phosphatase deficiency). In my second postdoc, I brought cloning techniques involving screening that depended on protein-DNA interactions to the laboratory and used these techniques to isolate the cDNAs for various transcription factors involved in immunoglobulin expression.

As a faculty member, I have continued to concentrate on the molecular basis of inherited disease, concentrating on naturally occurring genetic diseases of dogs and cats that can serve as homologues of human disease. My laboratory focusses on the identification of the primary genetic defect in diseases that have been captured in the National Referral Center for Animal Models of Human Genetic Disease (NIH OD P40-10939; PI: Vite). This Center establishes and maintains breeding colonies of models of human genetic diseases, with the mission to discover, understand, and develop treatments for naturally occurring hereditary disorders, and genetic predispositions to disease, in dogs and cats that are orthologous to those found in human patients. My laboratories studies lead to genetic tests that allow disease diagnoses in the colony within two days for birth, including cloning cDNA molecules necessary for gene delivery therapy approaches and providing information critical for designing and implementing therapeutic regimes, and also inform continued molecular characterization of molecular pathogenesis. As genetic technologies have advanced, we have also been able to determine the underlying gene defects in dogs and cats using DNA and clinical information from client-owned animals when the disease under study is not amenable (due to late age of onset or complex inheritance pattern) to establishment in a colony setting. Below are references that support our expertise in the study of simple and complex genetic disease in dogs and cats.

a) **Henthorn** PS, Somberg RL, Fimiani VM, Puck JM, Patterson DF and Felsburg PJ (1994) IL-2R gene microdeletion demonstrates that canine X-linked severe combined immunodeficiency is a homologue of the human disease. Genomics 23:69-64.
b) Werner P, Raducha MG, Prociuk U, Sleeper MM and **Henthorn** PS (2008) A novel locus for dilated cardiomyopathy maps to canine chromosome 8. Genomics 91:517-521.
c) Fyfe JC, Al-Tamimi RA, Liu J, Schäffer AA, Agarwala R and **Henthorn** PS (2011) A novel mitofusin 2 mutation causes canine fetal-onset neuroaxonal dystrophy. Neurogenetics 12(3):223-232.
d) Littman MP, Wiley CA, Raducha MG and **Henthorn** PS (2013) Glomerulopathy and mutations in

NPHS1 and KIRREL2 in soft-coated Wheaten Terrier dogs. Mammalian Genome 24(3-4):119-26. PMID: 23325127

B. Positions and Honors

Positions

1978-1984 Ph.D. Candidate - in the laboratory of Dr. Oliver Smithies
1984-1987 Postdoctoral Fellow - in the laboratory of Dr. Harry Harris
1987-1989 Research Associate - Howard Hughes Medical Institute - laboratory of Dr. Tom Kadesch
1990-1996 Assistant Professor of Medical Genetics - U. of Penn. School of Veterinary Medicine
1996-2005 Associate Professor of Medical Genetics - U. of Penn. School of Veterinary Medicine
2005-present Professor of Medical Genetics - U. of Penn. School of Veterinary Medicine
2008-present Chief, Section of Medical Genetics, U. of Penn. Sch. Vet. Med.

Honors and Awards

1978-1979 Wisconsin Alumni Research Foundation Fellowship
1978 Phi Beta Kappa
1979-1981 NIH Predoctoral Training Grant Fellowship
1984-1987 NIH Postdoctoral Training Grant Fellowship
1991 Young Investigator Award - American Society for Bone and Mineral Research
1996 Phi Zeta Veterinary Research Honorary Society
1998 Pfizer Award for Research Excellence

Other Experience and Professional Membership

2008-present Chief, Section of Medical Genetics, U. of Penn. Sch. Vet. Med.

C. Contributions to Science

The overarching theme of my career has been the discovery of the DNA variations underlying inherited disease in mammals. My early studies involved characterization of naturally occurring deletions in the human beta-globin gene locus causing various hemoglobin deficiency states. From there I moved to the study of canine and feline inherited disease both for use as homologues of human genetic diseases and for the direct benefit to cats and dogs. My initial work utilized breeding colonies of naturally-occurring canine and feline models of human genetic diseases that had been established to better understand disease pathogenesis, to develop therapies, and to develop biomarkers of disease severity and therapeutic efficacy. The candidate genes for many of these diseases were known, and could be cloned based on knowledge of the human disease. More recently, I have been able to make use of client-owned dogs and cats to study disease with a complex genetic basis and without obvious candidate genes. In this case, the work has had a larger "discovery" component.

As a geneticist, one studies the genetic basis of disease, which leads to more in depth studies of the disease, which are not genetic in nature. So as a geneticist, once underlying mutations are identified, one either carries on and studies other aspects of the disease, using other non-genetic approaches, or moves on to work out the underlying DNA changes on other diseases. I have done the latter, and thus have been involved in many diseases, providing critical information for the study of these disease, but not necessarily contributing a great deal to the body of knowledge about any disease in particular. (references below selected from 98 peer reviewed publications)

1. Human molecular genetics, cloning and sequencing, hemoglobinopathies, deletion analysis, cloning alkaline phosphatase cDNAs by protein expression in bacteria and antibody screening, and cloning transcription factors using DNA binding-sites as probes for protein-DNA interaction: In all of these cases, I used unusual approaches to clone cDNAs or previously unidentified deletion breakpoint regions.

   a. Vanin EF, **Henthorn** PS, Kioussis D, Grosveld F, Smithies O. Unexpected relationships between four large deletions in the human beta-globin gene cluster. Cell. 1983; 35(3 Pt 2):701-9. PubMed [journal] PMID: 6652684
   b. Mager DL, **Henthorn** PS. Identification of a retrovirus-like repetitive element in human DNA. Proceedings of the National Academy of Sciences of the United States of America. 1984; 81(23):7510-4. PubMed [journal] PMID: 6095301, PMCID: PMC392176
   c. Elder JT, Forrester WC, Thompson C, Mager D, **Henthorn** P, Peretz M, Papayannopoulou T, Groudine M. Translocation of an erythroid-specific hypersensitive site in deletion-type hereditary persistence of fetal hemoglobin. Molecular and cellular biology. 1990; 10(4):1382-9. PMID:1690839, PMCID: PMC362240

d. **Henthorn** P, Kiledjian M, Kadesch T. Two distinct transcription factors that bind the immunoglobulin enhancer microE5/kappa 2 motif. Science (New York, N.Y.).1990; 247(4941):467-70. PMID: 2105528

2. Canine (and human) X-linked SCID and initial trials for gene therapy using isolated CD34 positive bone marrow cells

   a. **Henthorn** PS, Somberg RL, Fimiani VM, Puck JM, Patterson DF, Felsburg PJ. IL-2R gamma gene microdeletion demonstrates that canine X-linked severe combined immunodeficiency is a homologue of the human disease. Genomics. 1994; 23(1):69-74. PMID: 7829104
   b. Puck JM, Deschênes SM, Porter JC, Dutra AS, Brown CJ, Willard HF, **Henthorn** PS.The interleukin-2 receptor gamma chain maps to Xq13.1 and is mutated in X-linked severe combined mmunodeficiency, SCIDX1. Human molecular genetics. 1993;2(8):1099-104. PMID: 8401490
   c. Suter SE, Gouthro TA, McSweeney PA, Nash RA, Haskins ME, Felsburg PJ, **Henthorn** PS. Isolation and characterization of pediatric canine bone marrow CD34+ cells. Veterinary immunology and immunopathology. 2004; 101(1-2):31-47PMID: 15261691
   d. Suter SE, Gouthro TA, O'Malley T, Hartnett BJ, McSweeney PA, Moore PF, Felsburg PJ, Haskins ME, **Henthorn** PS. Marking of peripheral T-lymphocytes by retroviral transduction and transplantation of CD34+ cells in a canine X-linked severe combined immunodeficiency model. Veterinary immunology and immunopathology. 2007;117(3-4):183-96. PMID: 17442404

3. Determining molecular defect in canine and feline lysosomal storage diseases and other human disease homologues used in gene therapy approaches.

   a. Casal ML, Scheidt JL, Rhodes JL, **Henthorn** PS, Werner P. Mutation identification in a canine model of X-linked ectodermal dysplasia. Mammalian genome 2005; 16(7):524-31. PMID: 16151697, PMCID: PMC3330241
   b. Fyfe JC, Kurzhals RL, Lassaline ME, **Henthorn** PS, Alur PR, Wang P, Wolfe JH, Giger U, Haskins ME, Patterson DF, Sun H, Jain S, Yuhki N. Molecular basis of feline beta-glucuronidase deficiency: an animal model of mucopolysaccharidosis VII. Genomics. 1999; 58(2):121-8. PMID: 10366443
   c. Ray J, Bouvet A, DeSanto C, Fyfe JC, Xu D, Wolfe JH, Aguirre GD, Patterson DF, Haskins ME, **Henthorn** PS. Cloning of the canine beta-glucuronidase cDNA, mutation identification in canine MPS VII, and retroviral vector-mediated correction of MPS VII cells. Genomics. 1998; 48(2):248-53. PMID: 9521879
   d. Fyfe JC, Kurzhals RL, Hawkins MG, Wang P, Yuhki N, Giger U, Van Winkle TJ, Haskins ME, Patterson DF, **Henthorn** PS. A complex rearrangement in GBE1 causes both perinatal hypoglycemic collapse and late-juvenile-onset neuromuscular degeneration in glycogen storage disease type IV of Norwegian forest cats. Molecular genetics and metabolism. 2007; 90(4):383-92. PMID: 17257876, PMCID: PMC2063609

4. Genetic characterization of congenital heart disease in dogs

   a. Werner P, Raducha MG, Prociuk U, Budarf M, **Henthorn** PS, Patterson DF. Comparative mapping of the DiGeorge region in the dog and exclusion of linkage to inherited canine conotruncal heart defects. The Journal of heredity. 1999; 90(4):494-8. PMID: 10485139
   b. Werner P, Raducha MG, Prociuk U, Ostrander EA, Spielman RS, Kirkness EF, Patterson DF, **Henthorn** PS. The keeshond defect in cardiac conotruncal development is oligogenic. Human genetics. 2005; 116(5):368-77. PMID: 15711798
   c. Werner P, Raducha MG, Prociuk U, Sleeper MM, Van Winkle TJ, **Henthorn** PS. A novel locus for dilated cardiomyopathy maps to canine chromosome 8. Genomics. 2008;91(6):517-21. PMID: 18442891, PMCID:PMC2486407

5. Molecular basis of complex genetic disease and disease of previously unknown etiology in dogs and cats:

   a. Fyfe JC, Al-Tamimi RA, Liu J, Schäffer AA, Agarwala R, **Henthorn** PS. A novel mitofusin 2 mutation causes canine fetal-onset neuroaxonal dystrophy. Neurogenetics. 2011; 12(3):223-32. PMID:21643798, PMCID: PMC3165057
   b. Massey J, Boag A, Short AD, Scholey RA, **Henthorn** PS, Littman MP, Husebye E, Catchpole B, Pedersen N, Mellersh CS, Ollier WE, Kennedy LJ. MHC class II association study in eight breeds of dog with hypoadrenocorticism. Immunogenetics. 2013; 65(4):291-7. PMID: 23358933
   c. Littman MP, Wiley CA, Raducha MG, **Henthorn** PS. Glomerulopathy and mutations in NPHS1 and

KIRREL2 in soft-coated Wheaten Terrier dogs. Mammalian genome. 2013;24(3-4):119-26 PMID: 23325127

d. Brons AK, **Henthorn** PS, Raj K, Fitzgerald CA, Liu J, Sewell AC, Giger U. SLC3A1 and SLC7A9 mutations in autosomal recessive or dominant canine cystinuria: a new classification system. Journal of veterinary internal medicine. 2013; 27(6):1400-8. PMID: 24001348, PMCID:PMC3946761

NCBI bibliography:
https://www.ncbi.nlm.nih.gov/pubmed/?term=henthorn+p

D. Research Support
Current

NIH-P40-OD-010939-32 (PI Vite) 9/30/2013-2/28/2017
"Referral Center - Animal Models of Human Genetic Disease"
To discover, understand, and treat naturally occurring hereditary disorders, and genetic predispositions to disease, in dogs and cats that are orthologous to those found in human patients.
Role: Co-Investigator, I oversee and direct discovery of disease-associated alleles, and design and implement molecular (DNA-based) tests, including such things as assessment of major histocompatibility loci for use in donor and recipient matching for transplantation work.

Scottish Deerhound Club of America (PI Henthorn) 11/1/2016- 6/30/2018
"Next Step With Cystinuria Research in Scottish Deerhounds"
The goals of the project are to perform whole genome sequencing on cystinuric and normal Scottish Deerhounds and compare to phenotypic data to to clarify the molecular etiology of this disease in the Scottish Deerhound breed, and compare it to findings in other dog breeds and cystinuric humans.
Role: PI

Recently Completed:

BCA Charitable Fund Henthorn (PI) 3/1/2015-12/31/2015
"Investigation of Type 3 Cystinuria in Bulldogs"
The major goal of this project is to determine if cystinuria in this breed is associated with polymorphisms in the SLC3A1 that have been previously identified in other breeds, and catalogue the phenotypic variation of the disease in this dog breed in order to clarify the molecular etiology of this disease.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Margret L. Casal

eRA COMMONS USER NAME (credential, e.g., agency login): casalml

POSITION TITLE: Associate Professor of Medical Genetics

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Zürich, Switzerland	Med. Vet.*	09/1984	Veterinary Medicine
University of Bern, Switzerland	Dr. phil. II**	01/1988	Virology/Pathology
University of Pennsylvania, Philadelphia, PA	Residency	07/1994	Medical Genetics
University of Pennsylvania, Philadelphia, PA	PhD	04/1999	Pathology/Gene therapy
*DVM equivalent; **Master's equivalent			

A. Personal Statement

I have been working with dog, cat, and mouse models of mucopolysaccharide disorders since arriving at the University of Pennsylvania in 1991. Initially, I was responsible for their veterinary care and assisted with reproductive/breeding plans. Thereafter, my graduate studies involved in utero gene therapy in mice with MPS VII, during which I continued to provide veterinary care for the large animal models, including reproductive emergencies such as Cesarean sections. I then became a faculty member with my own research but continued to provide veterinary care of the canine and feline models and continue to perform my own experiments in the MPS I and VII animals. I became board certified in reproduction in 2001 and am head of the Small Animal (feline and canine) Reproduction Clinic at the School of Veterinary Medicine, University of Pennsylvania. With my expertise in assisted reproductive techniques (e.g. oocyte harvest, embryo transfer, artificial insemination), I will continue to provide reproductive support in the production of the CRISPR-Cas9 cat models. My long-term goal is to expand these techniques to the dog to provide additional large animal models. I have the expertise, training, and medical knowledge to perform and assist with the proposed experiments.

1. **Casal ML**, Wolfe JH. (2001). *In utero* transplantation of fetal liver cells in the mucopolysaccharidosis type VII mouse results in low level chimerism but overexpression of ß-glucuronidase can delay onset of clinical signs. Blood 97:1625-1634.
2. Bradbury AM, Gurda BL, **Casal ML**, Ponder K, Vite C, Haskins ME. (2015) A review of gene therapy in canine and feline models of lysosomal storage disorders. Hum Gene Ther Clin Dev 26:27-37 PMID: 25671613 PMC4516914
3. Hinderer C, Bell P, Louboutin JP, Zhu Y, Yu H, Lin G, Choa R, Gurda BL, Bagel J, O'Donnell P, Sikora T, Ruane T, Wang P, Tarantal AF, **Casal ML**, Haskins ME, Wilson JM. (2015) Neonatal systemic AAV induces tolerance to CNS gene therapy in MPS I dogs and nonhuman primates. Molec Therapy 23:1298-1307. PMD: 26022732 PMC: 4817868.
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5. Hinderer C, Bell P, Katz N, Vite CH, Louboutin JP, Bote E, Yu H, Zhu Y, **Casal ML**, Bagel J, ODonnell P, Wang P, Haskins ME, Goode T, Wilson JM (2017) Evaluation of intrathecal routes of administration for

adeno-associated virus vectors in large animals. Hum Gene Ther. Oct 3. doi: 10.1089/hum.2017.026

B. Positions and Honors

Positions and Employment

1984-85 Fulltime Fellow in the Department of Pathology, University of Zürich, Switzerland
1985-86 Fulltime doctoral thesis research; Federal Institute of Vaccines, Basle, Switzerland; Doctor of Veterinary Medicine Degree (Dr med vet; Master's Degree equivalent; January 1988)
1986-88 Part-time (50%) veterinarian in charge of the research colony, Biological Central Laboratory and part-time (50%) staff veterinarian at the Department of Reproduction, University of Zürich
1988-91 Full time instructor and researcher at the Department of Reproduction, University of Zürich
1991-94 Resident in Medical Genetics, School of Veterinary Medicine, University of Pennsylvania
1994-99 PhD graduate student in the Pathology Graduate Group, University of Pennsylvania. PhD degree conferred. Topic "In utero gene therapy of murine mucopolysaccharidosis type VII"
1999 Fellowship in the Department of Hematology/Oncology, University of Illinois at Chicago.
1999-2009 Assistant Prof. of Medical Genetics, School of Veterinary Medicine, University of Pennsylvania
2009- Associate Prof. of Medical Genetics, School of Veterinary Medicine, University of Pennsylvania

Honors and Awards

1994 Randy Award - Devon Rex Breeders Association
1995 Phi Zeta Veterinary Honor Society - University of Pennsylvania
1995-98 National Science Research Award
2001 Diplomate, European College of Animal Reproduction
2003 K01 - NIAMS
2005 Residents' Excellence in Teaching Award, University of Pennsylvania
2006 Kenneth S. Brown MD Research Award, National Foundation of Ectodermal Dysplasias
2006 Pfizer Research Award for Research Excellence, University of Pennsylvania

C. Contributions to Science

1. My early publications were directed at identifying large animal models of human genetic disease. One of these discovered models led to one of my primary areas of research. All of these studies were and are funded by a P40 grant of which I am a co-PI (Previously NIH RR-002512, now OD010939). A few of these models are listed here. As part of this grant, I continue to define new canine and feline models of human disease that we identify in the clinics at our veterinary hospital and through submissions from referring veterinarians. Whenever possible my lab identifies the gene mutations and the biochemical defects. Large animal models of human genetic disease have become exceedingly important, as they provide a valuable bridge between proof of principle models such as tissue culture and mice and clinical trials in humans. These naturally occurring diseases faithfully replicate the disease in humans and are true orthologues. As opposed to mice, dogs and cats are long-lived with an immune system as diverse as a human's. Also, scaling up from a mouse to a human can pose a problem because of the extreme size difference (adult mouse 30 grams, average human adult 70 kg; 2333-fold difference), yet our dogs weigh on average 15 kg (4.7-fold difference).

a) Olivry T, Linder KE, Wang P, Bizikova P, Bernstein JA, Dunston SM, Paps JS, **Casal ML** (2012) Deficient Plakophilin-1 expression due to a mutation in *PKP1* causes ectodermal dysplasia-skin fragility syndrome in Chesapeake Bay retriever dogs. PLoS-One 7(2):e32072.
b) Mauldin EA, Wang P, Olivry T, Henthorn PS, **Casal ML**. (2017) Epidermolysis bullosa simplex in sibling Eurasier dogs is caused by a PLEC non-sense variant. Vet Dermatol. 2017 Feb;28(1):10-13.
c) **Casal ML**, Wang P, Mauldin EA, Lin G, Henthorn PS. (2017) A Defect in NIPAL4 Is Associated with Autosomal Recessive Congenital Ichthyosis in American Bulldogs. PLoS One. 25;12(1):e0170708.
d) Wang P, Henthorn PS, Galban E, Lin G, Takedai T, **Casal ML**. Canine GM2-gangliosidosis - Sandhoff disease associated with a 3-base-pair deletion in the HEXB gene. J Vet Intern Med, Nov 6. Doi: 10.1111/jvim.14862; 2017 [Epub ahead of print]. PMCID in progress.

2. X-linked hypohidrotic ectodermal dysplasia (XLHED) caused by a defect in the *EDA* gene is the most common form of ectodermal dysplasia in humans. It is characterized by missing and malformed teeth, lack of sweat, respiratory, and esophageal glands, After working up the initial case and establishing a model in our animal colony, together with my collaborators at the University of Lausanne in Switzerland, we tested the

efficacy of a recombinant protein (rEDA) for the treatment of ectodermal dysplasia. Because of our successes in this canine model, in a recent first clinical trial, five newborn children with X-linked ectodermal dysplasia have been treated. No side effects were noted and the children (like the dogs) tolerated the treatment very well. At this time, they are too young to examine the beneficial effects of the protein. We have completed our experiments examining the effects of in utero treatment with rEDA in our canine model and two papers have been submitted for publication.

a) **Casal, M. L.,** Lewis, J.R., Mauldin, E.A., Tardivel, A., Ingold, K., Favre, M., Paradies, F., Demotz, S., Gaide, O., Schneider, P. (2007). Significant correction of disease after postnatal administration of recombinant EDA in canine X-linked ectodermal dysplasia**.** Am J Hum Gen 81:1050-1056. PMCID: PMC2265652.
b) Mauldin, E. A., Gaide, O ,Schneider, P., **Casal, M. L.** (2009) Neonatal treatment with recombinant ectodysplasin prevents respiratory disease in dogs with X-linked ectodermal dysplasia. Am J Med Genet A. Sep;149A(9):2045-9. PMCID: PMC2754310.
c) Lewis, J. R., Reiter, A. M., Mauldin, E. A., **Casal, M. L.** (2010) Dental abnormalities associated with X-linked hypohidrotic ectodermal dysplasia in dogs Orthod Craniofac Res;13:40–47. PMCID: PMC2808637.
d) Kowalczyk C, Dunkel N, Willen L, **Casal ML**, Mauldin EA, Gaide O, Tardivel A, Badic G, Etter A, Favre M, Jefferson DM, Headon DJ, Demotz S, Schneider P. (2011) Molecular and therapeutic characterization of anti-ectodysplasin a receptor (edar) agonist monoclonal antibodies. J Biol Chem, 286:30769-79. PMCID: PMC3162438.

3. As stated in my personal statement, I have worked with the canine and feline models of mucopolysaccharidoses since the early 1990's. Besides assisting with the experiments at that time, I developed my own research during my graduate studies and postdoctoral period. During the past few years, I have increased my involvement again with the MPS disorders and oversee the lab that performs all of the biochemical analyses for MPS I, VI, and VII, as I am very familiar with all of the assays and interpretation of the results. I have been performing all of the post mortem exams including preparing the brain and the spinal cord for analysis. I have just finished another set of gene therapy experiments examining AAV vectors given to MPS VII dogs IV and intrathecally. Most of the data has been analyzed and we are preparing a paper. The next set of AAV experiments has been performed and the tissues are being analyzed. We have also obtained two additional grants to examine at the long-term biology of disease in MPS I dogs and to perform cognitive studies in MPS I dogs with and without IT AAV therapy. The first study is ongoing and the animal experiments have been completed for the second experiment. The data is being evaluated and shows improved cognition in the treated animals versus the non-treated MPS I dogs.

a) Gurda BL, De Guilhem De Lataillade A, Bell P, Zhu Y, Yu H, Wang P, Bagel J, Vite CH, Sikora T, Hinderer C, Calcedo R, Yox AD, Steet RA, Ruane T, O'Donnell P, Gao G, Wilson JM, **Casal M**, Ponder KP, Haskins ME (2016) Evaluation of AAV-mediated Gene Therapy for Central Nervous System Disease in Canine Mucopolysaccharidosis VII. Mol Ther 24:206-216.
b) Peck SH, **Casal ML**, Malhotra NR, Ficicioglu C, Smith LJ (2016) Pathogenesis and treatment of spine disease in the mucopolysaccharidosis. Mol Genet Metab 118:232-243. PMCID: PMC4970936
c) Hinderer C, Bell P, Louboutin JP, Katz N, Zhu Y, Lin G, Choa R, Bagel J, O'Donnell P, Fitzgerald CA, Langan T, Wang P, **Casal ML**, Haskins ME, Wilson JM (2016) Neonatal tolerance induction enables accurate evaluation of gene therapy for MPS I in a canine model. Mol Genet Metab. S1096-7192:30105-30106.
d) Hinderer C, Bell P, Katz N, Vite CH, Louboutin JP, Bote E, Yu H, Zhu Y, **Casal ML**, Bagel J, ODonnell P, Wang P, Haskins ME, Goode T, Wilson JM (2017) Evaluation of intrathecal routes of administration for adeno-associated virus vectors in large animals. Hum Gene Ther. Oct 3. doi: 10.1089/hum.2017.026

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/sites/myncbi/margret.casal.1/bibliography/45037007/public/?sort=date&direction=ascending

D. Additional Information: Research Support and/or Scholastic Performance
Ongoing research support:
R01 DK054481 (PI Casal) 5/1/14-4/30/18
Gene therapy for mucopolysaccharidosis type VII.

Novel gene therapies for the treatment of MPS VII using a canine model.
Role: PI

MPS I 16-GTP-01 Orphan Disease Center (PI Casal) 4/1/16 – 7/31/18
Biology of Disease study – canine MPS I. To investigate long term effect of MPS I in dogs.
Role: PI

MPS I-16-005-01 Orphan Disease Center (PI Lachlan Smith) 7/1/16 – 6/30/18
Novel therapies to improve bone formation in Mucopolysaccharidosis I Dogs. To examine the effects of lithium on bone development in dogs with MPS I.
Role: Co-investigator

Ultragenyx Pharmaceuticals (PI Lachlan Smith) 10/1/16 – 9/30/18
Treatment of canine MPS VII using recombinant enzyme IV. To test the ability of this large molecule to correct systemic disease with a focus on spine and joints.
Role: Co-investigator

Completed Research Support:
MPS I-15-001-01 Orphan Disease Center (PI Casal) 5/1/15 – 7/31/17
Improved Therapies for MPS I Grant Program. To investigate cognitive functions in MPS I dogs with and without AAV gene therapy and compared to normal dogs.
Role: PI

P40 OD010939 NCRR/NIH (PI Charles Vite) 4/1/13 – 11/30/17
Referral Center, Animal Models of Human Genetic Disease. To discover, develop and define animal models of human genetic disease. This grant has supported most of the dogs used for the study of genodermatoses.
Role: Co-investigator

EdimerPharma (PI Casal) 12/15/09 – 6/30/16
Use of recombinant EDA-A1 for the treatment of canine ectodermal dysplasia (XHED). This study parallels the first clinical trials in humans with the same disease.
Role: PI

AKC CHF (PI Casal) 6/1/13 – 5/31/14
Funding for elucidation of the molecular cause of lethal acrodermatitis in the bull terrier
Role: PI

ISVD (PI Casal) 7/1/11 – 6/30/13
International Society for Veterinary Dermatology: Ichthyosis in the Labrador retriever. Funds are provided to determine the gene and mutation causing this autosomal recessive form of a keratinization disorder.
Role: PI

Irish Wolfhound Seizure Study, Inc. (PI: Casal) 6/6/99 – 6/5/13
Funding for studies benefiting Irish Wolfhounds, in particular epilepsy and primary ciliary dyskinesia.
Role: PI