PI: **AGUIRRE, GUSTAVO David**	Title: Models for Therapy of Hereditary Retinal Degeneration	
Received: 11/02/2018	FOA: PA18-484 Clinical Trial:Not Allowed	Council: 05/2019
Competition ID: FORMS-E	FOA Title: NIH Research Project Grant (Parent R01 Clinical Trial Not Allowed)	
2 R01 EY006855-34	Dual:	Accession Number: 4234960
IPF: 6463801	Organization: UNIVERSITY OF PENNSYLVANIA	
Former Number:	Department: 5804 - Clinical Sciences & Adv	
IRG/SRG: DPVS	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 34: 498,724 Year 35: 498,724 Year 36: 498,724 Year 37: 498,724 Year 38: 498,724	Animals: Y Humans: N Clinical Trial: N Current HS Code: 10 HESC: N	New Investigator: N Early Stage Investigator: N
Senior/Key Personnel:	*Organization:*	*Role Category:*
GUSTAVO AGUIRRE	The Trustees of the University of Pennsylvania	PD/PI
KEIKO MIYADERA	The Trustees of the University of Pennsylvania	MPI
WILLIAM BELTRAN	The Trustees of the University of Pennsylvania	Other (Specify)-Other Significant Contributor
ARTUR CIDECIYAN	The Trustees of the University of Pennsylvania	Other (Specify)-Other Significant Contributor
KARINA GUZIEWICZ	The Trustees of the University of Pennsylvania	Other (Specify)-Other Significant Contributor
Alfred Lewin	UNIVERSITY OF FLORIDA	Other (Specify)-Other Significant Contributor
William Hauswirth PhD	UNIVERSITY OF FLORIDA	Other (Specify)-Other Significant Contributor
SAMUEL JACOBSON	The Trustees of the University of Pennsylvania	Other (Specify)-Other Significant Contributor

OMB Number: 4040-0001
Expiration Date: 10/31/2019

APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

3. DATE RECEIVED BY STATE	State Application Identifier

1. TYPE OF SUBMISSION*

○ Pre-application ● Application ○ Changed/Corrected Application

4.a. Federal Identifier
EY006855

b. Agency Routing Number

2. DATE SUBMITTED
2018-11-02

Application Identifier
10070196

c. Previous Grants.gov Tracking Number

5. APPLICANT INFORMATION

Organizational DUNS*: 0422507120000

Legal Name*: The Trustees of the University of Pennsylvania
Department: 5804 - Clinical Sciences & Adv
Division:
Street1*: Office of Research Services
Street2: 3451 Walnut Street, 5th Floor
City*: Philadelphia
County: Philadelphia
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 19104-6205

Person to be contacted on matters involving this application
Prefix: First Name*: ELIZABETH Middle Name: D Last Name*: PELOSO Suffix:
Position/Title: AssocVicePres/AssocViceProvost for Research
Street1*: 3451 Walnut Street
Street2: Franklin Building, 5th floor
City*: Philadelphia
County:
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 19104-6205
Phone Number*: 2157460234 Fax Number: 2158989708 Email: PennAORs@lists.upenn.edu

6. EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)** 1231352685A1

7. TYPE OF APPLICANT* O: Private Institution of Higher Education

Other (Specify):
Small Business Organization Type ○ Women Owned ○ Socially and Economically Disadvantaged

8. TYPE OF APPLICATION*

○ New ○ Resubmission
● Renewal ○ Continuation ○ Revision

If Revision, mark appropriate box(es).
○ A. Increase Award ○ B. Decrease Award ○ C. Increase Duration
○ D. Decrease Duration ○ E. Other *(specify)* :

Is this application being submitted to other agencies?* ○Yes ●No What other Agencies?

9. NAME OF FEDERAL AGENCY*
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER
TITLE:

11. DESCRIPTIVE TITLE OF APPLICANT'S PROJECT*
Models for Therapy of Hereditary Retinal Degeneration

12. PROPOSED PROJECT
Start Date* 12/01/2019
Ending Date* 11/30/2024

13. CONGRESSIONAL DISTRICTS OF APPLICANT
PA-002

SF 424 (R&R) APPLICATION FOR FEDERAL ASSISTANCE

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix: DR. First Name*: GUSTAVO Middle Name: D Last Name*: AGUIRRE Suffix:
Position/Title: PROFESSOR OF OPHTHALMOLOGY
Organization Name*: The Trustees of the University of Pennsylvania
Department: 5804 - Clinical Sciences & Adv
Division:
Street1*: [redacted]
Street2:
City*: PHILADELPHIA
County: Philadelphia
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 19104-6010
Phone Number*: [redacted] Fax Number: - Email*: gda@vet.upenn.edu

15. ESTIMATED PROJECT FUNDING

a. Total Federal Funds Requested*	$4,014,730.00
b. Total Non-Federal Funds*	$0.00
c. Total Federal & Non-Federal Funds*	$4,014,730.00
d. Estimated Program Income*	$0.00

16.IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?*

a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
DATE:

b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● I agree*

** The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

18. SFLLL or OTHER EXPLANATORY DOCUMENTATION File Name:

19. AUTHORIZED REPRESENTATIVE

Prefix: First Name* [redacted] Middle Name: Y Last Name*: [redacted] Suffix:
Position/Title*: Associate Director
Organization Name*: The Trustees of the University of Pennsylvania
Department: 8760 - Research Services
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County: PHILADELPHIA
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
ZIP / Postal Code*: 19104-6205
Phone Number* [redacted] Fax Number: 2158989708 Email*: PennAORs@lists.upenn.edu

Signature of Authorized Representative*

[redacted]

Date Signed*
11/02/2018

20. PRE-APPLICATION File Name:

21. COVER LETTER ATTACHMENT File Name:EY006855_Cover letter.pdf

424 R&R and PHS-398 Specific
Table Of Contents

OMB Number: 4040-0010
Expiration Date: 10/31/2019

Project/Performance Site Location(s)

Project/Performance Site Primary Location

❍ I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

Organization Name: The Trustees of the University of Pennsylvania
Duns Number: 0422507120000
Street1*:
Street2:
City*: Philadelphia
County: Philadelphia
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 19104-6010
Project/Performance Site Congressional District*: PA-002

Additional Location(s) File Name:

Funding Opportunity Number: PA-18-484. Received Date: 2018-11-02T11:54:23.000-04:00

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Other Project Information

1. Are Human Subjects Involved?* ○ **Yes** ● **No**

1.a. If YES to Human Subjects

Is the Project Exempt from Federal regulations? ○ Yes ○ No

If YES, check appropriate exemption number: _ 1 _ 2 _ 3 _ 4 _ 5 _ 6 _ 7 _ 8

If NO, is the IRB review Pending? ○ **Yes** ○ **No**

IRB Approval Date:

Human Subject Assurance Number

2. Are Vertebrate Animals Used?* ● **Yes** ○ **No**

2.a. If YES to Vertebrate Animals

Is the IACUC review Pending? ● Yes ○ No

IACUC Approval Date:

Animal Welfare Assurance Number A3079-01

3. Is proprietary/privileged information included in the application?* ○ **Yes** ● **No**

4.a. Does this project have an actual or potential impact - positive or negative - on the environment?* ○ **Yes** ● **No**

4.b. If yes, please explain:

4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? ○ Yes ○ No

4.d. If yes, please explain:

5. Is the research performance site designated, or eligible to be designated, as a historic place?* ○ **Yes** ● **No**

5.a. If yes, please explain:

6. Does this project involve activities outside the United States or partnership with international collaborators?* ● **Yes** ○ **No**

6.a. If yes, identify countries: Canada and Belgium

6.b. Optional Explanation: we provide assistance to vision research scientists

	Filename
7. Project Summary/Abstract*	EY006855_Project Summary_Abstract.pdf
8. Project Narrative*	EY006855_Project Narrative.pdf
9. Bibliography & References Cited	EY006855_Bibliography.pdf
10.Facilities & Other Resources	Facil Resources_LM.pdf
11.Equipment	Equipment_LM.pdf

Funding Opportunity Number: PA-18-484. Received Date: 2018-11-02T11:54:23.000-04:00

Inherited retinal diseases (IRDs) such as retinitis pigmentosa, Leber congenital amaurosis, cone-rod dystrophy, and Best macular dystrophy are devastating blinding diseases in people. While mutations in nearly 270 genes have been associated with different forms of IRDs to date, characterization of disease mechanisms and identification of therapeutic targets for many of these IRDs are yet to be developed. Encouraging clinical successes with gene replacement therapy have emerged in recent years for several forms of IRD in man, and some of these treatments have resulted from proof of principle studies carried out in dog models by our research group. At the [redacted] we have an established research capability and expertise that has allowed us to mechanistically assess over 20 canine strains and their crosses, each of which represent different forms of naturally-occurring IRDs. Using a subset of these canine models, we aim to better understand the molecular basis and pathogenic mechanisms of these unique IRDs, and evaluate new therapies to prevent or ameliorate disease. Thus renewal of the proposed program will allow continued advances in translational studies using canine models of IRDs, providing a sound basis for future development of new and effective therapies for human retinal degenerative diseases.

At our centralized resource facility, we will breed and maintain specific canine IRD strains with rigorously characterized phenotypes/genotypes. Investigators will be provided with mutant and age-matched control dogs either for independent or collaborative studies. The aim of these studies is to understand the molecular mechanisms involved in IRDs, and develop new therapies that can be evaluated on a short- or long-term basis. This centralized resource will also be used by multiple investigators to accomplish the research goals of their own NIH-funded grants. Lastly, hypothesis-driven studies by the PIs and collaborators will be aimed at characterizing new patient-relevant IRD models, studying their underlying cellular/molecular mechanisms, examining the role of inflammation and microglia/macrophages in IRDs for the identification of optimal therapeutic targets, optimizing targeting of different retinal cell types such as ON-bipolar cells, and testing the effect of a new protease in facilitating intravitreal AAV therapies. Our principal hypothesis is that the collaborative research using canine models from a centralized, well-maintained resource facility, supported by a team of investigators with expertise in both clinical ophthalmology and molecular/cell biology, will lead to critical proof-of-principle studies directed at developing safe and effective new therapies for IRDs in patients. The proposed program and studies are designed to fully address this hypothesis.

Inherited retinal diseases that lead to devastating blinding conditions were previously considered incurable, but a handful of them can now be treated with emerging new gene therapy options. However, before potential therapies can be made available for patients, it is critical that they are tested for effectiveness and evaluated for safety in appropriate clinically-relevant model systems. The research proposal aims to understand the mechanisms of genetic mutations causing retinal diseases, and to establish preclinical proof-of-principle by developing and testing new therapies that prevent or delay the retinal degeneration that leads to blindness.

FACILITIES AND OTHER RESOURCES

School of Veterinary Medicine, University of Pennsylvania

Background: All aspects of the dog studies at the core of this proposal are conducted at the School of Veterinary Medicine. In vivo studies will be carried out at the [redacted] of the School of Veterinary Medicine. All other studies will be done on the [redacted] campus of the School of [redacted] where laboratories assigned to MPIs Drs. Gustavo Aguirre and Keiko Miyadera are located.

1 [redacted] campus of School of Veterinary Medicine

Research Laboratory: Recently renovated laboratories (3277 ft^2) designed to function as a center for molecular studies of canine retinal diseases and their treatment. Facilities include: microtomy, cryomicrotomy, photomicrography, PCR and radioisotope laboratory, general equipment room, computer area for sequencing and data analysis. The laboratories consist of 5 modules with a central core equipment area. These facilities are dedicated to the ongoing funded studies dealing with various aspects of inherited retinal diseases and their treatment.

Clinical: Although only used in special circumstances, the facilities at [redacted] are suited for all aspects of clinical ophthalmic examinations, and include all equipment necessary for the examinations. More extensive clinical resources are present at the [redacted] ([redacted]), which is the site of the research dog colonies, and has dedicated: eye examination rooms, an operating suite for ocular microsurgery, a suite for retinal imaging, a suite for electroretinography, and rooms dedicated for visual behavior testing.

Computer: Networked Apple Macintosh and Dell computers in the offices and laboratories with on-line access to MEDLINE, Genbank, GDB/OMIM and other databases. A high end Dell Computer server supported by the Bioinformatics group is used for sequence analysis. Additionally, we have a subscription to Penn Medicine's High Performance Computing cluster which we are able to access through our desktop computers and use for sequence analysis-RNASeq and Whole Genome Sequencing.

Office: The MPI, the co-I's, and scientists and technicians have offices or desks within or immediately adjacent the laboratory or in the core office space of the Department.

Other: As members (Aguirre, Miyadera) or associate members of the P-30 funded Vision Research Center, the MPI, the co-I's have access and are heavy users of the Center's core resources. These include: Machine and Electronic shops, non-invasive image analysis, confocal microscopes, multi-electrode array unit, bioinformatics and biostatistics services.

2. [redacted]

Animal Facility: Facilities for animal housing, breeding, surgery, and necropsy are housed in the [redacted] of the University of Pennsylvania, in [redacted]. This purpose-built research/animal housing facility was constructed in 1989 specifically to house breeding colonies of dogs for use as research models of ocular disorders. Construction was undertaken as a joint effort of the [redacted], NEI/NIH, and the University of Pennsylvania. Approximately one-third of the construction budget for the [redacted] was provided as matching funds from NEI to the [redacted] under the terms of which the building is dedicated solely to the purposes of inherited retinal disease research.

Animals are held in indoor runs in 7 air conditioned, centrally heated kennel rooms. The largest three of these rooms serve as holding kennels for the adult breeding dogs; the smallest four are reserved exclusively for use as quarantine, isolation, whelping, and puppy rooms. All runs are equipped with self-feeders and self-waterers. The facility is designed and constructed to hold up to 200 post-weaning age dogs, depending on their size, plus pre-weaned pups in full compliance with AALAC standards and all regulatory requirements including NIH, USDA, the Pennsylvania Dog Law, and PENN's University Laboratory Animal Resources (ULAR).

Support facilities at the [redacted] include a clinical laboratory, animal examination and treatment suite (with full surgical facility), office space, washrooms for male and female employees, a laundry, separate store rooms for animal feed and general supplies, a mechanical room, loading dock and connecting corridor.

In 2004 the [redacted] underwent an expansion of ~5000 sq ft to facilitate translational studies, and upgrading of heating/cooling by converting from electric to natural gas. Four additional special kennel rooms were built in which small groups of dogs can be maintained for short or long-term studies in a light-controlled, isolation environment suitable for some BSL-2 gene therapy procedures. The addition also has a new operating suite, vision testing and electrophysiology rooms, and a general examination area. Equipment for the surgical suites include a Zeiss OPMI 6 operating microscope with Nightscope, enabling subretinal and intraocular procedures to be undertaken using infrared illumination in light sensitive retinas, thus preventing exposure of the retina to visible light during such procedures. Also available in the surgical suite is a phacoemulsification and vitreo-retinal surgery unit. Offices for research scientists are in adjoining rooms within the facility. A behavioral recording facility has been developed to allow objective obstacle course and Y-maze testing of dogs to measure and record visual performance under a wide range of illuminations. A third renovation paid for by the [redacted] and the University of Pennsylvania's Vice Provost for Research was completed in September, 2013, and upgraded all the air handling equipment, recoated/painted floors, walls and ceilings, and installed new aluminum cage runs in all the rooms.

The [redacted] fully equipped for diagnostic clinical examinations, electroretinography, and non-invasive OCT assessment of retinal microanatomy that enables evaluation of retinal integrity and rescue following gene therapy, or of complications.

Facilities for cryogenic preparation and storage of tissues include a continuously available Liquid Nitrogen supply, and 3 ultracold (-70°) freezers backed up by the LN_2 supply and monitored by the RDSF's security surveillance system.

The campus on which the [redacted] is a comprehensive academic component of the School of Veterinary Medicine. It houses [redacted] of the University of Pennsylvania [redacted] and a broad spectrum of research, diagnostic, administrative and support facilities; all available to our project if and as needed. Among these should be mentioned, specifically, the veterinary faculty of [redacted], a comprehensive diagnostic and clinical pathology laboratory, and NBC's administrative, maintenance and support staff.

Scientific Environment: The University of Pennsylvania has a large vision research community with ~50 investigators that participate in both a Vision Research Center and a Vision Training Program supported through the NEI, NIH. The core and training grants facilitate the research programs of scientists investigating the mechanisms of normal vision, characterizing how disease affects these mechanisms, using the insights gained from the basic research to develop sight-saving and sight-restoring treatments, and conducting clinical trials to evaluate what treatments work well. Both programs have a seminar series running on a weekly/biweekly basis which serves as the focus for interactions between vision research scientists. Important for the project is the close and long-standing collaboration that exists between the Schools of Veterinary Medicine and Medicine. Our laboratories are physically proximate in the same University campus, and such close working relationship has been instrumental, to a large extent, for the scientific advances made over the past decade.

Of equal importance is the excellent scientific support provided by the different modules of the Vision Research Center (VRC) as has been noted above. These have been used to design and analyze experiments and determine sample sizes needed for different studies (Biostatistics module), test optogenetic/optochemical tools for vision restoration in dogs using ex vivo MEA analysis on retinal explants (Electrophysiology module), develop image analysis software to analyze population densities of cones and ganglion cells, or manually segment ONL thickness measures (Image Analysis module), optimize image capture and analysis using the VRC-supported confocal microscopes (Shared Imaging and Instrumentation module), and design and construct specialized equipment for research studies, e.g. ganzfeld dome that accepts a dog head, Y-maze for vision testing and electronic controls, modification of operating microscope for using infrared illumination and viewing (Instrumentation/Electronics module).

EQUIPMENT

School of Veterinary Medicine, University of Pennsylvania

1, [redacted]s of School of Veterinary Medicine

Aguirre, Miyadera labs dedicated equipment: *Microscopes:* Zeiss Axioplan and Axioscope upright widefield fluorescence microscopes (2) equipped with SPOT digital cameras; Zeiss Universal microscope for epi-polarizing microscopy; Zeiss Axiovert 40 inverted widefield fluorescence microscope equipped with SPOT digital camera. *Molecular biology*: PCR machines 1 1 MJ Dyad, and Finnzyme PIKO; Nanodrop 1000 spectrophotometer; Ultra 80; RC-5C Superspeed; Sorvall 6J; Savant SS-11 Speed-vac; micro centrifuges (6); Model 50 Shaker Water Baths (2); Model 18EG Precision Ovens (2); floor Model 3526 Shaker; long- and short-wave UV light boxes and digital gel documentation system; Phosphor imager. Refrigerators and freezers: facilities for cryogenic storage of tissues include a continuously available liquid nitrogen supply and ultracold (-70°C) freezers (6) backed up by LN_2 or CO_2 and all have emergency power supplies; Kelvinator 2-door chromatography refrigerator (2); utility refrigerators (4); -20 freezers (4). *Specialized electronics*: Microelectrode (60) Array setup (Multi Channel Systems GmbH, Germany) with Plexon sorting and Matlab analysis software at Penn Vision Research Center Imaging and Electrophysiology core; Dell™ Poweredge 2500 linux server to process customized large scale analyses in-house; Li-COR Odyssey Fc Dual-Mode Imaging System with 700- and 800-nm channels and using the Image Studio Software; Heidelberg Spectralis HRA/sdOCT viewing station. *Tissue culture*: laminar flow hoods (2), cell culture incubators (3); Cryo-microtomy setup with 2 Thermo Fisher Microm cryostat (HM550 and NX70); standard microtomy set up with 2 Leica microtomes (2065 and RM2165).

Shared equipment: Leica SP5-II confocal and Leica SP8 confocal/multifocal microscopes and image analysis software (Leica LAS AF; Volocity, CellProfiler, Fiji/ImageJ) at Penn Vet imaging core facility; ABI 3700's and 3730 sequencers at core sequencing facility of Penn Genomics Institute; ABI 7500 qRT-PCR machine at veterinary school (core equipment); Buxton GP sterilizer/autoclave; Machine and electronic shops are available through Penn NIH/NEI Core Vision grant.

[redacted]: Heidelberg Spectralis HRA/sdOCT acquisition and viewing station; Espion ERG with custom made (canine-specific) ganzfeld dome, Colorburst mini ganzfeld and high intensity desktop ganzfeld domes; Zeiss OMPI 6 operating microscope; Bausch & Lomb Stellaris PC phacoemulsification and vitreo-retinal surgery unit; Clarity Medical Systems RetCam shuttle pediatric retinal imaging system; Keeler indirect ophthalmoscopes (2); Kowa hand held slit lamps (2); Genesis fundus camera (1); Tonopen (1) and TonoVet (1) tonometers. Objective vision testing apparatus (under continuous video monitoring) which include: an obstacle avoidance course for vision testing under scotopic, mesopic, and photopic conditions; a forced 2 choice Y maze with 4 different wavelength stimuli; a 3 monitor computer-based testing equipment that allows for determination of contrast sensitivity, color discrimination and visual acuity in dogs. A thin foot step detector mat and an automated food pellet dispenser are associated with each of the monitor stations. The dog receives an automatic food reward for approaching the monitor with the visual stimulus.

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED Senior/Key Person Profile (Expanded)

PROFILE - Project Director/Principal Investigator

Prefix: DR. First Name*: GUSTAVO Middle Name D Last Name*: AGUIRRE Suffix:

Position/Title*:	PROFESSOR OF OPHTHALMOLOGY
Organization Name*:	The Trustees of the University of Pennsylvania
Department:	5804 - Clinical Sciences & Adv
Division:	
Street1*:	[redacted]
Street2:	
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County:	Philadelphia
State*:	PA: Pennsylvania
Province:	
Country*:	USA: UNITED STATES
Zip / Postal Code*:	19104-6010

Phone Number*: [redacted] Fax Number: -

E-Mail*: gda@vet.upenn.edu

Credential, e.g., agency login: [redacted]

Project Role*: PD/PI Other Project Role Category:

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Aguirre_Biosketch_EY-06855_10-2018_no link.pdf

Attach Current & Pending Support: File Name:

Funding Opportunity Number: PA-18-484 . Received Date: 2018-11-02T11:54:23.000-04:00

PROFILE - Senior/Key Person

Prefix: DR. First Name*: KEIKO Middle Name Last Name*: MIYADERA Suffix:

Position/Title*: Assistant Professor of Ophthalmology
Organization Name*: The Trustees of the University of Pennsylvania
Department: 5804 - Clinical Sciences & Adv
Division:
Street1*: [redacted]
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State*: PA: Pennsylvania
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Credential, e.g., agency login: [redacted]

Project Role*: PD/PI Other Project Role Category:

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Miyadera_Biosketch_no link.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: DR. First Name*: WILLIAM Middle Name A Last Name*: BELTRAN Suffix:

Position/Title*: Professor
Organization Name*: The Trustees of the University of Pennsylvania
Department: 5804 - Clinical Sciences & Adv
Division:
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Street2:
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State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 19104-6010

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E-Mail*: wbeltran@vet.upenn.edu

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Project Role*: Other (Specify) Other Project Role Category: Other Significant Contributor

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Beltran_biosketch.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: DR. First Name*: ARTUR Middle Name V Last Name*: CIDECIYAN Suffix:

Position/Title*: RESEARCH PROFESSOR
Organization Name*: The Trustees of the University of Pennsylvania
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County: PHILADELPHIA
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
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E-Mail*: cideciya@mail.med.upenn.edu

Credential, e.g., agency login: [redacted]

Project Role*: Other (Specify) Other Project Role Category: Other Significant Contributor

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Cideciyan_NIHBiosketch revised.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: DR First Name*: KARINA Middle Name Last Name*: GUZIEWICZ Suffix:

Position/Title*: Research Assistant Professor of Ophthalmology
Organization Name*: The Trustees of the University of Pennsylvania
Department: 5804 - Clinical Sciences & Adv
Division:
Street1*: [redacted]
Street2:
City*: PHILADELPHIA
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State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
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Project Role*: Other (Specify) Other Project Role Category: Other Significant Contributor

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Guziewicz_10-2018_no link.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: DR. First Name*: SAMUEL Middle Name G Last Name*: JACOBSON Suffix:

Position/Title*: PROFESSOR
Organization Name*: The Trustees of the University of Pennsylvania
Department: 4352 - OP-Ophthalmology
Division:
Street1*: [redacted]
Street2:
City*: PHILADELPHIA
County: PHILADELPHIA
State*: PA: Pennsylvania
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 19104-2689

Phone Number*: [redacted] Fax Number: -

E-Mail*: jacobsos@mail.med.upenn.edu

Credential, e.g., agency login: [redacted]

Project Role*: Other (Specify) Other Project Role Category: Other Significant Contributor

Degree Type: Degree Year: 1977

Attach Biographical Sketch*: File Name: Jacobson biosketch.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: William Middle Name W. Last Name*: Hauswirth Suffix: PhD

Position/Title*: PROFESSOR
Organization Name*: UNIVERSITY OF FLORIDA
Department: Ophthalmology
Division:
Street1*: Box 100284, JHMHSC
Street2:
City*: Gainesville
County:
State*: FL: Florida
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 32610-3115

Phone Number*: [redacted] Fax Number:

E-Mail*: hauswrth@eye.ufl.edu

Credential, e.g., agency login:

Project Role*: Other (Specify) Other Project Role Category: Other Significant Contributor

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Hauswirth, WW Biosketch_11_01_18.pdf

Attach Current & Pending Support: File Name:

PROFILE - Senior/Key Person

Prefix: Dr. First Name*: Alfred Middle Name S. Last Name*: Lewin Suffix:

Position/Title*: Shaler Professor
Organization Name*: UNIVERSITY OF FLORIDA
Department: UNIVERSITY OF FLORIDA
Division:
Street1*: P.O. Box 100266
Street2:
City*: Gainesville
County:
State*: FL: Florida
Province:
Country*: USA: UNITED STATES
Zip / Postal Code*: 32610-0266

Phone Number*: [redacted] Fax Number:

E-Mail*: lewin@ufl.edu

Credential, e.g., agency login: [redacted]

Project Role*: Other (Specify) Other Project Role Category: Other Significant Contributor

Degree Type: Degree Year:

Attach Biographical Sketch*: File Name: Lewin NIHBiosketch.pdf

Attach Current & Pending Support: File Name:

:OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

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: Aguirre, Gustavo D.

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Professor of Medical Genetics and Ophthalmology

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 Pennsylvania, Philadelphia, PA		05/1964	Biology
University of Pennsylvania, Philadelphia, PA	VMD	05/1968	Veterinary Medicine
University of Pennsylvania, Philadelphia, PA		05/1970	Residency-Ophthalm
Johns Hopkins Univ. Wilmer Institute		06/1971	Fellowship-Retina
University of Pennsylvania, Philadelphia, PA	PhD	05/1975	Cell Biology

A. Personal Statement

I am a veterinarian with specialty training in ophthalmology, board certification in the American College of Veterinary Ophthalmologists, and a PhD in cell biology. For over 40 years I have been involved in studies of retinal degenerative diseases using animal models, particularly the dog. Studies are based on clinical phenotyping of potentially useful models, development of animal colony resources, gene discovery and therapy. Of the 17 known retinal disease mutations in dogs, 14 of them have been discovered by my laboratory [redacted] supported by NEI/NIH and [redacted]B funding serves as a small scale "Jackson Laboratory" of dog retinal diseases. Resources for collaborative investigations at the RDSF have resulted in developing the proof of principle studies that are required for translational work. As PI or co-investigator of several NIH and Foundation sponsored grants, I have created the environment and provided the basic groundwork for the studies that form the basis of this proposal. Critical to the success of this approach has been the establishment of strong and continuing collaborations with experts in viral vector design and clinical retinal specialists. This has resulted in translation to the clinic of two therapy projects (CNTF-ECT; *RPE65*-LCA) and two others that are in the planning phases for Phase 1 clinical trials (*CNGB3* achromatopsia; *RPGR*-XLRP). In addition to several highly successful proof of principle gene therapy studies, the studies carried out under this proposal will advance the field of retinal disease gene identification, identification of molecular mechanisms and pathways of disease, and therapies for these diseases.

1- Kijas, J.W. et al. Naturally-occurring rhodopsin mutation in the dog causes retinal dysfunction and degeneration mimicking human dominant retinitis pigmentosa *Proc. Natl. Acad. Sciences USA* 99:6328-6333, 2002. (PMC122948)
2- Zhu, L. et al. A naturally occurring mutation of the opsin gene (T4R) in dogs affects glycosylation and the stability of the G protein-coupled receptor. *J. Biol. Chem.* 279: 53828-53839, 2004. (PMC1351288)
3- Cideciyan, A.V. et al. Dynamics of retinal injury and repair after 'clinical' light exposure in the *rhodopsin* mutant dog model of retinitis pigmentosa. *Proc. Natl. Acad. Sci. USA* 102: 5233-5238, 2005. (PMC555975)
4- Cideciyan, A.V. et al. Mutation-independent rhodopsin gene therapy by knockdown and replacement with a single AAV vector. *Proc. Natl. Acad. Sci. U. S. A.* 115(36):E8547-E8556. (PMC6130384)

B. Positions and Honors

Positions and Employment

1973-1986 Assistant and Associate Professor of Ophthalmology, University of Pennsylvania School of Veterinary Medicine.
1986-1992 Professor of Ophthalmology, University of Pennsylvania Schools of Veterinary Medicine and Medicine.
1992-2004 Professor of Ophthalmology, College of Veterinary Medicine, Cornell University
2004-present Professor of Medical Genetics and Ophthalmology, University of Pennsylvania School of Veterinary Medicine.

Other experience and Professional Memberships

1992-2008 Editorial Board, Investigative Ophthalmology and Visual Science
1998-present Scientific Advisory Board, Foundation Fighting Blindness
1998-present Grant reviewer, Foundation Fighting Blindness
2009-present Editorial Board: American Journal of Translational Research, Archivos de la Sociedad Española de Oftalmologia, BMC Canine Genetics and Epidemiology

Honors

1979 Fight for Sight Citation in Basic Research (co-recipients: G. Chader, L. Liu G. Krishna)
1992 Fellow, College of Physicians of Philadelphia.
1993 Doctor of Philosophy (honoris causa), University of Göteborg, Göteborg, Sweden
2001 Foundation Fighting Blindness Trustee Award
2004 Paul Kayser International Award in Retina Research (Co-recipient)
2004 Scientist of the Year, Heart Sight$_{Miami}$/Foundation Fighting Blindness
2004 The Third Edition of the O.N.C.E. Int'l Prize for R&D in Biomedicine and New Technologies for the Blind
2009 Fellow, Association for Research in Vision and Ophthalmology
2009 Hope for Vision Fdn award
2012 The Kennel Club International Prize in Canine Health
2012 Member, Institute of Medicine (now National Academy of Medicine) of the National Academies
2013 Alcon Research Institute 2013 Award
2013 AVMA Lifetime Excellence in Research Award
2013 Foundation Fighting Blindness Board of Directors Award
2016 Louis Braille Special Recognition Award from Associated Services from the Blind and Visually Impaired
2017 2017 Proctor Medal of the Association for Research in Vision and Ophthalmology
2017 Fellow, American Association for the Advancement of Science

C. Contributions to Science

1. THE DOG AS AN INHERITED RETINAL DISEASE MODEL. My training in veterinary ophthalmology followed by postdoc training in retinal anatomy and physiology, and PhD in cell biology focused my early studies into identifying and characterizing new animal models of inherited retinal degenerations (RD). The studies clearly showed that the diseases, although clinically similar, differ in structure, function and causative gene defect. While the defective genes and mutations were unknown early on in my career, classical interbreed matings showed non-allelism for most of the disorders, although phenotypic variation was clearly present in mutations at the same locus. The variety of diseases present in dogs, and their similarity to the comparable human disease, established the dog as a valuable large animal model. For these studies I served as PI or Co-PI.

a. Aguirre, G.D. et al. Rod-Cone Dysplasia in Irish Setter Dogs: A Defect in Cyclic GMP Metabolism in Visual Cells. *Science* 201: 1133, 1978.
b. Aguirre G.D. and Acland G.M. Variation in Retinal Degeneration Phenotype Inherited at the *prcd* Locus. *Exp. Eye Res.* 46: 663, 1988.
c. Acland, G. et al. Non-allelism of Three Genes (*rcd* 1, *rcd* 2 and *erd*) for Early-Onset Hereditary Retinal Degeneration. Exp. Eye Res.49 : 983-998, 1989.
d. Goldstein, O. et al. IQCB1 and PDE6B mutations cause similar early onset retinal degenerations in two closely related terrier dog breeds. *Inv. Ophthalm. Vis. Sci.* 54:7005-7019, 2013. (PMC3809947)

2. RETINAL CELL BIOLOGY. To understand how abnormalities in the photoreceptor-RPE complex impacted on the disease, my laboratory has: a) developed methods to evaluate retinal function; b) applied methods to non-invasively assess the retina by OCT, and to evaluate the isolated photoresponses; c) characterized the rod and cone PRs using morphologic (LM/EM), IHC and *in situ* hybridization methods, and defined the extracellular

domains surrounding the normal and diseased visual cells; d) established the outer segment renewal characteristics of the rods, along with the topographic distribution of PR disease and degeneration; e) carried out biochemical studies to characterize abnormalities in cGMP metabolism, phospholipid biosynthesis and metabolism using both *in vivo* and organ culture approaches. Selected publications are:

a. Aguirre, G. and Stramm, L. The RPE: A Model System for Disease Expression and Disease Correction. *Prog. Retinal Res*, Osborne, N. and Chader, G., eds., 11:153-191, 1991.
b. Beltran, W.A. et al. *Inv. Ophthalm. Vis. Sci.* 50: 3985-3995, 2009. (PMC2718058)
c. Berta, Á.I. et al. Photoreceptor cell death, proliferation and formation of hybrid rod/S-cone photoreceptors in the degenerating *STK38L* mutant retina. *PLoS ONE* 6: e24074, 2011. (PMC3184085)
d. Beltran, W.A. et al. Canine retina has a primate fovea-like bouquet of cone photoreceptors which is affected by inherited macular degenerations. *PLoS ONE* 9:e90390, 2014. (PMC3944008)

3. DEVELOPING THE DOG GENETIC MAP AND MAPPING/MOLECULAR GENETICS STUDIES. In order to characterize the canine diseases at the molecular level, it was necessary to first develop the tools necessary to do modern day genomics in the dog as a model organism. We developed the first linkage map of the dog, and within 1 year mapped the first autosomal disorder in dogs which was the *prcd* retinal disorder. Overall, we have mapped >10 retinal disorders and identified the mutation in all. As canine cDNA arrays were not available commercially, we first developed a normalized canine retina- and brain-expressed sequence tag library, and used this resource to develop a cDNA microarray to examine gene expression in normal and mutant retinas, and mapped the uncharacterized ESTs to the canine genome which led to the identification of a novel retinal disease gene (*PRCD*) in dogs, and subsequently in human patients with RP.

a. Mellersh, C. S. et al. A linkage map of the canine genome. *Genomics* 46:326-336, 1997.
b. Acland, G. et al. Linkage analysis and comparative mapping of canine progressive rod-cone degeneration (*prcd*) establishes potential locus homology with retinitis pigmentosa (RP17) in humans. *Proc. Natl. Acad. Sci. USA*. 95:3048-3053, 1998. (PMC19692)
c. Zangerl, B. et al. Identical mutation in a novel retinal gene causes progressive rod-cone degeneration (prcd) in dogs, and retinitis pigmentosa in man. *Genomics* 88:551-563, 2006. (PMC3989879)
d. Genini, S. et al. Transcriptional profile analysis of RPGRORF15 frameshift mutation identifies novel genes associated with retinal degeneration. *Inv. Ophthalm. Vis. Sci.* **51:** 6038-6050, 2010. (PMC3061521)

4. PHENOTYPE GENOTYPE CORRELATION. Crucial to these studies is the ability to use multiple assessment measures to determine the genotype-phenotype correlation. In some cases, the correlation is precise, and same phenotype is present regardless of the mutation (e.g. *BEST1* mutations), while in others, e.g. cord1, there is an imprecise phenotype-genotype correlation. This is information is essential in order to establish disease metrics for gene therapy study and assess outcomes, or examine the mechanisms of disease at the molecular level.

a. Guziewicz, K.E. et al. Bestrophin gene mutations cause canine multifocal retinopathy, a novel animal model for Best disease. *Inv. Ophthalm. Vis. Sci.*. 48:1959-1967, 2007. (PMC1931491)
b. Guziewicz, K.E. et al. Molecular consequences of *BEST1* gene mutations in canine multifocal retinopathy predict functional implications for human bestrophinopathies. *Inv. Ophthalm. Vis. Sci.* 52: 4497-4505, 2011. (PMC3175949)
c. Kuznetsova, T. et al. Exclusion of RPGRIP1 ins44 from primary causal association with early-onset cone–rod dystrophy in dogs *Inv. Ophthalm. Vis. Sci.*. 53(9): 5486-5501, 2012. (PMC3422103)
d. Genini, S. et al. Up-regulation of Tumor Necrosis Factor superfamily genes in early phases of photoreceptor degeneration. *PLoS ONE* 8:e85408, 2013. (PMC3868615)

5. RETINAL GENE THERAPY. Once retinal disease causative mutations, our laboratory has focused on developing therapies that target RPE, rods, cone or both photoreceptor classes. We showed that targeting the RPE with AAV2 vectors and the human *RPE65* cDNA controlled by the CMV-CBA promoter restored function and preserved the retina as long as treatment was initiated prior to the degenerative phase in both patients and dog models. As well, we were the first to demonstrate that *CNGB3* achromatopsia could be successfully treated by gene therapy, and developed a "rescue" protocol for treating older retinas that we treated successfully by gene therapy, but cone function could not be rescued unless the photoreceptors were deconstructed. Lastly, we were the first ones to successfully treat *RPGR*-XLRP in an animal model, and now we have shown that treatment can be initiated at mid and late stages of the disease and still prevent the photoreceptors cells from degenerating.

a. Beltran, W.A. et al. Gene therapy rescues photoreceptor blindness in dogs and paves the way for treating human X-linked retinitis pigmentosa. *Proc. Natl. Acad. Sci. USA* 109(6): 2132-2137, 2012. (PMC3277562)
b. Cideciyan, A.V. et al. Human retinal gene therapy for Leber congenital amaurosis shows advancing retinal degeneration despite enduring visual improvement. *Proc. Natl. Acad. Sci. USA* 110(6): E517-E525, 2013. (PMC3568385)
c. Beltran W.A. et al. Expansion of the therapeutic window for retinal gene therapy: late intervention reverses photoreceptor degeneration and rescues vision *Proc Natl Acad Sci USA* 2015; 112, E5844-5853. (PMC4629324)
d. Guziewicz, K.E. et al. *BEST1* gene therapy corrects a diffuse retina-wide micro-detachment modulated by light exposure. *Proc. Natl. Acad. Sci. U. S. A.* 115(12):E2839-E2848, 2018. (PMC5866594)

Complete List of Published Work (>274 peer reviewed publications) in MyBibliography:

ncbi.nlm.nih.gov/sites/myncbi/gustavo.aguirre.1/collections/48535286/public/

D. Additional Information: Research Support and/or Scholastic Performance
Ongoing Research Support

Grant # PI name: U24-EY029890-01. Wolfe J, Beltran WA, Gamm DM (MPIs) 9/30/18-8/30/23
Sponsor: NIH-NEI
Title: Canine retinal disease models for translational photoreceptor replacement
The major goal of this multi-investigator, multi-center research project is to develop canine models in which to investigate the replacement of photoreceptors under disease conditions using iPSCs.
Role: Co-Investigator
Overlap: None.

Grant # PI name: EY017549, (Aguirre, G. and Beltran WA, MPIs) **Dates:** 09/01/16-08/31/21
NEI/NIH
Title: Translational Research for Retinal Degeneration Therapies
The major goals: A multi-investigator, multi-center research effort to develop and test gene-based retinal therapy in dog models of X-linked RP caused by mutations in *RPGR*. The study will develop and validate vectors, promoters, knockdown constructs and replacement cDNAs for therapy; establish therapy outcome measures in the models using morphologic and non-invasive functional and imaging that can be extrapolated to patients.
Role: PI
Overlap: None.

Grant # PI name: #R01EY-06855, (Aguirre,G.) **Dates**: 1/1/15-11/30/19
Sponsor: NEI/NIH
Title: Models of Hereditary Retinal Degeneration
The major goals: Develop canine models of inherited retinal degeneration (*erd, cd, prcd, rcd1*) and use these for molecular studies of the mechanisms underlying photoreceptor degeneration, and to develop therapies
Role: CoPI.
Together with FFB Research Center grant, grant provides partial support for the core resources of the RDSF.
Overlap: None.

Grant # PI name: [redacted] Aguirre, G and Beltran, WA) Co-Directors .
Dates: 4/1/14-3/31/19
Title: PENN Large Animal Model Translational and Research Center
The major goal: To provide partial support of the animal colonies with inherited retinal degeneration, test potential novel therapies, identify new animal models of retinal degeneration, and examine molecular mechanisms whereby mutations lead to photoreceptor degeneration.
Role: PI/Center Coordinator
Together with EY-06855, grant provides partial support for the core resources of the RDSF.
Overlap: None.

Grant # PI name: N/A Beltran WA (PD/PI) 06/01/18-05/31/20 .

Sponsor: [redacted]
Title: Gene therapy for RHO-adRP
The major goal: The goal of this study is to evaluate the toxicity and dose response of a specific gene therapy vector in dogs with *RHO* mutation.
Role: Co-investigator
Support from this Sponsored Research Agreement is for studies leading to an IND with the first generation vector, but do not cover any of the vector optimization and in vivo validation studies that are part of the current grant proposal and that will be needed to develop more efficacious vectors for long-term clinical applications.
Overlap: None.

R-24 EY 023937-01 (VanGelder, Russell) PI **Dates:** 9/1/14-8/31/19
Sponsor: NIH/NEI via University of Washington
Title: Photoswitchable channel blockers for treatment of blindness
Major Goals: The goal of this multi-investigator, multi-center research project is to develop small molecule photoswitches and test their safety and efficacy in providing visual recovery in animals (including canine models) with end stage retinal degeneration.
Role: Co-Investigator
Overlap: None.

Completed Research Support
Grant # PI name: R24-EY022012 Beltran W
(PD/PI) **Dates:** 3/1/12 - 02/28/18
NIH-NEI
Title: Translational Gene Therapy for Rhodopsin Autosomal Dominant Retinitis Pigmentosa.
The major goals of this multi-investigator, multi-center research effort are to develop and test gene-based retinal therapy in mouse and dog models of RHO-ADRP. The study will develop and validate vectors, promoters, knockdown constructs and replacement cDNAs for therapy; establish therapy outcome measures in the models using morphologic and non-invasive functional and imaging that can be extrapolated to patients.
Role: co-investigator

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

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: Miyadera, Keiko

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Assistant Professor of Ophthalmology

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)	END DATE MM/YYYY	FIELD OF STUDY
University of Tokyo, Japan	DVM	03/2005	Veterinary Medicine
University of Tokyo, Japan	Internship	09/2006	Small Animal Surgery
University of Cambridge, UK	PhD	01/2011	Molecular Biology
University of Pennsylvania, Philadelphia, PA	Postdoctoral	07/2012	Genetics & Ophthalmology
University of Pennsylvania, Philadelphia, PA	Residency	07/2015	Ophthalmology

A. Personal Statement

I am a clinician-scientist with specialty clinical training in veterinary ophthalmology and a PhD in molecular biology, with 13 years of research experience in the field of inherited retinal diseases in animals. My study subjects are canine models of retinal diseases homologous, both phenotypically and genetically, to diseases in patients. My research using these disease models span from characterizing the phenotype, searching for the underlying gene mutation, to developing and testing new therapies.

As a board-certified veterinary ophthalmologist, I am qualified and experienced with the diagnosis and treatment of ocular conditions in animals, a skill set that is essential in determining the phenotype with accuracy and assessing safety and efficacy of new treatments. I am also proficient in microsurgeries that are employed in certain therapies such as AAV injections. At our state-of-the-art Retinal Disease Studies Facility (RDSF) that has all the necessary tools, I routinely fulfill these clinical aspects of the studies.

As a researcher with a background in genetics and molecular biology, I am particularly interested in understanding the molecular basis of retinal conditions with relatively complex genetic etiologies. While most retinal diseases with known gene mutation have been characterized as straightforward monogenic traits, the involvement of other genes within and beyond the retinal interactome in modifying disease expression has received much focus for its broader impacts. To address this, I utilize the unique genetic background of the canine models that is sufficiently uniform to eliminate noises while segregating a disease allele that is identical by descent. With careful phenotyping and case selections, I have successfully identified gene mutations using powerful genetic tools such as genome-wide association study (GWAS) and whole-genome sequencing.

Throughout my research career, I have maintained a specific focus on a form of cone-rod dystrophy in dogs associated with a mutation in *RPGRIP1*. With subjects collected in Japan, I first showed substantial phenotypic variation and phenotype-genotype discordances. A GWAS carried out as part of my PhD in the UK allowed me to map a locus representing a disease onset modifier. I have since continued the study at the RDSF of UPenn Vet, establishing an expanded *RPGRIP1* mutant canine research colony. The reliable phenotyping and tissue resources available only from such a research setting have been indispensable for my work as the study developed into functional RNA/protein analyses. Of note, I have the particular advantage of being part of the Division of Experimental Retinal Therapies (ExpeRTs) at UPenn Vet, a collaborative group of eye researchers working towards the common goal to translate the canine works in treating blinding retinal diseases in human patients.

1. **Miyadera K**, Acland GM, Aguirre GD. Genetic and phenotypic variations of inherited retinal diseases in dogs: the power of within- and across-breed studies. Mamm Genome. 2012;23(1-2):40-61. PMID: 22065099; PMCID: PMC3942498.

2. Das RG, Marinho FP, Iwabe S, Santana E, McDaid KS, Aguirre GD, **Miyadera K**. Variabilities in retinal function and structure in a canine model of cone-rod dystrophy associated with RPGRIP1 support multigenic etiology. Sci Rep. 2017;9;7(1):12823. PMID: 28993665; PMCID: PMC5634483.

B. Positions and Honors

Positions and Employment

2015 - Assistant Professor of Ophthalmology, University of Pennsylvania, School of Veterinary Medicine, Philadelphia, PA

Other Experience and Professional Memberships

2004 - Member, Japanese Society of Animal Breeding and Genetics
2005 - Licensed Veterinarian, Japanese Ministry of Agriculture, Forestry and Fisheries
2011 - Member, Association for Research in Vision and Ophthalmology
2015 - Member, International Society for Eye Research
2016 - Diplomate, American College of Veterinary Ophthalmologists

Honors

2006 International Academic Exchange Studies Scholarship, University of Tokyo
2006 Scholarship, British Council Japan Association
2008 Senior Scholarship, Fitzwilliam College, Cambridge, UK
2010 Presidential Award, Japanese Society of Animal Breeding and Genetics
2010 Research Studentship, Cambridge Philosophical Society, Cambridge, UK
2012 Finalist, Members-in-Training Outstanding Poster Award, ARVO
2012 Career Development Award for Veterinary Residents, Foundation Fighting Blindness
2012 Scientific Travel Award, Van Sloun Foundation
2012 Fellowship, NEI/NIH "Fundamental Issues in Vision Research", Woods Hole, MA
2016 First Place, Poster Award, ISER (International Society for Eye Research), Tokyo, Japan

C. Contribution to Science

1. CANINE MODELS OF RETINAL DISEASES: The domestic dog has proven to be an excellent model in retinal disease studies in both gene discovery and development of new therapies. A variety of naturally-occurring inherited diseases in different dog breeds have been shown to be true clinical and genetic homologs of human diseases such as retinitis pigmentosa, cone-rod dystrophy, Leber congenital amaurosis, achromatopsia, and Best macular dystrophy. These models have contributed to a number of successful gene therapy trials, paving the way for translation of therapeutic options in human patients. To date, nearly 30 different forms of canine retinal diseases have been characterized at the molecular level, yet the role of each gene/mutation in retinal physiology and pathogenesis remains largely unexplored. At the RDSF of the University of Pennsylvania, we maintain these canine models and thrive to study the pathogenesis as well as new treatments while continuing to identify new models.

   a. **Miyadera K**. Inherited retinal diseases in dogs: advances in gene/mutation discovery. Dobutsu Iden Ikushu Kenkyu. 2014;42(2):79-89. PMID: 26120276; PMCID: PMC4480793.

   b. **Miyadera K**, Acland GM, Aguirre GD. Genetic and phenotypic variations of inherited retinal diseases in dogs: the power of within- and across-breed studies. Mamm Genome. 2012 Feb;23(1-2):40-61. PMID: 22065099; PMCID: PMC3942498.

2. CORD1 MODIFIERS: I have a long-standing pursuit of the molecular basis of a canine model of cone-rod dystrophy (cord1). This canine model has turned out to be rather genetically complex, uniquely standing out among other known canine retinal disease models that are all monogenic. The initial part of my work involved demonstrating the phenotypic variation as well as genotype-phenotype discordance with a mutation in a cilia gene *RPGRIP1* which was initially proposed as the sole cause of cord1. To account for the phenotypic variation among *RPGRIP1* mutant dogs, I carried out a GWAS and mapped a modifier

locus that influenced the age of disease onset; the mutation was subsequently found in *MAP9* which we identified immunohistochemically as new and additional cilia gene. There is increasing awareness that the protein interactome comprising multiple retinal genes and modifiers plays a major role in retinal physiology and pathogenesis in animals and humans. More recently, we have found the evidence for a third molecular player that seems to control the cone function. My ongoing works include studying the protein interaction between RPGRIP1, MAP9, and the third molecule in the wild type and disease state.

a. Das RG, Marinho FP, Iwabe S, Santana E, McDaid KS, Aguirre GD, **Miyadera K**. Variabilities in retinal function and structure in a canine model of cone-rod dystrophy associated with RPGRIP1 support multigenic etiology. Sci Rep. 2017;9;7(1):12823. PMID: 28993665; PMCID: PMC5634483.
b. Forman OP, Hitti RJ, Boursnell M, **Miyadera K**, Sargan D, Mellersh C. Canine genome assembly correction facilitates identification of a MAP9 deletion as a potential age of onset modifier for RPGRIP1-associated canine retinal degeneration. Mamm Genome. 2016;27(5-6):237-45. PMID: 27017229.
c. **Miyadera K**, Kato K, Boursnell M, Mellersh CS, Sargan DR. Genome-wide association study in RPGRIP1(-/-) dogs identifies a modifier locus that determines the onset of retinal degeneration. Mamm Genome. 2012;23(1-2):212-23. PMID: 22193413; PMCID: PMC3947618.
d. **Miyadera K**, Kato K, Aguirre-Hernández J, Tokuriki T, Morimoto K, Busse C, Barnett K, Holmes N, Ogawa H, Sasaki N, Mellersh CS, Sargan DR. Phenotypic variation and genotype-phenotype discordance in canine cone-rod dystrophy with an RPGRIP1 mutation. Mol Vis. 2009;11(15):2287-305. PMID: 19936303; PMCID: PMC2779058.

3. A NEW CANINE MODEL OF CONGENITAL STATIONARY NIGHT BLINDNESS (CSNB): We have clinically and molecularly characterized a new form of CSNB in collaboration with ophthalmologist colleagues in Japan. Based on electroretinogray and disease segregation, we have identified the disease form as the autosomal recessive, Schubert-Bornschein type complete CSNB. For the gene mutation discovery process, we carried out candidate gene screening, haplotype-based homozygosity mapping, GWAS, and whole-genome sequencing, leading to the identification of a new exonic mutation. I plan to carry out AAV gene therapy to obtain proof-of-concept as well as further study the molecular pathogenesis of this new large animal CSNB model that has promising translational potential.

a. Kondo M, Das G, Imai R, Santana E, Nakashita T, Imawaka M, Ueda K, Ohtsuka H, Sakai K, Aihara T, Kato K, Sugimoto M, Ueno S, Nishizawa Y, Aguirre GD, **Miyadera K**. A Naturally Occurring Canine Model of Autosomal Recessive Congenital Stationary Night Blindness. PLoS One. 2015;10(9):e0137072. PMID: 26368928; PMCID: PMC4569341.
b. Kondo M, Imai R, Nakashita T, Imawaka M, Ueda K, Ohtsuka H, Ueno S, Das G, **Miyadera K**, Aguirre G. Canine Model of Autosomal Recessive Complete-type Congenital Stationary Night Blindness. ARVO Annual Meeting, 2013, Seattle, WA.

Complete list of Published Work in MyBibliography
ncbi.nlm.nih.gov/myncbi/browse/collection/40151272/?sort=date&direction=descending

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

R01EY-06855, NEI/NIH Aguirre, Gustavo (PI) 1/1/15-11/30/19
"Models of Hereditary Retinal Degeneration" Develop canine models of inherited retinal degeneration (erd, cd, prcd, rcd1) and use these for molecular studies of the mechanisms underlying photoreceptor degeneration, and to develop therapies.
Role: Co-Investigator

[redacted] Miyadera, Keiko (PI) 1/1/17-6/30/19
"Canine model of night blindness to target retinal bipolar cells for new therapies" Obtain proof-of concept to target ON-bipolar cells to treat stationary night blindness and for optogenetics applications.

Role: PI

[redacted] Hirsch, Matthew (PI) 7/1/17-8/31/19
"Safe and Effective Therapy for Vision Loss in MPS1 Patients" Test AAV gene therapy by intracorneal injection in MPS1 canine models to obtain proof-of-concept to reverse corneal blindness.
Role: Sub-contractor

[redacted] Hirsch, Matthew (PI) 2/1/18-11/30/18
"AAV Gene Therapy for Muscular and Ocular Diseases" Develop AAV intracorneal gene therapy in dogs affected with MPS1 as proof-of-concept to reverse corneal blindness.
Role: Sub-contractor

[redacted] Miyadera, Keiko (PI) 11/9/15-6/30/19
"Clinical & safety evaluation of Burr-1 drops for the treatment of dogs with cataracts" Test safety and efficacy of a homeopathic plant extract in dissolving cataracts in canine patients.
Role: PI

Completed Research Support

[redacted] Miyadera, Keiko (PI) 3/1/16-2/28/17
"Molecular characterization of a multigenic canine model of retinal degeneration" Study protein-protein interaction of known molecular players of a multigenic form of canine cone-rod dystrophy.
Role: PI

[redacted] Miyadera, Keiko (PI) 7/16/12-7/15/15
"Molecular characterization of a genetic modifier controlling the age of onset in a canine model of RPGRIP1 cone-rod dystrophy" Identify modifiers affecting disease severity in an RPGRIP1 canine cone-rod dystrophy thereby establishing the first large animal model of retinopathy involving more than one gene.
Role: PI

[redacted] Aguirre, Gustavo (PI) 4/1/12-3/31/13
"Genome-wide association study in a novel canine model of congenital stationary night blindness" Map the chromosomal locus corresponding to a new canine model of naturally-occurring CSNB by GWAS. Identify the gene and the mutation responsible for CSNB by screening of positional candidate genes.
Role: Co-Investigator

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

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: William A. BELTRAN

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Professor of Ophthalmology (tenured)

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
Lycee Marcelin Berthelot, St Maur, France	Bac. C	1989	Sciences
Ecole Nationale Veterinaire, Alfort, France	DVM	1994	Veterinary Medicine
School of Medicine, Univ. of Paris, France	MS	1998	Biol & Medical Sciences
School of Medicine, Univ. of Paris, France	Uni. Dipl.	1999	Experimental. Surgery & Microsurgery
Ecole Nationale Veterinaire, Alfort, France	Dip. ECVO	2000	Veterinary Ophthalmology Residency
Cornell University	PhD	2006	Comparative Biomedical Sciences

A. Personal Statement

I am a clinician-scientist with board-certification in veterinary ophthalmology, and more than 17 years of research experience in the field of retinal degeneration. The research conducted in my laboratory is focused on characterizing canine models of inherited retinal degenerative diseases with a specific emphasis on models of ciliopathy (RPGR X-linked retinitis pigmentosa, NPHP5-LCA) and a model of RHO autosomal dominant retinitis pigmentosa (RHO-ADRP). Ongoing projects in my lab are aimed at characterizing the link between mutation and photoreceptor cell death, and testing novel treatment modalities for early/mid stage disease such as corrective gene therapy, and neuroprotective agents (CNTF, XIAP), as well as the use of optogenetic/optochemical tools for vision restoration in late stages of retinal degeneration. As such, my group has all the necessary scientific/technical/clinical expertise, and access to a unique large animal (canine) models, to conduct the work presented in this proposal. This includes but is not limited to: clinical assessment of retinal structure and function, intraocular surgery (e.g. subretinal injections, vitrectomies), histo-morphological and molecular evaluation of ocular and retinal tissues, electroretinography, and psychophysical vision testing. I have experience with multiple-PI grants and have recently led an NEI-funded R24 consortium effort to develop a gene therapy for *RHO*-adRP. As head of the PennVet Division of Experimental Retinal Therapies I have been exposed to working with biotech/pharmaceutical companies on preclinical projects aimed at characterizing new large/canine animal models and/or evaluating the efficacy and safety of novel retinal therapeutics. I was recently the Study Director for IND-enabling safety/efficacy studies in dogs for a gene therapy for XLRP that led to IND application and the launch of a human clinical trial (ClinicalTrials.gov Identifier: NCT03316560).

1. Beltran W.A., Cideciyan A.V., Guziewicz K.E., Iwabe S., Swider M., Scott E.M., Savina S.V., Ruthel G., Stefano F., Zhang L., Zorger R., Sumaroka A., Jacobson S.G., Aguirre G.D. Canine retina has a primate fovea-like bouquet of cone photoreceptors which is affected by inherited macular degenerations. *PLOS ONE*, 2014; 9:e90390. PMCID: PMC3944008.

2. Gaub, B.M., Berry, M.H., Holt, A., Reiner, A., Kienzler, M., Dolgova, N. Nikonov, S., Aguirre, G.D., Beltran, W.A., Flannery, J., Isacoff, E.Y. Restoration of visual function by expression of a light- gated mammalian ion channel in retinal ganglion cells or ON-bipolar cells. *Proceedings of the National Academy of Sciences of the USA*, 2014;111: E5574-5583, PMCID: PMC4335018.
3. Sudharsan R.S., Beiting D.P., Aguirre G.D., Beltran W.A. Involvement of innate immune system in late stages of inherited photoreceptor degeneration. *Scientific Reports*, 2017; 7 (1): 17897. PMCID: PMC5738376.
4. Cideciyan A.V., Sudharsan R., Dufour V.L., Massengill M., Iwabe S., Swider M., Lisi B., Sumaroka A., Marinho L.F., Appelbaum T., Rossmiller B., Hauswirth W.W., Jacobson S.G., Lewin A.S., Aguirre G.D., Beltran W.A. Mutation-independent Rhodopsin gene therapy by knockdown and replacement with a single AAV vector. *Proceedings of the National Academy of Sciences of the USA*, 2018: 115 (36): E8547-E8556. PMCID: PMC6130384

B. Positions and Honors
Employment:

2005-2006	Lecturer in Ophthalmology (University of Pennsylvania)
2006-2014	Assistant Professor of Ophthalmology (University of Pennsylvania)
2014-2018	Associate Professor of Ophthalmology (University of Pennsylvania)
2017-present	Director, Division of Experimental Retinal Therapies (University of Pennsylvania)
2018-present	Professor of Ophthalmology (University of Pennsylvania)

Other Experience and Professional Memberships

2001-	Member, Association for Research in Vision & Ophthalmology
2003-	Specialty board certification: European College of Veterinary Ophthalmologists
2005-	Member, Editorial board of the journal "Veterinary Ophthalmology"
2015-	Member, International Society for Eye Research
2016-	Member, Foundation Fighting Blindness, scientific advisory board
2017-	Fellow, Philadelphia College of Physicians

Honors:

1997	Schools of Medicine (Paris) and Veterinary Medicine (Alfort): Thesis award.
2000	Cornell University, Graduate Research Assistantship.
2004	Foundation Fighting Blindness: Career Development Award
2005	Merck/Merial Veterinary Research Award
2013	Merck Innovative Ophthalmology Research Award / ARVO Foundation
2013	Foundation Fighting Blindness Board of Directors Award
2016	Pfizer Ophthalmics Carl Camras Translational Award/ ARVO Foundation

C. Contributions to Science

1. Light-induced acceleration of disease in a canine model of autosomal dominant retinitis pigmentosa caused by a rhodopsin mutation. An area of investigation in my laboratory is to understand the mechanism by which light triggers and accelerates retinal degeneration in the canine model of *RHO*-autosomal dominant RP, as there is clinical evidence that such susceptibility to light also occurs in patients that carry some RHO mutations. We have excluded in the dog model the role of AP-1 activation as well as the occurrence of any ER-mediated stress, and have shown that following light exposure an acute burst of rod photoreceptor cell death is followed by a protracted loss.

a. D. Gu, W.A. Beltran, Z. Li, G.M. Acland, G.D. Aguirre. Clinical light exposure, photoreceptor degeneration and AP-1 activation: a cell death or cell survival signal in the rhodopsin mutant retina? *Investigative Ophthalmology and Visual Sciences*, 2007; 48: 4907-4918. PMCID; PMC2377016.
b. Marsili, S., Genini, S., Sudharsan, R., Gingrich, J., Aguirre, G.D., Beltran, W.A. Exclusion of the unfolded

protein response in light-induced retinal degeneration in the canine T4R RHO model of autosomal dominant retinitis pigmentosa. *PLOS ONE* 2015;10(2):e0115723. PMCID: PMC4335018.

c. Iwabe S., Ying G-S., Aguirre G.D., Beltran W.A. Assessment of visual function and retinal structure following acute light exposure in the light sensitive T4R rhodopsin mutant dog. Experimental Eye Research, 2016; 146:341-353, PMCID5004782.

d. Sudharsan R, Simone K.M., Anderson N.P., Aguirre G.D., Beltran W.A. Acute and protracted cell death in light-induced retinal degeneration in the canine model of rhodopsin autosomal dominant retinitis pigmentosa. *Investigative Ophthalmology and Visual Sciences*, 2017; 50:270-281. PMCID: PMC5464465.

2. Development of a gene therapy for the *RPGR* form of X-linked retinitis pigmentosa. For the past 10 years I have been a key member of a consortium of investigators working at developing a corrective gene therapy approach for *RPGR*-XLRP, a common and severe form of inherited retinal degeneration. I initially characterized a canine model of this disease, and defined the outcome measures that could be used to assess the response to therapeutic intervention. I then selected the viral vector/promoter that would provide efficient and safe levels of transduction of rods and cones. In 2012, I established with my collaborators the first proof of concept in two canine models of *RPGR*-XLRP that RPGR gene augmentation could prevent the onset and also stop the ongoing progression of photoreceptor cell loss if delivered at an early stage of disease. We have now recently published evidence demonstrating that the rescue effect lasts for at least 2.5 years, and can successfully prevent vision loss in the dogs even when the gene therapy is delivered at late stage disease (50-60% of photoreceptor loss). Under a sponsored research agreement with AGTC we successfully performed in the canine model the necessary preclinical safety studies. Based on this work, AGTC recently obtained IND approval to initiate the first clinical trial in the US for this early onset and severe form of blindness.

a. W.A. Beltran, P. Hammond, G. M. Acland, G. D. Aguirre. A frameshift mutation in RPGR exon ORF15 causes photoreceptor degeneration and early inner retina remodeling in a model of X- linked retinitis pigmentosa. *Investigative Ophthalmology and Visual Sciences*, 2006; 47: 1669-1681.

b. W.A. Beltran, A.V. Cideciyan, A.S. Lewin, S. Iwabe, H. Khanna, A. Sumaroka, V.A. Chiodo, D.S.Fajardo, A.J. Román, W.-T. Deng, M. Swider, T.S. Alemán, S.L. Boye, S. Genini, A. Swaroop, W.W. Hauswirth, S.G. Jacobson, G.D. Aguirre. Gene therapy rescues X-linked photoreceptor blindness in dogs and paves the way for treating RPGR form of human retinitis pigmentosa. Proc. Natl. Acad. Sci. USA, 2012; 109(6):2132-7. PMCID: PMC3277562.

c. Beltran W.A., Cideciyan A.V., Iwabe S., Swider M., Kosyk M.S., McDaid K., Martynyuk I., Ying G.-S., Shaffer S., Deng W.-T., Boye S.L., Lewin A.S., Hauswirth W.W., Jacobson S.G., Aguirre G.D. Successful arrest of photoreceptor and vision loss expands the therapeutic window of retinal gene therapy to later stages of disease. *Proceedings of the National Academy of Sciences of the USA*, 2015; 112(43):E5844-53. PMCID: PMC4629324.

d. Beltran W.A., Cideciyan A.V., Boye S.E., Ye G-J., Iwabe S., Dufour V.L., Marinho L.F., Swider M., Kosyk M.S., Sha J., Boye S.L., Peterson J.J., Witherspoon C.D., Alexander JJ., Ying G-S., Shearman M.S., Chulay J.D., Hauswirth W.W., Gamlin P.D., Jacobson S.G., Aguirre G.D. Optimization of retinal gene therapy for X-linked retinitis pigmentosa due to RPGR mutations. *Molecular Therapy*, 2017; 25 (8): 1866-1880. PMCID: PMC5542804.

3. **Retinal neuroprotection**. My early work focused on understanding the mechanism by which Ciliary neurotrophic factor (CNTF) rescues promotes the survival of photoreceptors and could be used as a novel therapy for inherited retinal degenerations. I initially showed that the receptor (CNTFRα) was expressed by photoreceptors in the retina of dogs as well as in several non-rodent mammalian species including human, suggesting that CNTF could trigger a rescue effect via a direct activation of a Jak-STAT induced pro-survival response in rods and cones. I further demonstrated that although intravitreal injections of CNTF promoted photoreceptor rescue in the canine PDE6B mutant, it failed to confer any protection in a canine RPGR mutant, and caused instead cell proliferation and retinal remodelling. More recently I demonstrated with my collaborators that a CNTF could be used in association with CNGB3 gene augmentation therapy to treat a canine model of achromatopsia that does not respond at later ages to corrective gene therapy alone.

a. W.A. Beltran, Q. Zhang, J. W. Kijas, D. Gu, H. Rohrer, J. A. Jordan, G. D. Aguirre. Cloning, mapping, and retinal expression of the canine Ciliary Neurotrophic Factor Receptor α (CNTFRα). *Investigative Ophthalmology and Visual Sciences*, 2003; 44:3642-3649.

b. W.A. Beltran, H. Rohrer, G. D. Aguirre. Immunolocalization of Ciliary Neurotrophic Factor Receptor α (CNTFRα) in mammalian photoreceptor cells. *Molecular Vision*, 2005; 11:232-244.

c. Beltran W.A., Wen R., Acland G.M., Aguirre G.D. Intravitreal injection of ciliary neurotrophic factor (CNTF) causes peripheral remodeling and does not prevent photoreceptor loss in canine RPGR mutant retina. *Experimental Eye Research*, 2007; 84:753-771. PMCID: PMC2709826

d. A.M. Komáromy, J.S. Rowlan, A.T. Parton Corr, S.L. Reinstein, S.L. Boye, A.E. Cooper, A. Gonzales, B. Levy, R. Wen, W.W. Hauswirth, W.A. Beltran, G.D. Aguirre. Transient photoreceptor deconstruction by CNTF enhances rAAV-mediated cone functional rescue in late stage CNGB3-achromatopsia. *Molecular. Therapy*, 2013; 21: 1131-1141. PMCID: PMC3677296

Complete list of Published Work in MyBibliography
Link: ncbi.nlm.nih.gov/myncbi/browse/collection/43642934/?sort=date&direction=ascending

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

Grant # PI name: U24-EY029890-01 Wolfe J, Beltran WA, Gamm DM (MPIs) **Dates:** 9/30/18-8/30/23
Sponsor: NIH-NEI
Title: Canine retinal disease models for translational photoreceptor replacement
The major goal of this multi-investigator, multi-center research project is to develop canine models in which to investigate the replacement of photoreceptors under disease conditions using iPSCs.
Role: MPI
Overlap: None

Grant # PI name: RO1-EY017549-11 Beltran WA & Aguirre GD (MPIs) **Dates:** 9/1/16 - 8/31/21
Sponsor: NIH-NEI
Title: Translational Research for Retinal Degeneration Therapies
The major goals of this multi-investigator, multi-center research project are to develop and test gene-based retinal therapy in dog models of NPHP5-LCA. The study will develop and validate vectors, promoters, knockdown constructs and replacement cDNAs for therapy; establish therapy outcome measures in the models using morphologic and non-invasive functional and imaging that can be extrapolated to patients.
Role: MPI
Overlap: None

Grant # PI name: [redacted] Beltran, WA & Aguirre GD (co-Directors) **Dates:** 4/1/14-3/31/19
Sponsor: [redacted]
Title: PENN Large Animal Model Translational and Research Center **"Medical Therapy Service"**
Partial support of animal studies to optimize currently tested therapies and develop/test new therapeutic approaches in dog models of inherited retinal degeneration (RD).
Role: co-Director
Overlap: None

Grant # PI name:N/A Beltran WA (PD/PI) **Dates:** 6/1/18-05/31/20
Sponsor [redacted]
Title: Gene therapy for RHO-adRP
The goal of this study is to evaluate the toxicity and dose response of a specific gene therapy vector in dogs with *RHO* mutation.
Role: Co-investigator
Overlap: None. Support from this Sponsored Research Agreement does not cover any of the vector optimization and in vivo validation studies that are part of this grant proposal.

R24EY023937-05 van Gelder R (PI) 9/1/14 - 8/31/19
NIH-NEI
Photoswitchable Channel Blockers for Treatment of Blindness

The goal of this multi-investigator, multi-center research project is to develop small molecule photoswitches and test their safety and efficacy in providing visual recovery in animals (including canine models) with end stage retinal degeneration.
Role: Module Principal Investigator (subcontract).

[redacted] Flannery J, Isacoff E, Beltran WA (co-PIs) 9/1/14 - 12/31/18
"Development of Optogenetic Tools with Increased Light Sensitivity for Vision Restoration"
The goal of this project is to test in dogs optogenetic tools tailored at rendering ON bipolar cells light-sensitive.
Role: co-Principal Investigator

Completed Research Support

R24-EY022012 Beltran W (PD/PI) 3/1/12 - 02/28/18
NIH-NEI
"Translational Gene Therapy for Rhodopsin Autosomal Dominant Retinitis Pigmentosa"
The goals of this multi-investigator, multi-center research effort are to develop and test gene-based retinal therapy in mouse and dog models of RHO-ADRP. The study will develop and validate vectors, promoters, knockdown constructs and replacement cDNAs for therapy; establish therapy outcome measures in the models using morphologic and non-invasive functional and imaging that can be extrapolated to patients.
Role: Program Director/Principial Investigator

PN2-EY018241 Isacoff E (PD/PI) 9/31/10 7/31/16
NIH-NEI
"Nanomedicine Development Center for the Optical Control of Biological Function"
The goals of this multi-investigator, multi-center research effort; are to develop and test optogenetic tools in mice and dogs to restore vision.
Role: Module PI

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

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: **ARTUR V. CIDECIYAN, Ph.D.**

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: **RESEARCH PROFESSOR OF OPHTHALMOLOGY**

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 Miami, Coral Gables, FL	B.S.	05/1986	Mechanical Engineering
University of Miami, Coral Gables, FL	M.S.	12/1988	Biomedical Engineering
University of Miami, Coral Gables, FL	Ph.D.	05/1992	Biomedical Engineering

A. Personal Statement

Throughout my career, I have been focused on understanding disease mechanisms and evaluating treatments in hereditary retinal degenerations. My time has always been divided between clinical research studies with patients and basic research studies with large and small animal models, often with duplicated non-invasive methodologies. This "back and forth" approach has been particularly fruitful in understanding the retinal disease caused by monogenic mutations. Specifically, I had the opportunity to perform collaborative studies with Dr. Aguirre and colleagues on the phenotypic comparison and treatment effects of dogs with retinal degeneration and their human counterparts. Specific genes examined include *RPE65, RHO, RPGR, BEST1*, and *NPHP5*. Research methods in my laboratory include imaging, electrophysiology and psychophysics as well as mathematical modeling and software development. My interest in retinal degenerative diseases and my extensive experience in clinical and pre-clinical examinations make me particularly well-suited to fulfill my role in the current proposal.

The following publications highlight my experience and qualifications for this project.

a. Cideciyan AV, Sudharsan R, Dufour VL, Massengill MT, Iwabe S, Swider M, Lisi B, Sumaroka A, Marinho LP, Appelbaum T, Rossmiller B, Hauswirth WW, Jacobson SG, Lewin AS, Aguirre GD, Beltran WA. Mutation-independent rhodopsin gene therapy by knockdown and replacement with a single AAV vector. *Proc Natl Acad Sci USA* 2018;115:E8547-E8556. PMC6130384

b. Guziewicz KE, Cideciyan AV, Beltran WA, Komáromy AM, Dufour VL, Swider M, Iwabe S, Sumaroka A, Kendrick BT, Ruthel G, Chiodo VA, Héon E, Hauswirth WW, Jacobson SG, Aguirre GD. BEST1 gene therapy corrects a diffuse retina-wide microdetachment modulated by light exposure. *Proc Natl Acad Sci USA* 2018;115:E2839-E2848. PMC5866594

c. Cideciyan AV, Jacobson SG, Beltran WA, Sumaroka A, Swider M, Iwabe S, Roman AJ, Olivares MB, Schwartz SB, Komaromy AM, Hauswirth WW, Aguirre GD. Human retinal gene therapy for Leber congenital amaurosis shows advancing retinal degeneration despite enduring visual improvement. *Proc Natl Acad Sci USA* 110:E517-25, 2013. PMC3568385

d. Cideciyan AV, Jacobson SG, Aleman TS, Gu D, Pearce-Kelling SE, Sumaroka A, Acland GM, Aguirre GD. In vivo dynamics of retinal injury and repair in the rhodopsin mutant dog model of human retinitis pigmentosa. *Proc Natl Acad Sci USA* 2005;102:5233-8. PMC555975

B. Positions and Honors

Positions and Employment

1993-1995 Research Assistant Professor of Ophthalmology, University of Miami, Miami, FL.
1994-1995 Research Assistant Professor of Biomedical Engineering, University of Miami, Miami, FL.
1995-2001 Research Assistant Professor of Ophthalmology, University of Pennsylvania, Philadelphia, PA.
2001-2010 Research Associate Professor of Ophthalmology, University of Pennsylvania, Philadelphia, PA.
2010-todate Research Professor of Ophthalmology, University of Pennsylvania, Philadelphia, PA.

Other experience and Service

1996-2008 Member, Graduate group, Bioengineering Dept., University of Pennsylvania, Philadelphia, PA.
1999 Site Visit Committee to NYU, Foundation Fighting Blindness
2001, 2003 Review panel, NSF
2004-todate *Ad Hoc* reviewer/advisor, Foundation Fighting Blindness
2004-todate *Ad Hoc* Member, NIH various Special Emphasis Panels
2004-todate *Ad Hoc* Member, NIH DPVS (or BDPE or VISC) Study Section
2007-todate Editorial Board Member, The Open Ophthalmology Journal
2016,2018 Guest Editor for Proceedings of the National Academy of Sciences USA
1992-todate *Ad Hoc* Reviewer for New England Journal of Medicine, Journal of Clinical Investigation, Proceedings of the National Academy of Sciences USA, Nature Communications, Journal of Physiology, Progress in Retina and Eye Research, Journal of Neuroscience, Human Molecular Genetics, Investigative Ophthalmology and Visual Science, Experimental Eye Research, Vision Research, Visual Neuroscience, and others.

Honors

1983 TBP, Engineering Honor Society
1986 ODK, National Honor Society
1986 Graduated Magna Cum Laude and General Honors
1988-1991 University of Miami Ph.D. Fellowship
1991 Student Paper Competition Finalist, IEEE/EMBS Conference
1996 Travel Award to Tubingen, Germany, ISCEV Conference
1997 Eberhard Dodt Memorial Award
2001 Research to Prevent Blindness William & Mary Greve Scholar
2008 Hope For Vision Visionary Scientists Award
2009 FFB Board of Trustees Award (Co-recipient)
2011 Research to Prevent Blindness Senior Scientific Investigator
2015 Silver Fellow, ARVO
2018 Proctor Award (co-recipient), ARVO

C. Contributions to Science

1. Understanding and treatment of Leber congenital amaurosis (LCA) caused by *RPE65* mutations

LCA refers to severe childhood onset forms of blindness now known to be caused by mutations in 20+ genes. In the late 1990s, initial descriptions of *RPE65* mutations causing human LCA, knockout mouse model and naturally occurring dog model were published nearly contemporaneously. Soon thereafter I was involved in two different multi-institutional collaborations taking different paths for the potential treatment of this rare disease. One approach used 9-cis retinoids in knockout mice to bypass the visual cycle that was blocked (a) and another approach used gene augmentation therapy in dog models in collaboration with Dr. Aguirre. In both approaches, my ERG photoresponse recordings and analyses using a model I had developed earlier provided in vivo evidence of photoreceptor basis of the vision improvements. Over the next decade I continued to be involved in many human, mouse and dog experiments, and contributed substantially to the publication of 32 publications describing different aspects of *RPE65*-LCA and its treatment. Noteworthy among them was my study demonstrating very large improvements in rod- and extrafoveal cone-based sensitivities shortly after human gene therapy (b). I also evaluated quantitatively medium term results demonstrating development of a pseudofovea in treated patients (c). Also important were my studies evaluating the long term effects of gene therapy in terms of vision and retinal degeneration in both humans as well as early- and late-treated dogs in collaboration with Drs. Aguirre and Beltran (d). These studies were significant not only for showing the counterintuitive combination of improved sensitivities co-localizing with progressive retinal degeneration in

gene therapy treated *RPE65*-LCA patients but also provided for the first time ever measures of photoreceptor layer thinning over time in any form hereditary retinal degeneration.

a. van Hooser JP, Aleman TS, He Y-G, Cideciyan AV, et al, Palczewski K. Rapid restoration of visual pigment and function with oral retinoid in a mouse model of childhood blindness. *Proc Natl Acad Sci USA* 97:8623-8628, 2000. PMC26998.

b. Cideciyan AV, Aleman TS, Boye SL, et al, Hauswirth WW. Human gene therapy for RPE65-isomerase deficiency activates the retinoid cycle of vision but with slow rod kinetics. *Proc Natl Acad Sci USA* 105:15112-15117, 2008. PMC256750.

c. Cideciyan AV, Hauswirth WW, Aleman TS, et al, Jacobson SG. Vision 1 year after gene therapy for Leber's congenital amaurosis. *New Eng J Med*, 361:725-727, 2009. PMC2847775.

d. Cideciyan AV, Jacobson SG, Beltran WA, et al, Aguirre GD. Human retinal gene therapy for Leber congenital amaurosis shows advancing retinal degeneration despite enduring visual improvement. *Proc Natl Acad Sci USA* 110:E517-25, 2013. PMC3568385.

2. Disease mechanism and progression of retinal degenerations caused by *ABCA4* mutations

Several key papers in the late 1990s showed that autosomal recessive forms of Stargardt disease was caused by mutations in *ABCA4* - a photoreceptor protein involved in the visual cycle. Over the last two decades, I published 25 papers related to various aspects of human phenotype resulting from *ABCA4* mutations. Initially, I showed in a large series of patients that mutations did not result in the slowing of the retinoid cycle until later stages of the disease; I provided quantitative details of human disease sequence starting with a diffuse accumulation of lipofuscin and ending with complete atrophy of the photoreceptors and RPE (a). Other studies defined the unusual parapapillary preservation and the effects of oral lutein supplementation. Later I published long term serial followup of peripheral function in a large group of patients with two *ABCA4* alleles and provided a predictive model linking specific alleles to disease severity (b). Most unexpected were my findings showing some point mutations resulting in greater disease severity than truncation mutations. I collaborated with Dr. Palczewski and colleagues to generate a dual knock-in mouse replicating a common human mutation and provided evidence for protein misfolding (c). More recently I published another major study where I followed longitudinally the expansion of the leading edge of earliest disease in a large number of patients with two *ABCA4* alleles and provided a predictive model for use in future clinical trials (d).

a. Cideciyan AV, Aleman TS, Swider M, Schwartz SB, et al, Jacobson SG. Mutations in ABCA4 result in accumulation of lipofuscin before slowing of the retinoid cycle: A reappraisal of the human disease sequence. *Hum Mol Genet*, 13:525-534, 2004. Does not fall under the public access policy.

b. Cideciyan AV, Swider M, Aleman TS, et al, Palczewski K. ABCA4 disease progression and a proposed strategy for gene therapy. *Hum Mol Genet*, 18:931-941, 2009. PMC2640207.

c. Zhang N, Tsybovsky Y, Kolesnikov AV, et al, Cideciyan AV, Palczewski K. Protein misfolding and the pathogenesis of ABCA4-associated retinal degenerations. *Hum Mol Genet* 24:3220-3237, 2015. PMC4424957.

d. Cideciyan AV, Swider M, Schwartz SB, Stone EM, Jacobson SG. Predicting progression of ABCA4-associated retinal degenerations based on longitudinal measurements of the leading disease front. *Invest Ophthalmol Vis Sci* 56:5946-5955, 2015. PMC4572941

3. Vulnerability of diseased retinas and development of reduced-illuminance imaging methods

It is well known that standard ophthalmic care involves bright lights and all ophthalmic equipment is subjected to strict standards to make sure that light damage does not occur. However, it is much less known that all light damage standards are based on normal retinas; potential vulnerability of diseased retinas are rarely considered. In a group of patients with *RHO* mutations I described the spatial distribution of disease and suggested that environmental light may be contributing to disease progression (a). Most dramatic evidence for vulnerability of diseased retinas came a few years later in a collaboration with Dr. Aguirre. In a naturally occurring canine model of retinal degeneration due to a *RHO* mutation, I showed that 'standard' fundus photographs resulted in severe retinal degeneration. I carefully titrated the light levels and defined the limits of retinal injury resulting from unexpectedly dim lights (b). To image human patients with vulnerable retinas, I developed two novel autofluorescence methods either by moving the excitation wavelength to the infrared region, or by reducing the light levels of shorter wavelength excitation (c). I used these reduced-illuminance methods in dozens of diseases and published the results. Recently, based on our results, Heidelberg Engineering produced software to allow other users to duplicate our methods, and one of these reduced-

illuminance methods was used as the main outcome measure in a large multi-national ProgStar study evaluating progression of Stargardt disease. Most recently, I published a novel method of imaging with infrared autofluorescence that allows greater contrast between atrophic and non-atrophic retinal regions (d).

a. Cideciyan AV, Hood DC, Huang Y, et al, Jacobson SG. Disease sequence from mutant rhodopsin allele to rod and cone photoreceptor degeneration in man. *Proc Natl Acad Sci USA*, 95:7103-7108, 1998. PMC22754.
b. Cideciyan AV, Jacobson SG, Aleman TS, et al, Aguirre GD. In vivo dynamics of retinal injury and repair in the rhodopsin mutant dog model of human retinitis pigmentosa. *Proc Natl Acad Sci USA*, 102:5233-5238, 2005. PMC555975.
c. Cideciyan AV, Swider M, Aleman TS, et al, Jacobson SG. Reduced-illuminance autofluorescence imaging in ABCA4-associated retinal degenerations. *J Opt Soc Am A,* 24:1457-1467, 2007. PMC2579898.
d. Cideciyan AV, Swider M, Jacobson SG. Autofluorescence imaging with near-infrared excitation: Normalization by reflectance to reduce signal from choroidal fluorophores. *Invest Ophthalmol Vis Sci*, 56:3393–3406, 2015. PMC4455314.

4. Contributions to understanding of differential light-scattering from different OCT layers

Optical coherence tomography (OCT) technology became clinically available in 1995. For OCT to become useful for the retinal degeneration community, it was paramount to determine the identity of different lamina producing different levels of light backscattering. I helped design, perform and analyze the experiments that defined for the first time an unbiased comparison of OCT and histology lamina in normal and degenerate retinas (a). Indeed, decades years later, this highly cited publication remains the only such unbiased estimate. Similar studies were performed in transgenic pigs (b) as well as many human patients. Through my collaboration with Drs. Beltran and Aguirre, I have also performed quantitative analyses of photoreceptor layers in naturally occurring canine models of retinal degeneration. Of note was our recent results evaluating spatio-temporal properties of retinal degeneration and its treatment across vast expanses of canine retina (c). The outer retina OCT signals corresponding to the inner and outer segments of rods and cones have remained controversial. Recently, I made a major contribution to this area by quantitatively describing the outer segment contributions in normal eyes and in the eyes of patients with blue-cone monochromacy (d).

a. Huang Y, Cideciyan AV, Papastergiou GI, et al, Jacobson SG. Relation of optical coherence tomography to microanatomy in normal and rd chickens. *Invest Ophthalmol Vis Sci*, 39:2405-2416, 1998. Does not fall under the public access policy.
b. Huang Y, Cideciyan AV, Aleman TS, et al, Jacobson SG. Optical coherence tomography (OCT) abnormalities in rhodopsin mutant transgenic swine with retinal degeneration. *Exp Eye Res* 70:247-251, 2000. Does not fall under the public access policy.
c. Beltran WA, Cideciyan AV, Lewin AS, et al, Hauswirth WW, Jacobson SG, Aguirre GD. Gene therapy rescues photoreceptor blindness in dogs and paves the way for treating human X-linked retinitis pigmentosa. *Proc Natl Acad Sci USA*, 109:2132-2137, 2012. PMC3277562.
d. Cideciyan AV, Hufnagel RB, Carroll J, et al, Jacobson SG. Human cone visual pigment deletions spare sufficient photoreceptors to warrant gene therapy. *Hum Gene Ther* 24:993–1006, 2013. PMC3868405.

5. Development of novel mathematical models relevant to understanding retinal function and structure

The application of a mathematical model to scientific observations tends to allow greater depth and rigor in hypothesizing about the details of underlying biological processes. My PhD dissertation included a complex model of an imaging system to better understand the sources of the tapetal-like reflex in carriers of X-linked RP. Early in my career I designed a method to record ERG photoresponses in human patients and developed a complex model to fit the resulting recordings (a). I have published the application of the recording method and its modeling in dozens of articles. Also early in my career, I was very interested in the kinetics of dark-adaptation and helped develop a complex model describing the underlying hypothesized retinoid biochemistry. This testing method and the model was used to discover so-called rod plateau in patients with delayed dark-adaptation (b) and quantified cone adaptation abnormalities in a rare disease that was thought to affect only rods (c). I also developed the first model of expected relationship between colocalized photoreceptor structure and function measured non-invasively in human subjects. This model was used initially to demonstrate the dissociation of function and structure in *RPE65*-LCA (d) and later to evaluate the efficacy of gene therapy treatment.

a. Cideciyan AV and Jacobson SG. An alternative phototransduction model for human rod and cone ERG a-waves: normal parameters and variation with age. *Vis Res* 36:2609-2621, 1996. Does not fall under the public access policy.
b. Cideciyan AV, Lamb TD, Pugh EN Jr, Huang Y, Jacobson SG. Rod plateaux during dark adaptation in Sorsby's fundus dystrophy and vitamin A deficiency. *Invest Ophthalmol Vis Sci*, 38:1786-1794, 1997. Does not fall under the public access policy.
c. Cideciyan AV, Zhao X, Nielsen L, Khani SC, Jacobson SG, Palczewski K. Null mutation in the rhodopsin kinase gene slows recovery kinetics of rod and cone phototransduction in man. *Proc Natl Acad Sci USA*, 95:328-333, 1998. PMC18214.
d. Jacobson SG, Aleman TS, Cideciyan AV, et al, Bennett J. Identifying photoreceptors in blind eyes due to RPE65 mutations: Prerequisite for human gene therapy success. *Proc Natl Acad Sci USA*, 102:6177-6182, 2005. PMC1087926.

Complete List of Published Work at NCBI: ncbi.nlm.nih.gov/pubmed/?term=ARTUR+CIDECIYAN

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

N/A Cideciyan (PI) 09/01/17-08/31/19 [redacted]
Study to evaluate QR-110 in LCA due to p.Cys998X mutation in CEP290
This is a multi-center clinical trial in patients with CEP290-LCA.
Role: Principal investigator

R01 EY017549 Aguirre (PI) 09/01/16-08/31/21 NIH/NEI
Translational research for retinal degeneration therapies
The goal of this study is to perform gene therapy in dogs with *NPHP5* mutations.
Role: Co-investigator

N/A Beltran (PI) 06/01/18-05/31/20 [redacted]
Gene therapy for RHO-adRP
The goal of this study is to evaluate the toxicity and dose response of a specific gene therapy vector in dogs with *RHO* mutation.
Role: Co-investigator

[redacted] Guziewicz (PI) 01/01/16-12/31/18 [redacted]
AAV-mediated therapy for Best vitelliform macular dystrophy
The goal of this study is to perform gene therapy in dogs with *BEST1* mutations.
Role: Co-investigator

Completed Research Support

R24-EY022012 Beltran W (Prog Dir) 3/1/12 - 02/28/18 NIH-NEI
"Translational Gene Therapy for Rhodopsin Autosomal Dominant Retinitis Pigmentosa"
The goals of this multi-investigator, multi-center research effort were to develop and test gene-based retinal therapy in mouse and dog models of RHO-ADRP. The study developed and validate vectors, promoters, knockdown constructs and replacement cDNAs for therapy; established therapy outcome measures in the models using morphologic and non-invasive functional and imaging that can be extrapolated to patients.
Role: Co-investigator

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

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: Karina E. Guziewicz

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: Research Assistant Professor of Ophthalmology

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 Environmental & Life Sciences, Wroclaw, Poland	B.Eng.	06/1998	Biology & Animal Sciences
University of Environmental & Life Sciences, Wroclaw, Poland	M.Sc.	10/2000	Genetics & Animal Sciences
Swiss Federal Institute of Technology (ETH), Zürich, Switzerland	Ph.D.	12/2004	Molecular Biology & Genetics
University of Pennsylvania, PA	Post-doc	04/2007	Cell Biology & Ophthalmic Genetics

A. Personal Statement

I am a Research Assistant Professor of Ophthalmology at the UPenn Department of Clinical Sciences & Advanced Medicine, with more than 10 years of research experience in the field of retinal degeneration. Over the past decade, my research has focused on identification and characterization of new animal models for human inherited retinal dystrophies (IRDs) to provide testing platforms for evaluation of novel strategies and modern molecular therapeutics for translational research in ophthalmology. Ongoing studies in my lab span a wide spectrum of interdisciplinary approaches, including characterization of new retinal phenotypes, gene and mutation discovery, elucidation of molecular mechanisms underlying IRDs, as well as design and testing of therapeutic strategies for prevention and/or restoration of vision. As a member of the UPennVet's Division of Experimental Retinal Therapies (ExpeRTs), I have been involved in preclinical research studies aimed at characterizing new animal models and evaluating the efficacy and safety of novel retinal therapeutics. To this end, I have ongoing collaborations with the Aguirre, Miyadera (UPenn Vet), and Jacobson, Cideciyan (UPenn Med) Labs, and have experience with multiple-PI grants. I have recently led a multi-investigator, multi-center research initiative aimed at developing and testing of a new RPE-directed gene therapy for *BEST1*-associated retinopathies. Our research efforts led to the identification of an important translational model for one of the most prevalent forms of juvenile maculopathies in man, Best vitelliform macular dystrophy (BVMD or Best disease). BVMD, caused by mutations in the RPE-specific gene *BEST1*, was thus far considered untreatable, and the lack of progress was mainly attributed to the dearth of reliable animal models to carry out the mechanistic studies. Using our canine Best disease model (#1), we have made significant advances in the understanding of the disease pathogenesis (#2), and developed a safe and effective AAV-based therapy for *BEST1*-associated macular dystrophies (#3 and #4). These proof-of-concept studies are currently under preclinical development. Thus, the present grant application builds logically on my prior work as a natural extension of my expertise in the identification and development of large animal models for translational research in ophthalmology.

1. **Guziewicz KE**, Zangerl B, Lindauer SJ, Mullins RF, Sandmeyer LS, Grahn BH, Stone EM, Acland GM, Aguirre GD. (2007) Bestrophin gene mutations cause canine multifocal retinopathy: a novel animal model for Best disease. *Invest Ophthalmol Vis Sci.* 48: 1959-1967; PMCID: PMC1931491;PMID17460247

2. **Guziewicz KE**, Sinha D, Gómez NM, Zorych K, Dutrow EV, Dhingra A, Mullins RF, Stone EM, Gamm DM, Boesze-Battaglia K, Aguirre GD. (2017) Bestrophinopathy: An RPE-photoreceptor interface disease. *Prog Retin Eye Res.* 58: 70-88; PMCID: PMC5441932; PMID28111324

3. **Guziewicz KE**, Zangerl B, Komáromy AM, Iwabe S, Chiodo VA, Boye SL, Hauswirth WW, Beltran WA, Aguirre GD. (2013) Recombinant AAV-mediated *BEST1* transfer to the retinal pigment epithelium: analysis of serotype-dependent retinal effects. *PLoS One* 8: e75666; PMCID: PMC3797066; PMID24143172

4. **Guziewicz KE**, Cideciyan AV, Beltran WA, Komáromy AM, Dufour VL, Swider M, Iwabe S, Sumaroka A, Kendrick BT, Ruthel G, Chiodo VA, Héon E, Hauswirth WW, Jacobson SG, Aguirre GD. (2018) *BEST1* Gene Therapy Corrects a Diffuse Retina-wide Microdetachment Modulated by Light Exposure. *Proc Natl Acad Sci USA* 115(12): E2839-E2848; PMCID: PMC5866594; PMID29507198

B. Positions and Honors

Employment

2004-05	Research Assistant, Institute of Animal Genetics, VETSUISSE, University of Berne, Switzerland
2007-08	Research Scientist, Laboratory of Retinal Cell Biology, University of Zürich, Switzerland
2008-16	Senior Research Investigator, School of Veterinary Medicine, University of Pennsylvania, PA
2016-	Research Assistant Professor of Ophthalmology, Department of Clinical Sciences & Advanced Medicine, School of Veterinary Medicine, University of Pennsylvania, PA

Honors

2000	*Summa Cum Laude* Award at the University of Environmental & Life Sciences, Wroclaw, Poland
2006	First Award for presentation at the 30th Conference of the International Society of Animal Genetics (ISAG), August 20-25th, 2006, Porto Seguro, Brazil
2012	ISER 2012 Fellowship Award for Young Investigator at the 20th Biennial Meeting of the International Society for Eye Research (ISER), July 21-25th, 2012, Berlin, Germany
2016	RD 2016 Fellowship Award for Young Investigator at the 17th International Symposium on Retinal Degeneration, September 19-24th, 2016, Kyoto, Japan

Other Experience and Professional Memberships

2002-08	Member of the International Society of Animal Genetics (ISAG)
2005-	Member of the Association of Research in Vision and Ophthalmology (ARVO)
2012-	Member of the International Society for Eye Research (ISER)
2013-	Member of the European Association for Vision and Eye Research (EVER)
2017-	Member of the Division of Experimental Retinal Therapies (University of Pennsylvania)

C. Contributions to Science

1. Development of animal models of human IRDs

Our research work focuses on the development and characterization of new animal models for human degenerative diseases of RPE and retina. Our overarching goals are to understand the pathomechanism underlying these conditions and design experimental therapies for prevention and restoration of vision. A large focus of my recent research work has been to develop and test therapeutic strategies for *BEST1*-associated retinopathies, a form of juvenile macular degeneration thus far considered untreatable. I initially identified the disease-causative mutations in the canine *BEST1* gene and characterized this disease model for translational applications in patients. Our recent studies on AAV-mediated *BEST1* gene augmentation therapy in canine models have proven pivotal in the translation to the ongoing preclinical trials. Seminal studies are described in the manuscripts below:

a. **Guziewicz KE**, Zangerl B, Lindauer SJ, Mullins RF, Sandmeyer LS, Grahn BH, Stone EM, Acland GM, Aguirre GD. (2007) Bestrophin gene mutations cause canine multifocal retinopathy: a novel animal model for Best disease. *Invest Ophthalmol Vis Sci.* 48: 1959-1967; PMCID: PMC1931491; PMID17460247

b. Zangerl B, Wickström K, Slavik J, Lindauer SJ, Ahonen W, Schelling C, Lohi H, **Guziewicz KE**, Aguirre GD. (2010) Assessment of canine *BEST1* variations identifies new mutations and establishes an independent bestrophinopathy model (*cmr3*). *Mol Vis.* 16: 2791-2804; PMCID: PMC3008713; PMID21197113

c. Tanaka N, Dutrow EV, Miyadera K, Delemotte L, MacDermaid CM, Reinstein SL, Crumley WR, Dixon CJ, Casal ML, Klein ML, Aguirre GD, Tanaka JC, **Guziewicz KE**. (2015) Canine CNGA3 Gene Mutations Provide Novel Insights into Human Achromatopsia-Associated Channelopathies and Treatment. *PLoS One* 10: e0138943; PMCID: PMC4583268; PMID26407004

d. **Guziewicz KE**, Cideciyan AV, Beltran WA, Komáromy AM, Dufour VL, Swider M, Iwabe S, Sumaroka A, Kendrick BT, Ruthel G, Chiodo VA, Héon E, Hauswirth WW, Jacobson SG, Aguirre GD. (2018) *BEST1* Gene Therapy Corrects a Diffuse Retina-wide Microdetachment Modulated by Light Exposure. *Proc Natl Acad Sci USA* 115(12): E2839-E2848; PMCID: PMC5866594; PMID29507198

2. Understanding retinal homeostasis in health and disease

Central to the development of lasting corrective therapies for IRDs is to understand the underlying disease mechanism. Along with the assessment of new animal models as translational platforms, this area of research constitutes the primary focus of my laboratory. In addition to *in vivo* models, our research team utilizes a range of *in vitro* and *in silico* model systems, including structural modeling and molecular dynamics simulations, to address basic biological questions and to test novel therapeutic approaches.

a. **Guziewicz KE**, Slavik J, Lindauer SJ, Aguirre GD, Zangerl B. (2011) Molecular consequences of *BEST1* gene mutations in canine multifocal retinopathy predict functional implications for human bestrophinopathies. *Invest Ophthalmol Vis Sci.* 52: 4497-4505; PMCID: PMC3175949; PMID21498618

b. Singh R, Kuai D, **Guziewicz KE**, Meyer J, Wilson M, Lu J, Smith M, Clark E, Verhoeven A, Aguirre GD, Gamm DM. (2015) Pharmacological Modulation of Photoreceptor Outer Segment Degradation in a Human iPS Cell Model of Inherited Macular Degeneration. *Mol Ther.* (11): 1700-1711; PMCID: PMC4817951; PMID26300224

c. Tanaka N, Dutrow EV, Miyadera K, Delemotte L, MacDermaid CM, Reinstein SL, Crumley WR, Dixon CJ, Casal ML, Klein ML, Aguirre GD, Tanaka JC, **Guziewicz KE**. (2015) Canine CNGA3 Gene Mutations Provide Novel Insights into Human Achromatopsia-Associated Channelopathies and Treatment. *PLoS One* 10: e0138943; PMCID: PMC4583268; PMID26407004

d. **Guziewicz KE**, Sinha D, Gómez NM, Zorych K, Dutrow EV, Dhingra A, Mullins RF, Stone EM, Gamm DM, Boesze-Battaglia K, Aguirre GD. (2017) Bestrophinopathy: An RPE-photoreceptor interface disease. *Prog Retin Eye Res.* 58: 70-88; PMCID: PMC5441932; PMID28111324

3. Retinal development and cell biology

The key to rapid advances in the development of modern translational platforms is to understand the basic processes involved in retinal development and visual pathways. Therefore, another area of our investigations involves studies on retinal development, architecture, function and epigenetic mechanisms underlying this aspects in health and disease. We have challenged the dogma that within the phylogenetic tree of mammals, haplorhini primates with fovea are the sole lineage in which the retina has a central bouquet of cones, and identified canine fovea-like area that shares not only anatomical features with the primate fovea, but also similar susceptibility to the retinal degenerations. Our recent molecular pathology studies in canine *BEST1* disease models revealed major structural abnormalities at the RPE-photoreceptor interface, implicating RPE microvillar ensheathment and cone-associated insoluble interphotoreceptor matrix that destabilize the close apposition between RPE and photoreceptors. We showed the absence of high-reaching RPE apical processes in the *BEST1* mutation-affected canine fovea-like area predisposes this region to its primary detachment in *BEST1*-associated maculopathies.

a. **Guziewicz KE**, Aguirre GD, Zangerl B. (2012) Modeling the Structural Consequences of *BEST1* Missense Mutations. *Adv Exp Med Biol.* 723: 611-618; PMCID: PMC3951900; PMID22183385

b. Genini S, **Guziewicz KE**, Beltran WA, Aguirre GD. (2014) Altered miRNA expression in canine retinas during normal development and in models of retinal degeneration. *BMC Genomics* 15: 172; PMCID: PMC4029133; PMID24581223

c. Beltran WA, Cideciyan AV, **Guziewicz KE**, Iwabe S, Swider M, Scott EM, Savina SV, Ruthel G, Stefano F, Zhang L, Zorger R, Sumaroka A, Jacobson SG, Aguirre GD. (2014) Canine retina has a primate fovea-like bouquet of cone photoreceptors which is affected by inherited macular degenerations. *PLoS One* 9: e90390; PMCID: PMC3944008; PMID24599007

d. **Guziewicz KE**, McTish E, Dufour VL, Zorych K, Dhingra A, Boesze-Battaglia K, Aguirre GD. (2018) Underdeveloped RPE Apical Domain Underlies Lesion Formation in Canine Bestrophinopathies. *Adv Exp Med Biol.* 1074: 309-215; PMCID: PMC6035728; PMID29721958

Complete list: *ncbi.nlm.nih.gov/pubmed/?term=guziewicz+ke*

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

[redacted] KE Guziewicz (PD/PI) 01/01/16-12/31/18

Title: 'AAV-mediated Therapy for Best Vitelliform Macular Dystrophy'
The goal of this multi-investigator, multi-center research initiative is to develop and test AAV-based gene therapy for *BEST1*-associated maculopathies. The studies are conducted in a validated large animal model, parallel to the investigations in patients and hiPSC-RPE-based *in vitro* model system, designed for maximizing the momentum for translation into the clinical applications.
Role: PD/PI

[redacted] GD Aguirre (PI) 04/01/14-03/31/19

Title: 'PENN Large Animal Model Translational and Research Center'
The goal is to provide support for the animal colonies with inherited retinal degenerations, identify new animal models of retinal dystrophies, and examine molecular mechanisms whereby mutations lead to the RPE and/or photoreceptor degeneration.
Role: Co-investigator

U24-EY029890 JH Wolfe, WA Beltran, DM Gamm (MPIs) 09/01/18-08/31/23
NEI/NIH
Title: 'Canine retinal disease models for translational photoreceptor replacement'
The goal of this multi-investigator, multi-center grant is to develop strategies for photoreceptor cell replacement in canine IRD models.
Role: Co-investigator

Completed Research Support

[redacted] GD Aguirre (PI), KE Guziewicz (co-PI) 05/01/12-04/30/15

Title: 'Gene replacement therapy in bestrophin-1 model: implications for human *BEST1*-disorders'
The goals are to characterize naturally-occurring models for human *BEST1*-associated retinopathies and asses gene augmentation in *cmr3* model.

[redacted] KE Guziewicz (PI) 07/01/11-06/30/13

Title: 'Therapeutic Intervention for *BEST1* disorders'
The major goal is to develop rAAV-mediated gene replacement therapy for canine Best disease models.

Pending Research Support

[redacted]

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

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: **SAMUEL G. JACOBSON, M.D, Ph.D.**

eRA COMMONS USER NAME (credential, e.g., agency login): [redacted]

POSITION TITLE: **PROFESSOR OF OPHTHALMOLOGY**

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, Chicago, IL	B.A.	1966	Humanities
University of Illinois, Chicago, IL	M.D.	1970	Medicine
University of London, London, U.K.	Ph.D.	1977	Psychophysics

A. Personal Statement

For the past 35+ years, I have worked exclusively as a clinician-scientist in the field of inherited retinal degenerations, specifically retinitis pigmentosa (RP). My laboratory focuses on human and animal genetic retinal degenerations in order to decipher the complex retinal response to genetic injury. We have elucidated mechanisms of retinal degeneration, resulting from mutation in genes such as *Rhodopsin*, *Peripherin/RDS*, *TULP1*, *RPGR*, *RPE65*, *CRB1*, *CRX*, *RDH12*, *USH* genotypes, *VMD2*, *BBS1*, *CHM*, *LCA5*, *NPHP6*, *IQCB1*, *SPATA7*, *AIPL1*, *EYS and NR2E3*. The ultimate goal is to develop safe and beneficial treatments for otherwise incurable hereditary retinal degenerations. For example, we discovered the ameliorative effects of vitamin A in Sorsby Fundus dystrophy and Late-onset Retinal Degeneration I was the Regulatory Sponsor and Principal Investigator for a continuing human gene therapy clinical trial for *RPE65*-LCA and am now involved with consortiums of investigators planning or performing treatment trials for other forms of syndromic and non-syndromic retinal degenerations.

B. Positions and Honors

Postgraduate Training and Fellowships:

1970-1971 Intern in Internal Medicine, Rush-Presbyterian St. Luke's Medical Center, Chicago, IL
1971-1972 Resident in Neurology, Rush-Presbyterian St. Luke's Medical Center, Chicago, IL
1972-1977 Honorary Clinical Assistant and doctoral student, The National Hospital, London, U.K.
1977-1980 Resident in Ophthalmology, Mass. Eye & Ear Infirmary, Harvard Med. School, Boston, MA
1977-1983 Research Affiliate, MIT, Department of Psychology, Cambridge, MA
1980-1982 Fellow, Moorfields Eye Hospital, Institute of Ophthalmology & Vision Research Unit, St.Thomas' Hospital, London, U.K.
1982-1983 Fellow, Mass. Eye & Ear Infirmary, Harvard Medical School, Boston, MA

Faculty Appointments:

1983-1995 Director, Retinitis Pigmentosa Center and Visual Physiology Service, Bascom Palmer Eye Institute, University of Miami, Miami, FL
1983-1989 Assistant Professor of Ophthalmology, Bascom Palmer Eye Institute, University of Miami School of Medicine, Miami, FL
1989-1995 Associate Professor of Ophthalmology, University of Miami
1995 Professor of Ophthalmology, University of Miami
1995- Professor of Ophthalmology, Director, Center for Hereditary Retinal Degenerations and Retinal Function Department, Scheie Eye Institute, University of Pennsylvania, Philadelphia, PA

Honors and Awards:

1968, 1969 Alpha Omega Alpha

1977 Queen Square Research Prize in Neurology
1991 RPB Dolly Green Scholar
1989- Scientific Advisory Board, Foundation Fighting Blindness
1992-5 Member, NIH Visual Sciences C Study Section
2001 RPB Senior Scientist Award
2001-2 FFB Board of Trustees Retinal Degeneration Research Award (co-recipient)
2004 Paul Kayser International Award in Retina Research (co-recipient)
2006 Alcon Research Institute Award
2006-9 Ruth and Milton Steinbach Award
2009 Board of Directors Award, Foundation Fighting Blindness (co-recipient)
2014 College of Physicians of Philadelphia
2015 Member (Honorary), La Sociedad Columbiana de Oftalmologia
2016 Fellow, Association of Research in Vision and Ophthalmology (ARVO)
2017 Keynote Speaker, FASEB
2017 Proctor Medal Awardee of ARVO (co-recipient)
2018 Champalimaud Vision Award (co-recipient)

C. Contributions to Science

As for experience to accomplish the goals in the current proposal, in the 1980's I advanced dark-and light-adapted chromatic perimetry techniques in computerized perimeters so that we could measure and map rod- and cone-mediated function across the visual field of patients with inherited retinal degenerations. At first this was in patients with unknown genotypes and subsequently in those with known molecular diagnoses. Always conscious of the practicality of the methods, I adapted a common and available automated perimeter, a 'fixture' in ophthalmic practices for glaucoma visual field monitoring at the time. This method has recently moved to other platforms (in addition to the 'gold standard' automated perimeter from 1986) and is a well-recognized psychophyscal method to measure outcome in natural history studies and clinical trials. When it was obvious that free-viewing perimetry was not suited for patients with certain retinal degenerations (e.g. Leber congenital amaurosis), I developed a full-field stimulus test (FST) that permitted us to quantify rod- and cone-mediated vision in those patients without steady fixation. FST was used to monitor patients in many studies leading up to and including our clinical trial of gene therapy for LCA caused by *RPE65* mutations and it is now used in other departments as an outcome for therapy in LCA and RP with severe visual loss. Further, we now have the ability to relate vision to quantitative measures of structure - a key to deciding which forms of RP are potentially treatable and with what strategies. We evolved the methodology and used it in RP, at first with fundus reflectometry to map visual pigments across the retina and more recently with retinal microstructure by optical coherence tomography (OCT). What is now named 'microperimetry' (previously fundus perimetry) began with our original and subsequent efforts to visualize small stimuli on the fundus of patients with unstable fixation and record their rod and cone-mediated responses to increase accuracy in inherited retinal degenerations. 'Microperimetry' (light- or dark-adapted) has now been adopted as an outcome in certain clinical trials.

Mechanisms of disease and then treatment

Without clues about mechanism, it has been very difficult to treat inherited retinal degenerations. We had clinical eye examinations, and visual function measures, but a major role was played in the 1990's (and before) by human histopathology of eye donor retinas. This provided insights into the structural basis of these difficult-to-fathom diseases. Two rare retinal degenerations, both autosomal dominant, became amenable to 'treatment' as a result of data from histopathology – Sorsby's fundus dystrophy (SFD; now *TIMP3* disease) and a disorder we named late-onset retinal degeneration (L-ORD; now *C1QNTF5* disease). Thickened sub-RPE deposits across the retina in these two entities led to testing the hypothesis that these retinas were suffering from a 'nutritional night blindness' and that an oral dose of vitamin A could alter function positively. Rod vision in both conditions was ameliorated by the short-term use of high doses of oral vitamin A and whetted the taste of this RP clinician for treatment that improved vision and not only slowed the natural history.

a. Jacobson SG, Cideciyan AV, Sumaroka A, Roman AJ, Wright AF. Late-onset retinal degeneration caused by C1QTNF5 mutation: sub-retinal pigment epithelium deposits and visual consequences. *JAMA Ophthalmol*. 2014;132,1252-1255.
b. Jacobson SG, Cideciyan AV, Wright E, Wright AF. Phenotypic marker for early disease detection in dominant late-onset retinal degeneration. *Invest Ophthalmol Vis Sci.* 2001;42, 1882-1890.
c. Kuntz CA, Jacobson SG, Cideciyan AV, Li Z-Y, Stone EM, Milam AH. Sub-retinal pigment epithelial

deposits in a dominant late-onset retinal degeneration. *Invest. Ophthalmol. Vis. Sci.*1996; 37, 1772-1782.

d. Jacobson SG, Cideciyan AV, Regunath G, Rodriguez FJ, Vanderburgh K, Sheffield VC, Stone EM. Night blindness in a TIMP3-associated Sorsby's fundus dystrophy is reversed by vitamin A. *Nature Genet.* 1995;11: 27-32.

Colocalizing function and structure

Bridging the gap between the rare occurrence of available histopathology of human diseased retina and the limits of magnification without cross-sectional view from ophthalmoscopy has been non-invasive optical imaging, specifically OCT. It was obvious to this RP clinician in the 1990's and to the present day that these data would add a dimension of understanding to retinal degenerations. OCT (and algorithms we developed to analyze quantitatively retinal lamination) became routine in our evaluation of retinal degenerations. Over the years of technical advances from time-domain to spectral domain instruments, my laboratory has kept up with the speedy technological improvements in OCT and pursued increased understanding of retinal structure as well as co-localized function and their relationships in the patients and in animal models. Differences and similarities among genotypes have been clarified, all the while seeking clarity of which of the many new human diseases are treatable and by what modalilty.

a. Jacobson SG, Matsui R, Sumaroka A, Cideciyan AV (2016) Retinal structure measurements as inclusion criteria for stem cell-based therapies of retinal degenerations. *Invest Ophthalmol Vis Sci.* 57(5):ORSFn1-9.

b. Jacobson SG, Sumaroka A, Luo X, Cideciyan AV (2013) Retinal optogenetic therapies: clinical criteria for candidacy. *Clin Genet.* 84, 175-82.

c. Jacobson SG, Aleman TS, Cideciyan AV, Sumaroka A, Schwartz SB, Windsor EAM, Traboulsi EI, Heon E, Pittler SJ, Milam AH, Maguire AM, Palczewski K, Stone EM, Bennett J. Identifying photoreceptors in blind eyes due to RPE65 mutations: Prerequisite for human gene therapy success. *Proc Natl Acad Sci USA.*2005;102, 6177-6182.

d. Huang Y, Cideciyan AV, Papastergiou GI, Banin E, Semple-Rowland SL, Milam AH, Jacobson, SG. Relation of optical coherence tomography to microanatomy in normal and *rd* chickens. *Invest. Ophthalmol. Vis. Sci.* 1998; 39, 2405-2416.

The era of gene augmentation therapy arrived

Together with others in a multi-disciplinary collaborative group of scientists and clinicians, we developed a treatment for a specific form of early-onset retinal degeneration, Leber congenital amaurosis (LCA) caused by *RPE65* mutations. We performed collaboaratively the original proof-of-concept work in the canine model of the disease, similar experiments in mutant mice and the pre-clinical safety and efficacy studies that led to the first-in-man clinical trial for *RPE65*-LCA.

a. Jacobson SG, Boye SL, Aleman TS, Conlon TJ, Zeiss CJ, Roman AJ, Cideciyan AV, Schwartz SB, Komaromy AM, Doobrajh M, Cheung AY, Sumaroka A, Pearce-Kelling SE, Aguirre GD, Kaushal S, Maguire AM, Flotte TR, Hauswirth WW. Safety in nonhuman primates of ocular AAV2-RPE65, a candidate treatment for blindness in Leber congenital amaurosis. Hum Gene Ther. 2006 Aug;17(8):845-58.

b. Jacobson SG, Acland GM, Aguirre GD, Aleman TS, Schwartz SB, Cideciyan AV, Zeiss CJ, Komaromy AM, Kaushal S, Roman AJ, Windsor EA, Sumaroka A, Pearce-Kelling SE, Conlon TJ, Chiodo VA, Boye SL, Flotte TR, Maguire AM, Bennett J, Hauswirth WW. Safety of recombinant adeno-associated virus type 2-RPE65 vector delivered by ocular subretinal injection. Mol Ther. 2006 Jun;13(6):1074-84.

c. Acland GM, Aguirre GD, Ray J, Zhang Q, Aleman TS, Cideciyan AV, Pearce-Kelling SE, Anand V, Zeng Y, Maguire AM, Jacobson SG, Hauswirth WW, Bennett J. Gene therapy restores vision in a canine model of childhood blindness. Nat Genet. 2001 May;28(1):92-5.

First-in-human gene therapy trials

I was PI of one of the first-in-human gene therapy clinical trials for *RPE65*-LCA. Our results documented safety, the exceptional improvement of vision in these patients post-treatment, the unexpected abnormality in the kinetics of the visual cycle after treatment, the development of a pseudo-fovea in a subset of these patients and its usefulness for daily life, the inability of the therapy to halt the progressive retinal degeneration in this disease and most recently the slow loss of efficacy. In other words, our observations were a call to the

community of workers in this field to rejoice in our cumulative advances in this treatment and solve some of these problems to make this remarkable therapy even more remarkable than its first iteration.

a. Jacobson SG, Cideciyan AV, Roman AJ, Sumaroka A, Schwartz SB, Heon E, Hauswirth WW. Improvement and decline in vision with gene therapy in childhood blindness. N Engl J Med. 2015; May 14;372(20):1920-6.
b. Cideciyan AV, Jacobson SG, Beltran WA, Sumaroka A, Swider M, Iwabe S, Roman AJ, Olivares MB, Schwartz SB, Komáromy AM, Hauswirth WW, Aguirre GD. Human retinal gene therapy for Leber congenital amaurosis shows advancing retinal degeneration despite enduring visual improvement. Proc Natl Acad Sci U S A. 2013 Feb 5;110(6):E517-25.
c. Jacobson SG, Cideciyan AV, Ratnakaram R, Heon E, Schwartz SB, Roman AJ, Peden MC, Aleman TS, Boye SL, Sumaroka A, Conlon TJ, Calcedo R, Pang JJ, Erger KE, Olivares MB, Mullins CL, Swider M, Kaushal S, Feuer WJ, Iannaccone A, Fishman GA, Stone EM, Byrne BJ, Hauswirth WW. Gene therapy for Leber congenital amaurosis caused by RPE65 mutations: safety and efficacy in 15 children and adults followed up to 3 years. Arch Ophthalmol. 2012 Jan;130(1):9-24.
d. Cideciyan AV, Aleman TS, Boye SL, Schwartz SB, Kaushal S, Roman AJ, Pang JJ, Sumaroka A, Windsor EA, Wilson JM, Flotte TR, Fishman GA, Heon E, Stone EM, Byrne BJ, Jacobson SG, Hauswirth WW. Human gene therapy for RPE65 isomerase deficiency activates the retinoid cycle of vision but with slow rod kinetics. Proc Natl Acad Sci U S A. 2008 Sep 30;105(39):15112-7.

Next steps being taken

The results of our *RPE65* progress have established that there can be a successful path from basic science discovery to proof-of-concept research to pre-clinical safety studies to evaluation of the human phenotype to human clinical trials. I have now investigated numerous hereditary retinal diseases in collaboration with multiple colleagues within and outside our center to assess the potential for treatment and determine the most sensitive outcomes to monitor the specific diseases during a clinical trial. These studies have provided the international retinal degeneration research communities with the groundwork to move towards clinical trials, whether gene therapy or other treatment modalities, for various genetic retinal degenerations.

a. Calzetti G, Levy RA, Cideciyan AV, Garafalo AV, Roman AJ, Sumaroka A, Charng J, Heon E, Jacobson SG. Efficacy outcome measures for clinical trials of USH2A caused by the common c.2299delG mutation. Am J Ophthalmol. 2018 Jun 25;193:114-129.
b. Jacobson SG, Cideciyan AV, Sumaroka A, Roman AJ, Charng J, Lu M, Choudhury S, Schwartz SB, Heon E, Fishman GA, Boye SE. Defining outcomes for clinical trials of Leber congenital amaurosis caused by GUCY2D mutations. Am J Ophthalmol. 2017 May;177:44-57.
c. Charng J, Cideciyan AV, Jacobson SG, Sumaroka A, Schwartz SB, Swider M, Roman AJ, Sheplock R, Anand M, Peden MC, Khanna H, Heon E, Wright AF, Swaroop A. Variegated yet non-random rod and cone photoreceptor disease patterns in RPGR-ORF15-associated retinal degeneration. Hum Mol Genet. 2016 Dec 15;25(24):5444-5459.
d. Cideciyan AV, Hufnagel RB, Carroll J, Sumaroka A, Luo X, Schwartz SB, Dubra A, Land M, Michaelides M, Gardner JC, Hardcastle AJ, Moore AT, Sisk RA, Ahmed ZM, Kohl S, Wissinger B, Jacobson SG. Human cone visual pigment deletions spare sufficient photoreceptors to warrant gene therapy. Hum Gene Ther. 2013 Dec;24(12):993-1006.

Complete List of Published Work in MyBibliography: I have published 328 peer-reviewed articles as of September, 2018, which can be seen at: https://www.ncbi.nlm.nih.gov/pubmed/?term=jacobson+sg

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

N/A Jacobson (PI) 03/01/18-02/28/19 [redacted]
Study to evaluate NR2E3-associated retinal degenerations as targets for therapy
The goal of this study is to determine outcomes for a potential future clinical trial in this rare disease
Role: Principal investigator

N/A Cideciyan (PI) 09/01/17-08/31/19 [redacted]
Study to evaluate [redacted] in LCA due to p.Cys998X mutation in CEP290
This is a multi-center clinical trial in patients with CEP290-LCA.
Role: Co-investigator

R01 EY017549 Aguirre (PI) 09/01/16-08/31/21 NIH/NEI
Translational research for retinal degeneration therapies
The goal of this study is to perform gene therapy in dogs with *NPHP5* mutations.
Role: Co-investigator

N/A Beltran (PI) 06/01/18-05/31/20 [redacted]
Gene therapy for RHO-adRP
The goal of this study is to evaluate the toxicity and dose response of a specific gene therapy vector in dogs with *RHO* mutation.
Role: Co-investigator

[redacted] Guziewicz (PI) 01/01/16-12/31/18 [redacted]
AAV-mediated therapy for Best vitelliform macular dystrophy
The goal of this study is to perform gene therapy in dogs with *BEST1* mutations.
Role: Co-investigator

N/A Boye (PI) 03/01/14-12/31/18 [redacted]
Study to evaluate GUCY2D-LCA as a target for gene therapy
The goal of this study is to develop a strategy to achieve a future clinical trial in this form of LCA disease
Role: Co-investigator

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

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: Hauswirth, William W.

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

POSITION TITLE: Rybaczki-Bullard Professor of Ophthalmology

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
Stanford University, Stanford, CA	BS	06/1966	Chemistry
Oregon State University, Corvallis, OR	PhD	01/1971	Chemistry-Biochemistry
The Johns Hopkins University, Baltimore, MD	Postdoc	08/1975	Biochemistry-Mol. Biol.

A. Personal Statement

Dr Hauswirth has a long-term interest in the delivery and testing of potentially therapeutic genes for Dominant, Recessive and X-Linked Retinitis Pigmentosa, Leber Congenital Amaurosis, Achromatopsia, Blue Cone Monochromacy, Usher's Disease, Macular Degeneration, Diabetic Retinopathy, Glaucoma and Optic Neuropathies in natural and transgenic animal models of each human disease. He has been/is principal investigator on numerous NIH and private foundation grants supporting this work. He was also coPI on a related clinical trial grant for LCA2 in which the patients began treatment in 2007 and for which NIH funded follow-up continues presently. He is also currently PI on a multicenter NIH grant to bring gene therapy for Achromatopsia into clinical testing. Over the past six years he has also generated successful proof of principle gene therapy in animal models for the A3 and B3 forms of Achromatopsia, the RPGR form of X-linked RP, rhodopsin autosomal dominant RP, the ND4 form of Leber Hereditary Optic Neuropathy, Best Macular Dystrophy, Usher 1B, LCA1 and MERTK disease. Overall, more than 390 peer reviewed papers have been published. Through NIH, University and Biotech collaborations the A3 and B3 forms of Achromatopsia, the RPGR form of X-linked RP, the ND4 form of Leber Hereditary Optic Neuropathy, LCA1, MERTK disease as well as X-linked Retinoschisis are either in early phase gene therapy clinical trials. Finally, he collaborates with more than 70 PI's around the world, by designing and providing AAV vectors (~100 per year) for disorders affecting essentially all parts of the eye.

B. Positions and Honors

Positions and Employment

1974 Assistant Professor, Department of Biochemistry, Johns Hopkins University, Baltimore, MD
1976-1980 Assistant Professor, Department of Immunology and Medical Microbiology, College of Medicine, University of Florida, Gainesville, FL
1980-1985 Associate Professor, Department of Immunology and Medical Microbiology,
1983-1984 Visiting Scholar, Department of Pathology, Stanford University, Stanford, CA
1985-present Professor, Department of Molecular Genetics, University of Florida, Gainesville, FL
1986-present Rybaczki-Bullard Professor, Department of Ophthalmology, University of Florida, Gainesville, FL

Other Professional Experience and Honors

1986-1990	Member, Molecular Biology Study Section, NIH.
1987	Welcome and SERC Professor, University of Edinburgh, Scotland.
1990	CNR Professor, Dept. of Molecular Biology, CNR Institute of Genetics, Pavia, Italy
1993	CNRS Professor, Center for Molecular Genetics, Univ. Paris, Gif-sûr-Yvette, France
1996	Visiting Fellow, Wolfson College, Institute of Molecular Medicine, Oxford University, U.K.
1999	Ruth and Milton Steinbach Award
2001	Alcon Award for Vision Research
2002	Trustee's Award for Retina Research, Foundation Fighting Blindness
2004	Paul Kayser International Award for Retinal Research
2005	HeartSight Scientist of the Year, Hope for Vision Foundation
2007	Career Achievement Award in Vision Research, ARVO-OCMB
2009, 11	2009 and 2011 Board of Director's Award, Foundation Fighting Blindness
2009	Florida Scientist of the Year (Florida Trend Magazine)
2009	Third Most Important Science Discovery of 2009 (Time Magazine)
2011	Work cited in "A Decade of Breakthroughs", Science/AAAS, sciencemag.org
2011	International Gold Award of the Chinese Ophthalmological Society
2013	Llura Liggett Gund Award for Lifetime Achievement, Fdn. Fighting Blindness
2014	Human Gene Therapy 25th Anniversary Pioneer Award
2014	Ophthalmology Innovator of the Year Award, OIS/AAO
2016	Invited speaker: Vatican Conference on Children's Regenerative Medicine
2018	2018 co-awardee of the Champalimaud Award for Vision Science

C. Contributions to Science. (Selected from more than 390 peer-reviewed publications)

1. Demonstration for the first time that cone targeted retinal diseases could be cured in animal models work has led to funding for gene therapy clinical trials for the B3 and A3 forms of human Achromatopsia. Hauswirth was PI on the mouse projects and the key vector collaborator on the dog project.

   Alexander JJ, Umino Y, Chang B, Min SH, Li Q, Timmers AM, Hawes NL, Pang JJ, Barlow RB, **Hauswirth WW**. Restoration of cone vision in a mouse model of achromatopsia. *Nat Med.* 2007 Jun;13(6):685-7 PMID:7515894. PMC3985124

   Komáromy AM, Alexander JJ, Rowlan JS, Garcia MM, Chiodo VA, Kaya A, Tanaka JC, Acland GM, **Hauswirth WW**, Aguirre GD. Gene therapy rescues cone function in congenital achromatopsia. *Hum Mol Genet.* 2010 Jul 1;19(13):2581-93. PMC2883338.

2. Demonstration that RPE65 gene therapy restores retinoid cycle activity and vision in humans for a significant period of time but not indefinitely and leads to pseudo-fovea formation in some patients. This longitudinal shows unexpected aspects of LCA2 gene therapy, both in terms of success and limitations. Hauswirth was the preclinical PI and then coPI with Sam Jacobson on the clinical work.

   Cideciyan AV, **Hauswirth WW**, Aleman TS, Kaushal S, Schwartz SB, Boye SL, Windsor EA, Conlon TJ, Sumaroka A, Roman AJ, Byrne BJ, Jacobson SG. Vision 1 year after gene therapy for Leber's Congenital Amaurosis. *N Eng J Med.* 2009 Aug 13;361(7):725-7. PMC2847775.

   Cideciyan AV, Jacobson SG, Beltran WA, Sumaroka A, Swider M, Iwabe S, Roman AJ, Olivares MB, Schwartz SB, Komáromy AM, **Hauswirth WW**, Aguirre GD. Human retinal gene therapy for Leber congenital amaurosis shows advancing retinal degeneration despite enduring visual improvement. *Proc Natl Acad Sci USA.* 2013 Feb 5;110(6):E517-25. PMC3568385.

   Cideciyan AV, Aguirre GK, Jacobson SG, Butt O, Schwartz SB, Swider M, Roman AJ, Sadigh S, **Hauswirth WW**. "Pseudo-fovea formation after gene therapy for RPE65-LCA." Invest Ophthalmol Vis Sci. 2014 Dec 23. 56:16-23 PMID:25537204 PMC4303042

   Samuel G. Jacobson, M.D., Ph.D., Artur V. Cideciyan, Ph.D., Alejandro J. Roman, M.Sc., Alexander Sumaroka, Ph.D., Sharon B. Schwartz, M.S., C.G.C., Elise Heon, M.D. and **William W. Hauswirth**, Improvement and decline in vision with gene therapy in childhood blindness: New Eng. J. Med. 2015 372:1920-1926 PMID:25936984. PMC4450362

3. Demonstration of successful gene therapy for the RPGR form of X-linked RP in dogs has led to corporate funding for an XLRP gene therapy clinical trial. Demonstration that Dominant Rhodopsin RP can be successfully treated by gene therapy in a dog model. Demonstration of successful gene therapy for Best1 disease in a dog model. Hauswirth is the lead academic collaborator.

   Beltran WA, Cideciyan AV, Lewin AS, Iwabe S, Khanna H, Sumaroka A, Chiodo VA, Fajardo DS, Román AJ, Deng WT, Swider M, Alemán TS, Boye SL, Genini S, Swaroop A, **Hauswirth WW**, Jacobson SG, Aguirre GD. Gene therapy rescues photoreceptor blindness in dogs and paves the way for treating human X-linked retinitis pigmentosa. *Proc Natl Acad Sci USA*. 2012 Feb 7;109(6):2132-7. PMC3277562.

   Cideciyan AV, Sudharsan R, Dufour VL, Massengill MT, Iwabe S, Swider M, Lisi B, Sumaroka A, Marinho LF, Appelbaum T, Rossmiller B, **Hauswirth WW**, Jacobson SG, Lewin AS, Aguirre GD, Beltran WA. "Mutation-independent rhodopsin gene therapy by knockdown and replacement with a single AAV vector. Proc Natl Acad Sci U S A. 2018 Sep 4;115(36):E8547-E8556. doi: 10.1073/pnas.1805055115. Epub 2018 Aug 20. PMID:30127005

   Guziewicz KE, Cideciyan AV, Beltran WA, Komáromy AM, Dufour VL, Swider M, Iwabe S, Sumaroka A, Kendrick BT, Ruthel G, Chiodo VA, Héon E, **Hauswirth WW**, Jacobson SG, Aguirre GD. "BEST1 gene therapy corrects a diffuse retina-wide microdetachment modulated by light exposure." Proc Natl Acad Sci U S A. 2018 Mar 20;115(12):E2839-E2848. doi: 10.1073/pnas.1720662115. Epub 2018 Mar 5. PMID:29507198

4. Demonstration that capsid mutations of surface exposed multiple AAV serotypes have unique and useful in vivo properties in transducing mammalian photoreceptors for the purpose of enhancing the efficacy and safety of retinal gene therapy will have multiple applications in future gene therapy clinical trials. Hauswirth was PI on this work.

   Petrs-Silva, H, Dinculescu, A, Li, Q, Min, S, Chiodo, V. Pang, J.J., Zhong, L. , Zolotukhin, S., Srivastava, A., Lewin, A.S and **Hauswirth, W.W**. "High efficiency transduction of the mouse retina by tyrosine-mutant AAV serotype vectors" Mol Ther. 17:463-471 (2009). PMID: 19066593 PMC2835095

   Pang JJ, Dai X, Boye SE, Barone I, Boye SL, Mao S, Everhart D, Dinculescu A, Liu L, Umino Y, Lei B, Chang B, Barlow R, Strettoi E, **Hauswirth WW**. Long-term retinal function and structure rescue using capsid mutant AAV8 vector in the rd10 mouse, a model of recessive retinitis pigmentosa. *Mol Ther*. 2011 Feb;19(2):234-42. PMC3034861.

   Boye SL, Peshenko IV, Huang WC, Min SH, McDoom I, Kay CN, Liu X, Dyka FM, Foster TC, Umino Y, Karan S, Jacobson SG, Baehr W, Dizhoor A, **Hauswirth WW**, Boye SE. A"AV-mediated gene therapy in the guanylate cyclase (RetGC1/RetGC2) double knockout mouse model of Leber congenital amaurosis." *Hum Gene Ther*. 2013 Feb;24(2):189-202. PMC3581260.

   Du W, Tao Y, Deng WT, Zhu P, Li J, Dai X, Zhang Y, Shi W, Liu X, Chiodo VA, Ding XQ, Zhao C, Michalakis S, Biel M, Zhang Z, Qu J, **Hauswirth WW**, Pang JJ. "Vitreal Delivery of AAV Vectored Cnga3 Restores Cone Function in CNGA3-/-/Nrl-/- Mice, an All-Cone Model of CNGA3 Achromatopsia."Hum Mol Genet. 2015 Apr 8. pii: ddv114. [Epub ahead of print] PMID:25855802 PMC4459390

Complete List of Published Work in MyBibliography:
ncbi.nlm.nih.gov/sites/myncbi/william.hauswirth.1/bibliography/41145607/public/?sort=date&direction=ascending

D. Additional Information: Research Support and/or Scholastic Performance

All Ongoing

Grant Identifier: R24 EY022023 (Hauswirth) 6/01/13-5/31/19
Sponsor: NEI
Title of Grant: rAAV-CNGB3 Gene Therapy for Achromatopsia: Translational Research Studies
Goals of Grant: Develop an AAV mediated gene therapy for the B3 form of Achromatopsia through the IND approval stage for a Phase I/II gene therapy clinical trial. Four clinical sites are involved.
Role on Grant: PI

Grant Identifier: NA (Hauswirth) 1/01/13-12/31/21
Sponsor: [redacted]
Title of Grant: Gene Therapy for Blue Cone Monochromacy (BCM)
Goals of Grant: Carry out efficacy and safety studies for BCM gene therapy in rat and mouse models
Role on Grant: PI

Grant Identifier: R01 EY017549 (Aguirre) 9/01/17-8/31/22
Sponsor: NEI
Title of Grant: Translational Research for Retinal Degeneration Therapies
Goals of Grant: Produce custom AAV vectors for testing gene therapy in RP and LCA dog models.
Role on Grant: coPI

Grant Identifier: R01 EY026268 (Lewin) 9/01/17-8/31/22
Sponsor: NEI
Title of Grant: Models of Geographic Atrophy
Goals of Grant: Produce custom AAV vectors for testing gene therapy for GA on rodent models.
Role on Grant: coPI

Grant Identifier [redacted] 8/01/18-7/31/19
Title of Grant: Gene Therapy for ABCA4 Stargardt Disease
Goals of Grant: Develop and test AAV dual vectors for ABCA4 disease gene therapy in the knock-out mouse
Role on Grant: PI

Grant Identifier: R24 EY027285 (Peterson-Jones) 4/01/18-1/31/23
Sponsor: NEI
Title of Grant: Translational Gene Therapy for Cngb1 Retinitis Pigmentosa
Goals of Grant: Through vector potency, vector safety and patient natural history studies file an IND to initiate a clinical trial for Cngb1 RP
Role on Grant: coPI

OMB No. 0925-0001 and 0925-0002 (Rev. 09/17 Approved Through 03/31/2020)

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: Lewin, Alfred S.

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

POSITION TITLE: Shaler Richardson Professor

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 Chicago	B.A.	1973	Biological Sciences
University of Chicago	Ph.D.	1978	Biology
University of Basel, Switzerland	Postdoctoral	1979	Biochemistry

A. Personal Statement

Since the mid-1990's, my laboratory has been attempting to develop gene therapies for diseases of the retina and optic nerve. These include autosomal dominant retinitis pigmentosa (adRP), X-linked retinitis pigmentosa (XLRP), age related macular degeneration (AMD) and Leber Hereditary Optic Neuropathy (LHON). We focus on gene transfer using adeno-associated virus (AAV) for developing treatments in mouse, pig and dog models of adRP. With respect to this project, we have designed small interfering RNA (siRNA) capable of degrading the mRNA for rhodopsin from humans. These are cloned as small hairpin RNAs (shRNAs) or as artificial microRNAs (miRNAs) in AAV in association with a cDNA for human rhodopsin that is resistant to the cognate RNA inhibitor. We have tested these in cell culture, then in mouse models of adRP. We sent the most potent of these to the Beltran Group at the University of Pennsylvania for testing in T4R rhodopsin adRP dogs. While we have had success in using the tools of gene therapy to delay or prevent retinal degeneration in rodent models of adRP (Ref. 1), it is my conviction that a large animal model with cone rich central retinas, such as the the T4R dog, is required to address essential questions regarding delivery dosage and outcome measurements. For this reason, this collaboration is essential for the development of gene therapy for autosomal dominant retinitis pigmentosa. In this application, we start with an shRNA-resistant *RHO* combination AAV vector that has proven effective in preventing retinal degeneration in T4R *RHO* dogs (Ref. 2). In the proposed experiments, we plan to increase the production of rhodopsin protein, determine if there are significant off-target effect of the siRNA, and determine if the AAV vector can prevent ongoing retinal degeneration in the dog model. These steps will help us develop a safe and effective therapy for RHO adRP.

Recent relevant publications:

1. Mao H, Gorbatyuk MS, Rossmiller B, Hauswirth WW, Lewin AS. Long Term Rescue of Retinal Structure and Function in P23H RHO Transgenic Mice by Rhodopsin RNA Replacement with a Single AAV Vector. *Hum Gene Ther. 23*:356-366 (2012). PMID: 22289036; PMC3327607

2. Cideciyan, AV, Sudharsan, R, Dufour, VL, Masengill, MT, Iwabe, S, Swioder, M, Lisi, B, Sumaroka, A. Marinho, LF, Applebaum, T, Rossmiller, B, Hauswirth, WW, Jacobson, SG, Lewin, AS, Aguirre, GD, Beltran, WA. Mutation-independent rhodopsin gene therapy by knockdown and replacement with a single AAV vector. 2018, Proc. Natl. Acad. Sci. (USA) doi: 10.1073/pnas.1805055115. [Epub ahead of print] PMID: 30127005

3. Lewin AS, Rossmiller B, Mao H. Gene Augmentation for adRP Mutations in RHO. Cold Spring Harb Perspect Med. 2014 ;4(9):a017400. doi: 10.1101/cshperspect.a017400. PubMed PMID: 25037104.

4. Mao H, James T Jr, Schwein A, Shabashvili AE, Hauswirth WW, Gorbatyuk MS, Lewin AS. AAV delivery of wild-type rhodopsin preserves retinal function in a mouse model of autosomal dominant retinitis pigmentosa. *Hum Gene Ther.* 2011 May;22(5):567-75; PMID: 21126223; PMCID: PMC3131806.

B. Positions and Honors

Positions and Employment

1981-1987	Assistant Professor of Chemistry, Indiana University, Bloomington, IN
1984	Visiting Professor, Université de Paris-Sud
1987-1994	Associate Professor, Dept. Immunology & Medical Microbiology, University of Florida
1994-	Professor, Molecular Genetics and Microbiology, College of Medicine, University of Florida

Other Experience and Professional Memberships

1995-96	Member, Advisory Committee on Cell Biology, American Cancer Society
1997-98	Member, Advisory Panel on Cell Cycle and Growth Control, American Cancer Society
2001-	Editorial Board, *Mitochondrion*
2001-08	Member NIH SSS-Y study section
2005-	Editorial Board, *Molecular Vision*
2007-	Editorial Board, *PLoS ONE*
2008-12	Member of IMST-E review panel (NIH)
2009-	Member, grant review panel on AMD, BrightFocus Foundation
2010-18	Editorial Board, *Experimental Eye Research*
2012-16	Member DPVS review panel (NIH)
2017-	Deputy Editor *Current Gene Therapy*
2018	Member of grant review panel Foundation Fighting Blindness
2018	Grant review panel on Gene and Cell Therapy, Deutsche Forschungsgemeinschaft

Honors

1987-92	Established Investigator of the American Heart Association
2002	Shaler Richardson Professorship in Ophthalmic Sciences, University of Florida
2003	Jules Stein Living Tribute Award, RP International
2005-06	Doctoral Dissertation Mentoring Award
2008,2009	Exemplary Teacher Award, University of Florida College of Medicine
2011	Board of Directors Award, Foundation Fighting Blindness
2015	Elizabeth Anderson Macular Degeneration Research Award, BightFocus Foundation
2018-21	University of Florida Research Foundation Professorship

C. Contribution to Science

1. As a pre-doctoral student at the University of Chicago I made the first physical map and transcript map of mitochondrial DNA and characterized the consequences of the *petite* mutant of yeast on mitochondrial DNA. These were the early days of eukaryotic molecular biology, and mitochondrial DNA was the first type of eukaryotic genome to be well characterized. As a postdoctoral fellow at the University of Basel, I characterized the import pathway of nuclear encoded subunits of cytochrome oxidase and F1 ATPase from the cytoplasm into mitochondria, and the *PNAS* paper arising from this work remains one of my most cited papers. I continued this line of research as an Assistant Professor at Indiana University and at the University of Florida and expanded into the synthesis of peroxisomal proteins in yeast.

5. Lewin A, Morimoto R, Rabinowitz M. Restriction enzyme analysis of mitochondrial DNAs of petite mutants of yeast: classification of petites, and deletion mapping of mitochondrial genes. *Mol Gen Genet.* 1978 Jul 25;163(3):257-75. PubMed PMID: 355853.

6. Lewin AS, Gregor I, Mason TL, Nelson N, Schatz G. Cytoplasmically made subunits of yeast mitochondrial F1-ATPase and cytochrome c oxidase are synthesized as individual precursors, not as polyproteins. *Proc Natl Acad Sci* U S A. 1980 Jul;77(7):3998-4002. PMID: 6254007; PMCID: PMC349755.

7. Burns DJ, Lewin AS. The rate of import and assembly of F1-ATPase in Saccharomyces cerevisiae. *J Biol Chem.* 1986 Sep 15;261(26):12066-73. PubMed PMID: 2875070.

8. Lewin AS, Hines V, Small GM. Citrate synthase encoded by the CIT2 gene of Saccharomyces cerevisiae is peroxisomal. *Mol Cell Biol.* 1990 Apr;10(4):1399-405. PMID: 2181273; PMCID: PMC362242.

2. I have participated in a long term project with the Hauswirth (UF) and Aguirre/Beltran (UPenn) labs to develop gene therapy for X-linked retinitis pigmentosa associated with mutation in the RPGR-ORF15 gene. My group developed shRNAs that would have been used to diminish expression of mutant RPGR, had that been required in addition to supplementation. We also developed the vector system that was ultimately used in the AAV vector used in the canine model of the disease. AAV mediated gene therapy was effective at halting progression of the disease not only when delivered in young animals, but also in older dogs in which loss of photoreceptors was already underway.

9. Beltran WA, Boye SL, Boye SE, Chiodo VA, Lewin AS, Hauswirth WW, Aguirre GD. rAAV2/5 gene-targeting to rods:dose-dependent efficiency and complications associated with different promoters. Gene Ther. 2010 Sep;17(9):1162-74 PubMed PMID: 20428215; PubMed Central PMCID: PMC2914811.

10.Beltran WA, Cideciyan AV, Lewin AS, Iwabe S, Khanna H, Sumaroka A, Chiodo VA, Fajardo DS, Román AJ, Deng WT, Swider M, Alemán TS, Boye SL, Genini S, Swaroop A, Hauswirth WW, Jacobson SG, Aguirre GD. Gene therapy rescues photoreceptor blindness in dogs and paves the way for treating human X-linked retinitis pigmentosa. Proc Natl Acad Sci U S A. 2012 Feb 7;109(6):2132-7. PubMed Central PMCID: PMC3277562.

11. Beltran WA, Cideciyan AV, Lewin AS, Hauswirth WW, Jacobson SG, Aguirre GD. Gene augmentation for X-linked retinitis pigmentosa caused by mutations in RPGR. Cold Spring Harb Perspect Med. 2014 Oct 9;5(2):a017392. doi:10.1101/cshperspect.a017392. Review. PubMed PMID: 25301933.

12. Beltran WA, Cideciyan AV, Iwabe S, Swider M, Kosyk MS, McDaid K, Martynyuk I, Ying GS, Shaffer J, Deng WT, Boye SL, Lewin AS, Hauswirth WW, Jacobson SG,Aguirre GD. Successful arrest of photoreceptor and vision loss expands the therapeutic window of retinal gene therapy to later stages of disease. Proc Natl Acad Sci U S A. 2015, 112(43):E5844-53 PubMed PMID: 26460017.

3. We have used AAV-ribozyme technology and conditional knockout technology to create a mouse model that recapitulates certain cardinal features of geographic atrophy, including alterations to Bruch's membrane, hypertrophy followed and atrophy of the RPE and death of photoreceptors in regions of RPE atrophy. This model has been useful in testing pharmacological therapy and gene therapy for dry AMD and in studying the importance of autophagy in the RPE. I believe that it will be of importance in testing gene therapy to prevent the development of advanced AMD.

13. Mao H, Seo S, Biswal MR, Li H, Conners M, Nandyala A, Jones K, Le YZ, Lewin AS. Mitochondrial Oxidative Stress in the Retinal Pigment Epithelium Leads to Localized Retinal Degeneration. Invest Ophthalmol Vis Sci. 2014 55(7):4613-27 PubMed PMID: 24985474.

14. Ildefonso CJ, Jaime H, Rahman MM, Li Q, Boye SE, Hauswirth WW, Lucas AR, McFadden G, Lewin AS. Gene delivery of a viral anti-inflammatory protein to combat ocular inflammation. *Hum Gene Ther.* 2015 26(1):59-68. PMID: 25420215; PMCID: PMC4303190.

15. Ildefonso CJ, Jaime H, Brown EE, Iwata RL, Ahmed CM, Massengill MT, Biswal MR, Boye SE, Hauswirth WW, Ash JD, Li Q, Lewin AS. Targeting the Nrf2 Signaling Pathway in the Retina With a Gene-Delivered Secretable and Cell-Penetrating Peptide. Investigative ophthalmology & visual science. 2016; 57(2):372-86. PubMed [journal] PMID: 26842755.

16. Ildefonso CJ, Jaime H, Biswal MR, Boye SE, Li Q, Hauswirth WW, Lewin AS. Gene Therapy with the Caspase Activation and Recruitment Domain (CARD) Reduces the Ocular Inflammatory Response. *Mol Ther.* 2015 Feb 20. doi: 10.1038/mt.2015.30. PubMed PMID: 25698151.

4. With John Guy and Bill Hauswirth, I have helped develop AAV vectors for the treatment of Leber Hereditary Optic Neuropathy (LHON), one of the most common diseases caused by mitochondrial DNA mutations. We took two approaches: The first is to transfer mitochondrial genes to the nucleus, changing the genetic code and adding a targeting peptide for import of the proteins into mitochondria. This approach has led to the first gene therapy clinical trials for a mitochondrial disease. The second approach is to delivery wild-type mitochondrial genes to the mitochondria using an AAV capsid that has a mitochondrial targeting peptide attached to some of its subunits. This technology has powerful implication for the treatment of mitochondrial diseases for the development of animal models for these diseases. I find this research particularly rewarding because it makes practical application of discoveries I helped to make as a graduate student and postdoc.

17.Yu H, Koilkonda RD, Chou TH, Porciatti V, Ozdemir SS, Chiodo V, Boye SL, Boye SE, Hauswirth WW, Lewin AS, Guy J. Gene delivery to mitochondria by targeting modified adeno associated virus suppresses Leber's hereditary optic neuropathy in a mouse model. *Proc Natl Acad Sci* U S A. 2012 May 15;109(20):E1238-47. PMID: 22523243; PMCID: PMC3356643.

18. Yu H, Ozdemir SS, Koilkonda RD, Chou TH, Porciatti V, Chiodo V, Boye SL, Hauswirth WW, Lewin AS, Guy J. Mutant NADH dehydrogenase subunit 4 gene delivery to mitochondria by targeting sequence-modified adeno-associated virus induces visual loss and optic atrophy in mice. *Mol Vis.* 2012;18:1668-83. PMID: 22773905; PMCID: PMC3388991.

19. Koilkonda RD, Yu H, Chou TH, Feuer WJ, Ruggeri M, Porciatti V, Tse D, Hauswirth WW, Chiodo V, Boye SL, Lewin AS, Neuringer M, Renner L, Guy J. Safety and effects of the vector for the Leber hereditary optic neuropathy gene therapy clinical trial. *JAMA Ophthalmol.* 2014 Apr 1;132(4):409-20. PMID: 24457989; PMCID: PMC4266107.

20. Hong Yu, Rajeshwari D. Koilkonda, Tsung-Han Chou, Vittorio Porciatti, Arpit Mehtab, Ian D. Hentallc, Vince A. Chiodo, Sanford L. Boye, William W. Hauswirth, Alfred S. Lewin, and John Guy, Consequences of zygote injection and germline transfer of mutant human mitochondrial DNA in mice. *Proc. Natl. Acad Sci. USA* doi: 10.1073/pnas.1506129112. PubMed PMID: 26438859; PubMed Central PMCID: PMC4620890.

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/sites/myncbi/1zQG7pxaauOAA/bibliography/41449449/public/?sort=date&direction=descending

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

NIH/NEI
1R01EY026268-01 Lewin (PI) 7/1/2016-11/30/2020
Testing Gene Therapy in Mouse Models of Geographic Atrophy

This is a translational research project to develop gene therapy for the advanced dry form of (AMD) using viral vectors to deliver genes for antioxidant and anti-inflammatory proteins in two independent mouse models of this disease.
Role: PI
Overlap: None

1R01EY02699901 Yan (PI) 8/1/2016-7/31/2021
National Institutes of Health (Lewin sub-project from UTMB)
mTORC1-TFEB pathway
Role: Co-I
Overlap: None

1R01EY027754 Ding (PI) 10/1/2017-9/30/2019
National Institutes of Health
The Role of Endoplasmic Reticulum Calcium Channels in Cone Degeneration Resulting from CNG Channel Deficiency (Lewin sub-project from OUHSC)
Role: Co-I
Overlap: None

[redacted] Beltran (PD/PI) 06/01/2018-05/31/2020
Gene Therapy for RHO adRP
The University of Florida component of this project is to purify and provide AAV2/5-RHO820-shRNA820 to co-investigators at the University of Pennsylvania; to test the efficacy of this vector in a P23H hRHO transgenic mouse model of adRP and to human cell lines to exclude or confirm the most likely candidates for off target effects.
Role: Co-I
Support from this Sponsored Research Agreement is for studies leading to an IND with the first generation vector, but do not cover any of the vector optimization and in vivo validation studies that are part of the current grant proposal and that will be needed to develop more efficacious vectors for long-term clinical applications.
Overlap: None.

Completed Research Support

R24-EY022012 Beltran (PD/PI) 03/01/2012-02/28/2018
NIH-NEI
Translational Gene Therapy for Rhodopsin Autosomal Dominant Retinitis Pigmentosa
The goals of this multi-investigator, multi-center research effort were to develop and test gene-based retinal therapy in mouse and dog models of RHO-ADRP. The study developed and validate vectors, promoters, knockdown constructs and replacement cDNAs for therapy; established therapy outcome measures in the models using morphologic and non-invasive functional and imaging that can be extrapolated to patients.
Role: Co-I
Overlap: None

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 1

ORGANIZATIONAL DUNS*: 0422507120000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Trustees of the University of Pennsylvania

Start Date*: 12-01-2019 **End Date*:** 11-30-2020 **Budget Period:** 1

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	DR.	GUSTAVO	D	AGUIRRE		PD/PI					28,440.00	8,674.00	37,114.00
2 .	DR.	KEIKO		MIYADERA		PD/PI					44,010.00	13,423.00	57,433.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **94,547.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Fellow	12			51,324.00	4,619.00	55,943.00
1	Research Coordinator	1.8			9,466.00	2,887.00	12,353.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**68,296.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**162,843.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-18-484 . Received Date: 2018-11-02T11:54:23.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 1

ORGANIZATIONAL DUNS*: 0422507120000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Trustees of the University of Pennsylvania

Start Date*: 12-01-2019 **End Date*:** 11-30-2020 **Budget Period:** 1

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,900.00
2. Foreign Travel Costs	
Total Travel Cost	**2,900.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 1

ORGANIZATIONAL DUNS*: 0422507120000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Trustees of the University of Pennsylvania

Start Date*: 12-01-2019 **End Date*:** 11-30-2020 **Budget Period:** 1

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	39,125.00
2. Publication Costs	1,250.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Other Costs	292,606.00
Total Other Direct Costs	**332,981.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**498,724.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Research	61	498,724.00	304,222.00
		Total Indirect Costs	**304,222.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Louis Martillotti, (212) 264-2069

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**802,946.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	802,946.00

L. Budget Justification*: File Name: Budget Justification_10_31_18 FINAL.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 2

ORGANIZATIONAL DUNS*: 0422507120000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Trustees of the University of Pennsylvania

Start Date*: 12-01-2020 **End Date*:** 11-30-2021 **Budget Period:** 2

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	DR.	GUSTAVO	D	AGUIRRE		PD/PI					28,440.00	8,674.00	37,114.00
2 .	DR.	KEIKO		MIYADERA		PD/PI					44,010.00	13,423.00	57,433.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **94,547.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Fellow	12			51,324.00	4,619.00	55,943.00
1	Research Coordinator	1.8			9,466.00	2,887.00	12,353.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**68,296.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**162,843.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-18-484 . Received Date: 2018-11-02T11:54:23.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 2

ORGANIZATIONAL DUNS*: 0422507120000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Trustees of the University of Pennsylvania

Start Date*: 12-01-2020 **End Date*:** 11-30-2021 **Budget Period:** 2

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,900.00
2. Foreign Travel Costs	
Total Travel Cost	**2,900.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 2

ORGANIZATIONAL DUNS*: 0422507120000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Trustees of the University of Pennsylvania

Start Date*: 12-01-2020 **End Date*:** 11-30-2021 **Budget Period:** 2

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	39,125.00
2. Publication Costs	1,250.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Other Costs	292,606.00
Total Other Direct Costs	**332,981.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**498,724.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Research	61	498,724.00	304,222.00
		Total Indirect Costs	**304,222.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Louis Martillotti, (212) 264-2069

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**802,946.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	802,946.00

L. Budget Justification* File Name: Budget Justification_10_31_18 FINAL.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 3

ORGANIZATIONAL DUNS*: 0422507120000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Trustees of the University of Pennsylvania

Start Date*: 12-01-2021 **End Date*:** 11-30-2022 **Budget Period:** 3

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	DR.	GUSTAVO	D	AGUIRRE		PD/PI					28,440.00	8,674.00	37,114.00
2 .	DR.	KEIKO		MIYADERA		PD/PI					44,010.00	13,423.00	57,433.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **94,547.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Fellow	12			51,324.00	4,619.00	55,943.00
1	Research Coordinator	1.8			9,466.00	2,887.00	12,353.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**68,296.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**162,843.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-18-484 . Received Date: 2018-11-02T11:54:23.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 3

ORGANIZATIONAL DUNS*: 0422507120000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Trustees of the University of Pennsylvania

Start Date*: 12-01-2021 **End Date*:** 11-30-2022 **Budget Period:** 3

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,900.00
2. Foreign Travel Costs	
Total Travel Cost	**2,900.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 3

ORGANIZATIONAL DUNS*: 0422507120000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Trustees of the University of Pennsylvania

Start Date*: 12-01-2021 **End Date*:** 11-30-2022 **Budget Period:** 3

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	39,125.00
2. Publication Costs	1,250.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Other Costs	292,606.00
Total Other Direct Costs	**332,981.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**498,724.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Research	61	498,724.00	304,222.00
		Total Indirect Costs	**304,222.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Louis Martillotti, (212) 264-2069

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**802,946.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	802,946.00

L. Budget Justification*: File Name: Budget Justification_10_31_18 FINAL.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 4

ORGANIZATIONAL DUNS*: 0422507120000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Trustees of the University of Pennsylvania

Start Date*: 12-01-2022 **End Date*:** 11-30-2023 **Budget Period:** 4

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	DR.	GUSTAVO	D	AGUIRRE		PD/PI					28,440.00	8,674.00	37,114.00
2 .	DR.	KEIKO		MIYADERA		PD/PI					44,010.00	13,423.00	57,433.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **94,547.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Fellow	12			51,324.00	4,619.00	55,943.00
1	Research Coordinator	1.8			9,466.00	2,887.00	12,353.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**68,296.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**162,843.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-18-484 . Received Date: 2018-11-02T11:54:23.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 4

ORGANIZATIONAL DUNS*: 0422507120000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Trustees of the University of Pennsylvania

Start Date*: 12-01-2022 **End Date*:** 11-30-2023 **Budget Period:** 4

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	Funds Requested ($)*
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,900.00
2. Foreign Travel Costs	
Total Travel Cost	**2,900.00**

E. Participant/Trainee Support Costs	Funds Requested ($)*
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 4

ORGANIZATIONAL DUNS*: 0422507120000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Trustees of the University of Pennsylvania

Start Date*: 12-01-2022 **End Date*:** 11-30-2023 **Budget Period:** 4

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	39,125.00
2. Publication Costs	1,250.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Other Costs	292,606.00
Total Other Direct Costs	**332,981.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**498,724.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Research	61	498,724.00	304,222.00
		Total Indirect Costs	**304,222.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Louis Martillotti, (212) 264-2069

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**802,946.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	802,946.00

L. Budget Justification*: File Name: Budget Justification_10_31_18 FINAL.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

OMB Number: 4040-0001
Expiration Date: 10/31/2019

RESEARCH & RELATED BUDGET - SECTION A & B, Budget Period 5

ORGANIZATIONAL DUNS*: 0422507120000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: The Trustees of the University of Pennsylvania

Start Date*: 12-01-2023 **End Date*:** 11-30-2024 **Budget Period: 5**

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1 .	DR.	GUSTAVO	D	AGUIRRE		PD/PI					28,440.00	8,674.00	37,114.00
2 .	DR.	KEIKO		MIYADERA		PD/PI					44,010.00	13,423.00	57,433.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **94,547.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Fellow	12			51,324.00	4,619.00	55,943.00
1	Research Coordinator	1.8			9,466.00	2,887.00	12,353.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**68,296.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**162,843.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

Funding Opportunity Number: PA-18-484 . Received Date: 2018-11-02T11:54:23.000-04:00

RESEARCH & RELATED BUDGET - SECTION C, D, & E, Budget Period 5

ORGANIZATIONAL DUNS*: 0422507120000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Trustees of the University of Pennsylvania

Start Date*: 12-01-2023 **End Date*:** 11-30-2024 **Budget Period:** 5

C. Equipment Description

List items and dollar amount for each item exceeding $5,000

Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	
Total Equipment	

Additional Equipment: File Name:

D. Travel	**Funds Requested ($)***
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,900.00
2. Foreign Travel Costs	
Total Travel Cost	**2,900.00**

E. Participant/Trainee Support Costs	**Funds Requested ($)***
1. Tuition/Fees/Health Insurance	
2. Stipends	
3. Travel	
4. Subsistence	
5. Other:	
Number of Participants/Trainees	**Total Participant Trainee Support Costs**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K, Budget Period 5

ORGANIZATIONAL DUNS*: 0422507120000
Budget Type*: ● Project ○ Subaward/Consortium
Organization: The Trustees of the University of Pennsylvania

Start Date*: 12-01-2023 **End Date*:** 11-30-2024 **Budget Period:** 5

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	39,125.00
2. Publication Costs	1,250.00
3. Consultant Services	
4. ADP/Computer Services	
5. Subawards/Consortium/Contractual Costs	
6. Equipment or Facility Rental/User Fees	
7. Alterations and Renovations	
8 . Other Costs	292,606.00
Total Other Direct Costs	**332,981.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**498,724.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1 . Research	61	498,724.00	304,222.00
		Total Indirect Costs	**304,222.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Louis Martillotti, (212) 264-2069

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**802,946.00**

J. Fee	Funds Requested ($)*

K. Total Costs and Fee	Funds Requested ($)*
	802,946.00

L. Budget Justification*: File Name: Budget Justification_10_31_18 FINAL.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Budget Justification

OVERVIEW

This grant proposal represents a project that has been active and highly productive since it was changed 33 years ago from a cooperative agreement (UO1-EY006855) to an RO1. Since 2013, when the project relocated back to the University of Pennsylvania (PENN) from Cornell University (to where it was transferred in 1992), there has been a substantial decrease in the funding level, which threatens the viability and success of the program. While restoring funding to pre-2012 levels would certainly be ideal, we acknowledge that it is likely not realistic. However, we feel that it is important to outline the changes in funding that have been made since 2013 to highlight the negative effects that these continued reductions have on the program. We realize that this is not a conventional way of introducing a Budget Justification, but is the only means we have of presenting our case to the SRG members and NEI's Office of Extramural Research.

EY-006855 was moved from Cornell to PENN on grant year 28. The budget request associated with the transfer of institution was $440,745 direct cost, and represented a major decrease in funding due to prior budget reductions associated with the downturn in the economy in the 2007-2011 time period. The award was further reduced to $354,661 direct costs even though there was no reduction in effort, scope or budgetary needs. With the competing renewal submitted in 2014 for grant years 29-33, we asked for direct costs for the first year of $478,782, and justified this higher level of funding by indicating that: ***a-*** *the current budget only has ~$1,500 for research supplies, and very limited or no funding for additional non-personnel categories other than animal care. As this project is not only a resource for vision scientists, but also carries out cutting edge investigator-initiated research, adequate funding for the research activities is necessary;* ***b-*** *an adequate level of funding is required to maintain a stable colony of research dogs that can serve the needs of multiple investigators, and ensure the continued viability of the program by having a robust semen banking and reproductive health program. A reduced budget will have a huge negative impact on this resource. Therefore, if this grant is to fulfill its role as a vision research community resource, as well as a science-based project, then adequate funding to fulfill these functions is necessary*. Despite the justifiable need, the funding awarded was reduced to $427,725, and this level has been held fixed for the 5 years of the award. Even though NIH Guide Notice NOT-OD-12-036 indicates that there will be no annual cost of living/inflationary adjustments in the salary category, salary increases in fact are mandated by the University. Consequently, the effect of inflation and actual/mandated salary increases result in a current funding levels that are much less than what was awarded in 2014.

Based on real annual cost increases and budget decreases, we have been able to sustain the project by reducing the proportionate cost to EY006855 for maintaining the research colonies and infrastructure at the Retinal Disease Studies Facility (RDSF) to ~42%. The latter adjustments have been made possible because of **1**) separate funding received from NEI/NIH (e.g. R01EY017549 "Translational Research for Retinal Degeneration Therapies", G.D. Aguirre P.I.; U24-EY029890-01 " Canine retinal disease models for translational photoreceptor replacement", Wolfe, Beltran, Gamm, mPI) that include line items for partial support of the RDSF infrastructure and/or the core nucleus of breeding dogs necessary for the research; **2**) ongoing complementary funding since 1994 from the [redacted] for gene therapy projects that have arisen from, been made possible by, and depend upon EY006855 grant; **3**) support of infrastructure through other Federal and non-federal grants. Note that there is no scientific or budgetary overlap among the programs described above, although each provides part of the fiscal support for the [redacted] these programs are detailed in the *Research Strategy* section. Important to note that while we will continue to strive for outside funding, it is not guaranteed, and any lapse in funding will jeopardize sustainability, thereby risking loss of valuable resources

In regards to the proposed budget:

- No requests are made for Equipment, or for Alterations and Renovations.
- The budget will not increase after -01 because of the $500,000/year cap. When adjusted for inflation, the budget requested is well below that requested ($478,782) when the competing renewal submitted in 2014. If there is any reduction in budget, we request a yearly increase until the cap is reached to at least help offset inflationary increases in all areas of the budget.
- The budget requests a 5 year period of funding. This period is essential for two main reasons. Firstly, the extended canine breeding cycle, lifetime, and the timeframes of the diseases make such a period necessary to realistically plan breeding programs, and schedule availability of dogs at appropriate ages and stages of disease for studies. Secondly, research investigators (see *Letters of Support*) using the dogs need a reasonable assurance that the resource will continue to be available before they can commit to undertaking

prospective studies.
• Certain costs of maintaining infrastructure services at RDSF will be provided by the NEI-mandated return of indirect costs to support this aspect of the project. As noted in the Significance section of *Research Strategy* "With the change to an RO1 (in 1991), the NEI director and NEI grants management office stipulated that *all indirect costs* associated with this project including dog care and operations of the animal care facility (RDSF) be "returned to the project by the PENN Vet School to pay for facility operations (e.g. heating, cooling, water, building rental, maintenance, minor repairs, 3rd party services)." In practical terms, ~$0.50 of every dollar of direct costs are returned by the School of Veterinary Medicine (representing 82% of the indirect cost recovery rate of 61% of direct costs), and these are returned **only** for facility operations (not research or direct animal care). This mechanism maximizes the NEI's return on investment, serves numerous other vision research investigators, and is very cost-effective. This is detailed more fully in the non-personnel costs section under 'Animal Care at [redacted]

PERSONNEL ($162,844)
Gustavo Aguirre, VMD, PhD (PI in multiple PD/PI project) is Professor of Medical Genetics and Ophthalmology in the Schools of Veterinary Medicine and Medicine, University of Pennsylvania. Since 1976, Dr. Aguirre has been involved in directing/co-directing this NEI/NIH sponsored project. Dr. Aguirre has continuing responsibilities for guiding the scientific direction of the proposed grant work in the areas of retinal anatomy, pathology, electrophysiology, genetics and establishing treatment outcome measures. He will be responsible for the overall management of the colony that includes planning the breedings, molecular genetic testing, and model discovery, as well as interacting with investigators who will be using the facility and models. Dr Aguirre's time effort commitment to the project will be [redacted] cal. mos) with fringe benefit rate (30.5%) set by the University of Pennsylvania. Based on NIH Guide Notice NOT-OD-12-036, there will be no annual cost of living increases.

Keiko Miyadera, DVM, PhD (PI in multiple PD/PI project) was originally project scientist at the time of the last competing renewal. In -29 grant year, she was changed to Key Personnel associated with her successfully becoming a Diplomate, American College of Veterinary Ophthalmologists and her expanded roles in the project. Her background training in molecular genetics and veterinary ophthalmology has made her an exceptional addition to the program. She has led the studies on csnb and the development of gene therapy approaches that target gene augmentation to ON-bipolar cells. Additionally, she was the clinician who identified and characterized the CNGA3 achromatopsia model, and was the one who mapped the disease locus for early-onset autosomal recessive retinal degeneration and identified the *CCDC66* mutation. She will work with Dr. Aguirre in the large animal studies and perform subretinal or intraocular injections, participate in the clinical examinations and in vivo retinal imaging, and histology and molecular biology assessment. She will be responsible for the surgical/medical components of many of the treatment studies, including those that deal with other investigators in academia and pharmaceutical industry. In addition, she will co-supervise animal and general medical care of the research colony dogs, and will interact with University, Federal and State regulatory agencies to ensure compliance with all animal care regulations. Dr. Miyadera will have [redacted] time effort commitment ([redacted] cal. mos). Based on NIH Guide Notice NOT-OD-12-036, there will be no annual cost of living increases.

[redacted] (postdoctoral research fellow) [redacted] has joined the project as a research fellow. He has been instrumental in mapping and characterizing/several retinal disease loci in dogs among which include the *PPT1* non-syndromic retinal degeneration, and identifying the frameshift mutation in the *ABCA4* model of Stargardt disease. His responsibilities will include development and implementation of genotyping tests for colony dogs, and gene and protein expression studies in support of research studies, and will direct the next generation sequencing part of the work. Dr. Murgiano will have [redacted] time effort commitment ([redacted] cal. mos). Based on NIH Guide Notice NOT-OD-12-036, there will be no annual cost of living increases charged to this grant.

[redacted] research coordinator; [redacted] time effort [redacted] cal. mos.). Coordinates with scientists and other investigators that use dogs, both within the facility and outside. This involves extensive record keeping, communications and coordination of activities of the PIs, Senior Animal Care & Experimental Technician and other scientists, and arranging for deliveries of animals and tissues under the terms of this project. [redacted] will arrange the transfer of animals from [redacted] to other sites within the project, and ascertain all necessary paperwork complies with University, State and Federal regulations is in order. Based on NIH Guide Notice NOT-OD-12-036, there will be no annual cost of living increases.

TRAVEL ($2,900)

Costs are requested for one of the PI's and the research fellow to travel ($1,450/person) to attend the annual ARVO or a comparable scientific meeting such as ASGCT. The meetings are the premier forum for presentation of results of research studies on the colony by the PIs and scientific collaborators. It is here that planning discussions take place with scientific collaborators for experiments using the colony resources during the upcoming year. This request is based on current expenditures.

OTHER DIRECT COSTS ($332,981)

Note that the individual costs under this category are highlighted below in bold.

• *Postdoctoral Research Fellow health insurance* (**$4,800**)

• *Animal Care at [redacted] Facility* (**$284,856**)

This line item represents the direct costs only of breeding and maintaining the canine colonies that form the heart of the project supported by grant EY006855, and conducting PI-directed studies detailed in the *Research Strategy* section. The project colonies are housed at the [redacted] of the School of Veterinary Medicine, University of Pennsylvania. This building was constructed in 1989 as a collaborative effort between the [redacted] and NEI/NIH specifically to house those dogs being studied as models of hereditary retinal degeneration. In 2004 the facility underwent an ~5000 sq ft expansion to facilitate translational studies, and the main source of power for heating/cooling was changed from electricity to natural gas. Four additional special kennel rooms were built in which small groups of dogs can be maintained for short or long-term studies in a light-controlled, isolation environment suitable for some BSL-2 gene therapy procedures. A subsequent renovation was completed in September, 2013 which replaced all air handlers, painted all walls, floors and ceilings, and upgraded the caging in the originally constructed animal rooms. The building and its facilities also are comprehensively described in the *Vertebrate Animals* section.

The project funded by grant EY006855 has been the single greatest user of this facility taking into account the numbers of animals held, number of breedings and whelpings and other procedures performed. The balance of the RDSF's capacity is taken up by other projects, primarily the [redacted] and, to a lesser extent, projects to other investigators that are funded by NEI/NIH or non-Federal sources; almost all the studies involve gene and other therapies for inherited retinal degenerations. Because of the increase in such funding in recent years, together with the budget decreases that have taken place in the last 3 years, it has been possible to reduce the proportionate cost of this facility to the EY006855 project. With the budget requested in this application, we anticipate that the proportionate use of the RDSF by the EY006855 project will be ~42-44% for tenure (2019-2024) of the project. As detailed in the Table in a subsequent page, the percentage reflects the specific animal care costs related to this project and does not include costs that are specific to other grant/projects that use the RDSF.

The dogs occupy runs in 4 main climate controlled kennel rooms, 4 smaller rooms where light cycles and intensities can be controlled independently, a separate puppy room, a whelping room, an isolation and a quarantine room. The dogs live in this building 24 hours per day, 7 days per week, every week of the year. Their needs for food, water and a warm, safe, sanitary and comfortable environment have to be met on a similar schedule. This is dictated not only by the animals' basic biological requirements and humane concerns, but by a variety of regulatory authorities, including NIH, the US Dept. of Agriculture (USDA), the Pennsylvania Dog Law, the University of Pennsylvania Unit for Laboratory Animal Research (ULAR) and Institutional Animal Care & Use Committee (IACUC), NIH's Office of Laboratory Animal Welfare (OLAW), and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).

The total cost of operating the [redacted] comprises fixed, semi-variable and variable expenses. Of these, the major fixed costs are electricity and natural gas consumption, which are used for the building's Heating Ventilation and Cooling (HVAC) as well as for general lighting and power. Regardless of the number of animals held or produced, these costs will remain essentially unchanged. **These costs are NOT included as a line item in the budget.** As noted in the *Significance* section of the Research Strategy and in the *Overview* section of the Budget Justification, the NEI director and NEI grants management office mandated in 1991 that *all indirect costs* be returned to the project by the University of Pennsylvania to pay for facility operations. These

include heating, cooling, water treatment, building rental, maintenance, minor repairs, 3rd party services. In practical terms, $0.50 of indirect costs/$1 in direct costs are returned for the facility operations which do **not** include research or direct animal care.

The remaining charges are directly associated with the operation of the [redacted] For example, labor charges for animal caretaking are the major semi-variable cost. This is high because of the need to schedule staff to be present every day of the year, including weekends, vacations and legal holidays. This issue is not a matter of choice but of biological and legal necessity. These costs are semi-variable because they can only be altered by large changes in the work load. For instance, changes in colony size of less than 25% will have no appreciable effect on labor costs. To achieve a 25% reduction in these costs might require a ~50% reduction in the animal census and colony production. The only linearly variable costs are for supplies such as dog food and vaccines. Although reductions in colony size and production will achieve proportionate reductions in these costs, they only account for a relatively small part of the total operating budget (see Table below).

Since most operating costs are fixed or semi-variable, proportionate savings cannot be achieved by a simple reduction either in the number of animals held or the number of litters produced. For instance, a modest 10% reduction in overall costs requires a ~25-30% reduction in the number of animals held. Similarly, a 25% reduction in these costs requires a 50% reduction in the animal census. This would cripple the viability of the colonies and impinge seriously on the research of all that depend on these animals. A significant reduction in colony size would also have long-term deleterious effects on the colony, creating problems of inbreeding and loss of reproductive vigor. Because of the long generation time of dogs, it would take years to recover from such a reduction, and could risk loss of some of the breeding strains. Consequently, it is important to maintain this colony at a viable productive level that is consistent from year to year.

The total direct operating budget for RDSF, i.e. not just grant EY006855, for the current fiscal year is detailed below:

Category	Total Cost	Charged to EY-006855	EY-06855 %
1) Labor charges, animal caretaking (Animal caretaker salaries + University of Pennsylvania employee benefits)	584,487	245,485	42%
2) Animal Feed	37,261	15,650	42%
3) Animal, veterinary supplies and medicines	46,028	8,300	18%
4) Veterinary & Diagnostic Laboratory Fees, VHUP & Hospital Charges	36,530	4,900	13%
5) Anim research support supplies:blood-tissue collection and chemicals/carcass disposal	26,302	10,521	40%
TOTAL (Direct Operating Budget)	$730,608	**$284,856**	

Note: The costs detailed above represent the actual current expenses. Some line items, e.g. 1), 2) and 5), are proportional to the utilization of the facility for EY-006855-specific projects. Other categories, however, have a lower proportional utilization as higher costs are incurred by other projects

Projected costs for the number of dogs that need to be held, are based on notional Full-Time Dogs (**FTDs**) where a FTD is held for 365 days a year. Thus short-term studies can utilize many more dogs and will be comparable in cost to a long-term study. This estimates the number of nonredundant dogs that will need to be held to meet the animal needs for the projected studies. Factors included in this calculation include: 1) the numbers of dogs needed for individual experiments; 2) the number of breeding dogs needed to produce the required normal and mutant dogs; 3) the length of time each dog will need to be held; 4) current cost factors at the [redacted]

On average, the per diem costs for the dogs is $13/day. This includes charges for breeding, whelping, administering medications, and covers technical support for surgical and other special procedures such as ERG, OCT and vision testing. Compared with other animal care facilities, even at the University of Pennsylvania, this cost

is well below the standard per diem fees. The reason is that other facilities charge 'a la carte', and costs rapidly escalate and can more than double the per diem cost for extended or complex studies that need much technical support.

In summary, we wish to stress the following issues:

- The estimated expenses in this line item represent a lean budget with no room for reduction without seriously impairing the project's performance and viability.
- Indirect costs of operating the [redacted], including such charges as electricity and natural gas, building rental from the [redacted] services and supplies, and 3rd party service and maintenance agreements, will be met by the School of Veterinary Medicine, University of Pennsylvania from Indirect Costs received under the EY006855 award.
- The budgets outlined above depend on the [redacted] operating at its present level, and on grant EY06855 being fully funded at the projected level. Projections of colony performance, our ability to maintain these invaluable mutant lines, our capacity to meet the needs of other investigators, and our ability to do PI-initiated research, critically depend both on initial funding at the level requested and on a consistent level of funding from year to year.

Part of the expenses in this category [*Animal Care at* [redacted] ***$284,856)***] also include several essential services; note that they are listed below to illustrate these expenses. The actual costs are included **within** the different categories in the Table above.

• *Reproduction service/semen collection/freezing*

As noted in *Research Strategy* (Aims 1 and 2), a semen-banking program has been instituted as a safety policy against loss of critical genotypes, and semen is stored at two separate locations in the University of Pennsylvania that are 35 miles apart. Semen banking is also used to maintain mutant strains for which there is no demand [e.g. macular coloboma (CEA), vitreo-retinal dysplasia and a new *PDE6B* mutant allele] or very limited demand [*prcd*, *rcd2*, *crd3*, *erd* (*STK38L*), S-antigen mutation/late-onset RD]. In the former case, the 'colony' consists of a semen-banked resource that can be used to re-derive breeders to subsequently produce affected dogs for research on an as-needed basis. In the latter case, we are able to maintain a small nucleus of breeding males to reduce overall costs, and maintain genetic heterogeneity by using frozen semen from totally unrelated males that have the same affected genotype at the disease locus of interest. As well, we have a strict regimen in place for monitoring the reproductive health of the colony. To carry out the general reproduction oversight of the colony, and collect semen for banking and reuse, we use Veterinary Reproductive Services, a group highly experienced in the area of reproduction. [$2,250 included within Categories 3) and 4) above]

• *Cryogenic supplies and storage*

A comprehensive program for cryogenic storage of tissues from colony animals in four ultralow (-70^0) temperature freezers and/or in liquid nitrogen is at the [redacted] and the PIs' laboratory at the University of Pennsylvania. This program represents an essential part of the project and requires a continuous supply of liquid N2 and dry ice. In addition to their use in semen banking, cryogens are necessary for protocols requiring tissues to be frozen rapidly; for storage of tissues prior to shipping to collaborative investigators; for shipping of tissues to investigators; for long term storage of eyes (eye bank), and tissue samples (blood, spleen, retinas, etc) stored as DNA/RNA resource. Liquid nitrogen or CO_2 supplies connected to our ultralow (-70^0) temperature freezers function as a backup system to prevent loss of cold in the case of electrical or mechanical failure. The cost is based on current usage expenses specifically for this project, and includes cryogenic supplies (Liquid nitrogen, CO_2 tanks, and dry ice). [$3,750 included within Categories 5) above]

• *Histopathology services*

This line item consists of costs for histopathology services for processing eyes for routine morphological evaluation by [redacted] (costs range between $150-200/eye depending on the number of "cuts" taken from each eye and the number of sections and special stains used). Routine histopathology is used in support of *Aim 1* to ascertain consistent phenotype of the retinal disease in the different strains, and to examine ocular/non-ocular tissues from animals in studies with academia or pharmaceutical company scientists or from animals that had unexpected deaths during the studies. [$2,774 included within Categories 4) and 5) above]

• *Laboratory/animal research supplies* (**$39,125**)

Laboratory research supplies: molecular, biochemical and cellular studies (**$27,200**)

These supplies are specific for the PIs'-initiated research studies that are part of this proposal. The budget estimates for this category ($27,200) are based on over 15 years experience in this and other NIH funded grants in which similar studies have been carried out. These costs are illustrated in this section and include costs for Illumina CanineHD Whole Genome Genotyping arrays for GWAS to be done for retinal disease model discovery (Aim 3①) and performed by Geneseek. Costs are $100/sample; 48 samples (cases and controls; $4,800) to be done in -01 and -02 years. Also as part of Aim 3①, whole genome sequencing (WGS) will be carried out at the [redacted] ($9,200/4 samples), and Sanger sequencing from regions of interest to identify variants of interest and carry out homozyosity mapping ($3200). As well, general laboratory supplies ($3,800: Eppendorf tubes, pipettman tips, pipettes (disposable), gloves, plastic tubes for general use, general chemicals, miscellaneous (for small items, e.g. Saran wrap, Kimwipe, Parafilm, cryoboxes, trays, weighing boats, cryotubes), and supplies for histologic studies ($6,200: glass slides, chemicals for tissue fixation, antibodies, processing for cryosections (OCT embedding) or plastic embedding).

Note: The molecular studies will not be done concurrently, but sequentially. We anticipate that GWAS will be undertaken by Q4 of -01 year with WGS in the -01-03 years, while, sequencing and cellular studies of disease characterization and biochemical/protein studies will be carried out during all 5 years of the proposal. The costs of GWAS and WGS of -01 year will be redistributed to the other categories in subsequent years as studies involving protein and gene expression (qRT-PCR, RNA seq) will be used more extensively.

Animal research supplies **($11,925**)

With the exception of the costs listed below, this category includes all animal research supplies used by both the PIs', and other investigators for specific research studies that use the colony dogs and are not covered under the categories listed in the Costs Table in previous pages. This category includes modification of subretinal injectors (cost of the device is $160, and we anticipate using 15 devices/year, $2400). General anesthesia supplies including injectables, inhalation anesthetics and consumables (cost $3,600/year), and medications used for euthanasia, analgesia, and topical/systemic antibiotics and anti-inflammatory drugs used pre- and post-operatively ($2,000). As well, the cost for producing monomethoxy poly(ethylene glycol)-poly(ε-caprolactone (mPEG-PCL) water-soluble self-assembling nanomicelles [38.6 ± 0.6 nm] encapsulating hydrophobic tamoxifen for the neuroprotection and cell survival studies ($3925; see quotation included in the budget). There are no charges for the [redacted]hat will be used in the vitreo-retinal lysis studies to facilitate intravitreal vector administration as the product is provided free of charge by the company [redacted] through a collaborative agreement (see *Letters of Support* section).

• *Service contracts/equipment repairs* (**$2,950**)

This line item includes the service-maintenance contract for four ultralow (-70^0) temperature freezers ($350/freezer/yr; $1400/year). In addition, calibration charges and estimated repairs for Espion ERG system, video cameras and monitors for vision testing, PCR machines, nanodrop spectrophotometers, digital microscopes and computers. The charges ($1550) are proportionate to the use of these instruments by grant EY-006855.

• *Publication Costs* (**$1,250**)

Estimates of document preparation costs are included and represent costs for artwork, and an allowance for scientific journal page charges or publication fees. Examples of publication fees are those charged by *PLoS ONE* ($1,350), *PNAS* printed research article ($1,800), *PNAS Plus* ($2,400) and BMC Genomics ($1793.50); we have published studies in all four journals in the past 3 years that were supported in part by EY-006855. The amount requested ($1,250) is the proportionate cost of the publication to EY-006855.

RESEARCH & RELATED BUDGET - Cumulative Budget

	Totals ($)	
Section A, Senior/Key Person		472,735.00
Section B, Other Personnel		341,480.00
Total Number Other Personnel	10	
Total Salary, Wages and Fringe Benefits (A+B)		814,215.00
Section C, Equipment		
Section D, Travel		14,500.00
1. Domestic	14,500.00	
2. Foreign		
Section E, Participant/Trainee Support Costs		
1. Tuition/Fees/Health Insurance		
2. Stipends		
3. Travel		
4. Subsistence		
5. Other		
6. Number of Participants/Trainees		
Section F, Other Direct Costs		1,664,905.00
1. Materials and Supplies	195,625.00	
2. Publication Costs	6,250.00	
3. Consultant Services		
4. ADP/Computer Services		
5. Subawards/Consortium/Contractual Costs		
6. Equipment or Facility Rental/User Fees		
7. Alterations and Renovations		
8. Other 1	1,463,030.00	
9. Other 2		
10. Other 3		
Section G, Direct Costs (A thru F)		2,493,620.00
Section H, Indirect Costs		1,521,110.00
Section I, Total Direct and Indirect Costs (G + H)		4,014,730.00
Section J, Fee		
Section K, Total Costs and Fee (I + J)		4,014,730.00

Funding Opportunity Number: PA-18-484 . Received Date: 2018-11-02T11:54:23.000-04:00

PHS 398 Cover Page Supplement

OMB Number: 0925-0001
Expiration Date: 03/31/2020

1. Vertebrate Animals Section

Are vertebrate animals euthanized? ● Yes ○ No

If "Yes" to euthanasia

Is the method consistent with American Veterinary Medical Association (AVMA) guidelines?

● Yes ○ No

If "No" to AVMA guidelines, describe method and provide scientific justification

2. *Program Income Section

*Is program income anticipated during the periods for which the grant support is requested?

○ Yes ● No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

*Budget Period *Anticipated Amount ($) *Source(s)

PHS 398 Cover Page Supplement

3. Human Embryonic Stem Cells Section

*Does the proposed project involve human embryonic stem cells? ○ Yes ● No

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://grants.nih.gov/stem_cells/registry/current.htm. Or, if a specific stem cell line cannot be referenced at this time, check the box indicating that one from the registry will be used:

Specific stem cell line cannot be referenced at this time. One from the registry will be used.

Cell Line(s) (Example: 0004):

4. Inventions and Patents Section (Renewal applications)

*Inventions and Patents: ○ Yes ● No

If the answer is "Yes" then please answer the following:

*Previously Reported: ○ Yes ○ No

5. Change of Investigator/Change of Institution Section

❑ Change of Project Director/Principal Investigator

Name of former Project Director/Principal Investigator

Prefix:

*First Name:

Middle Name:

*Last Name:

Suffix:

❑ Change of Grantee Institution

*Name of former institution:

PHS 398 Research Plan

OMB Number: 0925-0001
Expiration Date: 03/31/2020

Introduction	
1. Introduction to Application (for Resubmission and Revision applications)	
Research Plan Section	
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Specific Aims

Translational studies utilizing canine models of photoreceptor (PR) and retinal pigment epithelium (RPE) diseases have greatly accelerated the development and testing of new therapeutic strategies, thereby enabling their transition to human clinical trials (e.g. *RPE65*-LCA[1], *CNGB3*-achromatopsia[2], and *RPGR*-XLRP[3]). Our expanding portfolio of these canine models and our exciting progress emphasizes their importance in the translational continuum, as they have clearly demonstrated that therapeutic outcomes predict the response and long-term outcome observed in human clinical trials[4,5]. With one treatment already on the market[6], and the spectrum of potentially treatable PR and RPE diseases in patients increasing (e.g. *RHO*-adRP [7-9] and Best disease[10,11]), these successes have largely been possible through grant EY-006855. In 1991, the project was changed from a cooperative agreement to an RO1 by Dr. Carl Kupfer, NEI Director, to foster collaborative studies between experienced independent investigators through the use of a centralized resource that promoted and enhanced the success of these collaborations. In parallel with the therapeutic studies, the program encouraged investigation of the molecular mechanisms of patient-relevant model diseases to identify and test experimentally appropriate therapeutic targets. Lastly, efforts were directed to identify new models of relevance to human inherited retinal diseases (IRDs) that lacked the appropriate animal models to investigate the molecular pathogeneses and therapies.

The overarching goal of this project is to accelerate the development and pre-clinical evaluation of new and effective approaches to treat IRDs in patients by the efficient utilization of specific naturally-occurring canine IRD models. Historically, this project developed a well-defined suite of models; established a research facility [redacted]at the University of Pennsylvania) to house them and facilitate research investigations; instituted a comprehensive series of collaborative research programs to characterize the models at the cellular and molecular levels; and initiated efforts directed at therapy and prevention. This cost-effective strategy centralizes the physical and model resources at a site where the PIs and associates have the requisite expertise in the animal diseases to assess responses to therapy, and to develop appropriate outcome measures to establish safety and efficacy.

We **hypothesize** that collaborative research using a variety of canine models housed in a centralized well-maintained resource colony, and with an experienced team of investigators, will lead to critical proof-of-principle studies directed at developing safe and effective new therapies for IRDs in patients. This is supported by previous successes that utilized these models and provided significant groundwork for ongoing, human clinical trials directed at IRD therapies (see *Progress Report*). The current application is tightly focused on the use of these model systems to accelerate development of potential therapies for relevant human diseases.

To achieve this goal, the following Specific Aims will be implemented:

Aim 1. Maintain models critical for development of novel therapies. Canine strains affected with defined IRDs, together with appropriate controls, will be maintained, bred, and made available for ongoing and new research studies. To achieve this aim, we will carefully monitor the general health and reproductive capacity of the colony; verify the consistency of disease expression and inheritance in each mutant strain to ensure phenotypic and genotypic stability; and implement effective breeding plans to ensure the continued viability and availability of the mutant strains. The productivity of each strain, above the baseline necessary for strain continuance, will be adjusted in response to the needs of investigators/studies.

Aim 2. Continue existing or initiate new independent or collaborative studies with outside investigators directed at: **a)** understanding the molecular mechanisms of disease in the canine models; and **b)** evaluating short- and long-term outcomes of potential new therapies.

Aim 3. PI-directed studies to understand the genetic and molecular mechanisms of diseases, and evaluate potential methods of disease prevention, therapy or amelioration. Special emphasis will be placed on studies to: **a)** identify new naturally-occurring canine forms of IRD, and investigate the cell biologic mechanisms critical to the pathogenesis of the diseases; **b)** examine the role of inflammation and microglia/macrophages in retinal degenerative processes, and identify stages and therapeutic targets amenable to disease modulation; **c)** optimize gene augmentation therapies targeting ON-bipolar cells in stationary night blindness; and **d)** test the efficacy of [redacted] to increase vector entry into retina and facilitate intravitreal AAV therapies. Together with studies that will evaluate combinatorial therapies to complement gene augmentation, these studies will provide important supporting data to the ongoing human clinical trials, and inform on neuroprotective and other strategies to prevent further PR degeneration and enhance visual outcomes.

Research Strategy

Significance and Premise

EY-006855 is not a conventional RO1: In 1978, the project "Models for Therapy of Hereditary Retinal Degenerations" started as an NEI-sponsored contract (NO1-EY82142, NO1-EY12111) that changed to a cooperative agreement (UO1-EY006855) in 1984. At the time the agreement was submitted for competitive renewal in 1989, the decision was made by then NEI Director, Dr. Carl Kupfer, to designate the project for future review through the RO1 mechanism (grant EY-006855), and this process has been continued since 1991. Even though the proposal may not be considered a 'traditional' RO1 by the SRG, our progress in the current, and all previous funding periods, indicates that it is an incredibly effective mechanism to support a broad range of hypothesis-driven, investigator-initiated science. At the time of conversion to an RO1, the NEI director and NEI grants management office stipulated that *all indirect costs* associated with this grant be returned to the project by UPenn's Veterinary School to pay for facility operations (e.g. heating, cooling, water, building rental, maintenance, minor repairs, 3rd party services, etc). In practical terms, $0.50 of indirect costs/$1 in direct costs are returned for facility operations (not research). This mechanism maximizes the NEI's return on investment, serves numerous other vision research investigators, and is very cost-effective. Two downsides specific to this mechanism are: 1) budget cuts have a disproportionate adverse impact as there are no other funds to cover the facility operations costs; 2) the direct costs for this project have decreased considerably over the past 7 years thus significantly decreasing the funds available, not only for the research but also for infrastructure support.

The importance of canine IRD models: The achievements funded by this project are documented by the number of peer-reviewed scientific publications (32 since the last review) and abstracts (see *Progress Report Publication List*), and strongly attest to the value of canine models. Moreover, the [redacted] at the University of Pennsylvania supports a number of independently funded programs, and the successful extension of basic and proof-of-principle studies undertaken with the canine models have led to human clinical trials. Specifically, these canine studies enabled clinical trials for CNTF-ECT devices for early/late stage RP, AMD[12-14], and *CNGB3*-achromatopsia[15], and provided the large animal validation[16-18] for the [redacted] epiretinal implant now available commercially in the US and EU. Most notably, proof-of-concept gene therapy studies utilizing the canine models led to clinical trials for *RPE65*-LCA[1,19,20] that are now commercialized[6], as well as *RPGR*-XLRP[3,21,22] and *CNGB3*-achromatopsia[2,23] (both currently in Phase 1/2[24,25]). Further, recent progress in gene therapy for canine adRP[7-9] and Best disease[10,11] has put these diseases on the path to Phase 1 clinical trials. The *Letters of Support* section clearly illustrates the value of the canine models in the translational path from bench to clinic (e.g. see letters from [redacted] in supporting basic science research (e.g. [redacted]) or enabling the work of other funded NIH projects (e.g. [redacted]).

Based on the progress made thus far in going "From the Cage to the Bedside", our ***PREMISE*** is that the development and pre-clinical evaluation of new and effective approaches to treat IRDs in patients can be accelerated by the efficient utilization of specific naturally-occurring canine IRD models. The dog and the canine eye offer advantages for a broad range of translational studies quite apart from the merits of any individual disease model. Because of its lifespan and the time course of model diseases, the dog is an intermediate model between smaller laboratory animals and primates. Furthermore, the similar size of canine and human eyes permits the use of identical surgical approaches for injection of vector or drug using the same volumes or device implantation (e.g. retinal prostheses or for delivery of therapeutic agents) that are identical to those intended for human trials[1,14,17]. Additionally, the instruments/methods for *in vivo* outcome assessments are the same. Lastly, we have identified a fovea-like region in the canine retina with similar cone density as the human fovea. Based on the susceptibility of the canine fovea-like region to inherited macular diseases[26], it makes an ideal model system in which to study macular degenerations and their therapies. It is critical to emphasize, however, that regardless of their translational value, the canine models are *not alternatives* to other laboratory model systems, including rodents. Rather, they are a complementary and synergistic model, serving as an intermediate between rodents and man that provides an excellent test bed to develop or test new therapies. The history of the field clearly demonstrates that progress towards therapy of patients has been served best by judicious use of a comprehensive set of model systems, including rodent, canine and others.

The advantage of maintaining canine mutants as a centralized resource: Many investigators lack the physical resources or expertise to integrate larger species like dogs into their research program. This limitation is readily overcome for basic cell/tissue-based studies by having a facility that produces disease models of known genotype and phenotype and that can provide staged tissues to investigators at specific times for their studies. However, therapy studies require the ready availability of a sufficiently large number of animals of an

appropriate age that must be maintained for the period necessary to evaluate treatment efficacy and safety. In addition, the necessary expertise to assist in implementing the therapy and assessing outcomes using measures that are appropriate to the model and the treatment paradigm must be available to facilitate their future use in patients. From both financial and scientific perspectives, the EY-006855 program represents a unique resource and a highly cost-effective way of making these mutants available for research. Specifically, scientists are relieved of the financial, administrative, and time-consuming effort involved in the breeding and long-term maintenance of dogs for individual research studies. The per diem costs for dogs at most institutions is far higher than the equivalent costs in our facility, and does not include the essential ancillary support services. Furthermore, it is very unlikely that the track record of breeding success in this colony could be matched in individual investigators' institutions. Notably, we have established a track record of initiating studies in-house (i.e. within the EY-006855 project) that have led to separately funded projects that rely on this resource for infrastructure and/or support of the core nucleus of breeding animals. Examples of ongoing grants include an RO1 to Dr. Aguirre (EY-017549; *NPHP5*-LCA gene therapy) and a U24 to Drs. Wolfe/Beltran/Gamm (Multiple PI; EY-029890; Canine retinal disease models for translational photoreceptor replacement), and of completed grants to Drs. Komaromy (EY-019304; cone-directed gene therapy) and Beltran (R24EY-022012; gene therapy for *RHO*-adRP). In addition, modules for a larger NIH project by Drs. Van Gelder (R24EY-023937; Photoswitchable channel blockers for treatment of blindness; ongoing) also requires this centralized facility resources (*Progress Report* section).

This project provides the resource for access to canine models for IRD investigators that could very well be compared, albeit in a smaller scale, to what the Jackson Lab provides for mouse models. In some cases, this project has acted as a straightforward provider of canine tissue samples to independent investigators at multiple institutions. However, consistent with the increased emphasis on translational programs targeting human therapies, this project provides the resources to scientists in both academia and industry and has developed a series of multi-institutional collaborative programs that allows a concerted highly synergistic attack that is tightly focused on the twin goals of understanding the pathogeneses of these disorders, and developing safe and effective therapies.

EY-006855 is more than a resource but a translational science-based project: The role and accomplishments of this project in the past 4 years as a resource for IRD translational studies have been described above. Although critical, this represents a small though very important part of the overall work. During this time, we have also carried out a number of investigator-initiated studies that have resulted in multiple publications and which have focused on model discovery, characterization and molecular mechanisms of disease, identification of cell survival vs cell death pathways, and disease prevention and therapy. Summary details of these are presented in the *Progress Report* and all publications are listed in the *Progress Report Publication List*. Notable among these accomplishments are: identification of the underlying gene mutation responsible for stationary night blindness[27] (*LRIT3*) and two early-onset IRD models (*CCDC66* and *PPT1*); establishment of an oligogenic *RPGRIP1/MAP9*-CRD model [28,29]; examination of miRNA expression changes[30] and dysregulation of cell cycle genes[31]; characterization of the cell death kinetics in *RHO*-adRP[9]; establishment of the role of inflammation in early-[32] and late-stage[33] diseases; characterization of disease metrics for *NPHP5*-LCA[34] and *BEST1*-Best disease[35] as outcome measures for gene therapy; pharmacologic modulation in human iPS cells and *BEST1* mutant dogs[36]; and development of optogenetic tools for non-PR cells[37].

Clinical relevance: Treatment of human IRDs, conditions that at one time were considered incurable has become more attainable for certain forms of IRD. We have demonstrated short- and long-term outcome efficacy of gene therapy for *CNGB3*-achromatopsia[38], *RPGR*-XLRP[21,22,39], *NPHP5*-LCA[40-42], and *BEST1*-Best disease[10]. Studies supported by EY-006855 were crucial in approval of Phase 1/2 clinical trials for *CNGB3*-achromatopsia[38] (NCT02599922) and *RPGR*-XLRP[21,22,39] (NCT03316560). Such progress encourages further steps to be taken. To do so requires the resources and expertise that have been developed and maintained through EY-006855. The track record of this project in providing appropriate model systems, assembling and supporting appropriate teams of investigators with needed resources, and facilitating such collaborative research programs has amply demonstrated that we have the capability, experience and expertise to carry forward such studies, and that canine models provide a resource that will continue to be a vital asset in the quest for safe and effective therapies for IRD in patients.

Innovation

This research program provides an innovative and powerful strategy to translate basic research findings into human retinal therapies using a suite of canine models that bridge the gap between small rodent models and patients. The continuous addition of novel models to our portfolio of well-characterized canine models

provides an important pipeline that will continue to contribute to our understanding of disease-causing mechanisms, pathogenesis, and progression of inherited IRDs, and serve as valuable models for pre-clinical trials to test potential therapies. In addition, our characterization of previously unknown naturally-occurring models of IRDs clinically identified from among the broad canine population provides the potential for finding new mutations related to eye disease in dogs and humans. This not only broadens the knowledge of genes and mutations related to blindness, but also provides opportunities for testing new genetic and surgical applications. Finally, our unique and cost-effective centralized facility supports many collaborative projects, both in industry and academia as well as several existing, pending or prospective NEI-funded projects playing a critical role in synergistically advancing innovative therapeutic strategies for IRDs.

Approach

PROGRESS REPORT: MARCH 5, 2014-PRESENT. The *Aims* (abbreviated) were: ***1***-maintain models critical for development of novel therapies; ***2***-continue existing collaborative and independent studies with outside investigators to understand molecular mechanisms of disease and evaluate potential therapies; ***3***-initiate and promote new collaborative and independent investigations to better understand the genetics and disease mechanisms, and evaluate approaches for disease prevention or modification (targeting therapies to PRs; identifying new IRD models; cell death-survival molecular signals). We have *successfully accomplished all Aims*; results are summarized below, and relevant papers or abstracts are cited (see *Progress Report Publication List*):

• In support of *Aims 1-3*, we have distributed dogs or tissues or made planned breedings for investigators in industry (3 times to 3 different companies) and academia (36 times to 21 different investigators). The number of academic investigators noted above **excludes** those associated with the PIs and associates (Drs. Beltran/ Guziewicz) or collaborators at UPenn (Drs. Jacobson/Cideciyan) or Univ Florida (Drs. Hauswirth/Lewin). Specific details of investigators, project, and animals distributed are presented at the end of the *Resource Sharing Plan* section.

• In support of *Aims 2* and *3* (molecular mechanisms of disease and potential therapies), published studies supported by EY-006855 have: developed methods to assess visual function in disease[8], and as outcome measures after gene therapy[22]; examined miRNA expression changes[30] and dysregulation of cell cycle genes resulting in PR proliferation[31] as potential therapeutic targets in three non-allelic models of early-onset disease; defined the cell death kinetics in *RHO*-adRP[9] and excluded the unfolded protein response[43] from involvement in this process; established the role of inflammation in PR degeneration by showing up-regulation of inflammatory genes and innate immune response in early-[32] and late-stage[33] diseases; established disease metrics for *NPHP5*-LCA[34] as essential outcome measures for gene therapy; and defined the molecular and structural consequences of bestrophin mutations in RPE as outcome measures for gene therapy[35].

• In support of *Aims 2, 3* (evaluate potential therapies, targeting PRs and RPE), studies examined pharmacologic modulation of outer segment degradation in human iPS cells and in dogs with bestrophin gene mutations[36]; collaborated with groups to develop optogenetic tools to restore retinal function in ganglion and ON-bipolar cells[37]; demonstrated short- and long-term outcome efficacy of gene therapy for achromatopsia[38], X-linked RP[21,22,39], *NPHP5*-LCA[40-42] and *BEST1*-Best disease[10]. Most significantly, studies supported by EY-006855 were crucial in the FDA approval of Phase 1/2 clinical trials for *CNGB3*-achromatopsia[38] (NCT02599922) and *RPGR*-X-linked RP[21,22,39] (NCT03316560).

• In support of *Aim 3* (new naturally-occurring IRD models), we have identified the gene and mutation responsible for stationary night blindness[27] (*LRIT3*), and two early-onset IRD models in Portuguese water dogs (*CCDC66*) and Miniature Schnauzers (*PPT1*); in the latter breed, the novel gene mutation causes a non-syndromic retinal degeneration rather than neuronal ceroid lipofuscinosis. Additionally, we have now established a model for oligogenic retinal degeneration for which a mutation in *RPGRIP1* is essential but not sufficient to account for all the phenotypic variabilities in an IRD strain[28,29]. Finally, using whole genome sequencing (WGS), we have identified a 'C' insertion in exon 28 of *ABCA4* that results in a frameshift/premature stop codon; the mutation causes outer retinal degeneration and has already been introduced into the colony.

SCIENTIFIC RIGOR: The methods used in this proposal have been in use and validated through many publications[3,8-10,21,22,28,34,38,42] and include detailed record keeping of research colony animals, their phenotype assessment and ancillary testing performed. All this is peer-reviewed both at the technical level (e.g. entries by animal care techs are reviewed by the senior technician) as well as investigator level (e.g. recordings, imaging, clinical assessments made by postdoctoral researchers are always peer-reviewed by the PIs). The PIs taking

part in the assessment of therapeutic outcome will be blinded to the genotypes of each animal as well as therapeutic strategies assigned to each eye. Disease-associated genotypes are always verified by molecular testing, either in-house or at an ISO17025 certified laboratory. To further emphasize scientific rigor, it is important to note that we have been approved by the FDA to carry out 'GLP-like' work in the recently completed IND-enabling studies by [redacted] that led to the Phase 1/2 clinical trial for *RPGR*-XLRP. This approval covers everything from animal record keeping, therapeutic intervention, outcome assessment, and reporting. This process is now formally established in all of our research protocols. For the PI-directed studies, we work with [redacted] Biostatistics core of UPenn Vision Res. Center) using biological replicates (n=3 eyes or dogs) that have sufficient power to detect differences in parameters, e.g. ONL thickness, ERG amplitudes, that are both statistically and biologically significant. Our approach is based on the premise that a statistically significant effect in a valid experimental animal model has to be robust and biologically relevant. For example, we do not consider that preserving 1 row of PR nuclei will be sufficient to support further pre-clinical and clinical development. Consequently, if ONL thickness is used as an outcome measure of structural rescue, with an n=3, we achieve sufficient power in a paired (one-sided) situation.

BIOLOGICAL VARIABLES: All the animals used in the proposed studies are bred, raised and maintained in the same research animal facility. They are housed in the same conditions (light cycles, temperature, and humidity) and cared for by the same long-term staff. Lastly, they receive the same food and prophylactic medical care. For the studies proposed, we will use both males and females, but the ages will depend on the disease being investigated and the treatment protocol. Any sex and/or age-related effects will be clearly documented, analyzed and reported.

Aim 1. Maintain models critical for the development of novel therapies. Specific strains of dogs affected with defined IRDs, together with appropriate controls, will be maintained and bred, and made available for ongoing and new research studies. Details on the mutant strains and the facility are comprehensively described elsewhere in this proposal (see *Budget Justification*, *Vertebrate Animals*, *Facilities and Other Resources*).

Overview: This colony is a unique genetic resource where specific strains of dogs, both normal and affected, have been developed and the diseases characterized. These are special dogs and need special care. They are kept in accordance with IACUC, USDA and NIH guidelines, but the minimum care needed to maintain this colony is far greater than for most dog colonies. A semen-banking program has been instituted as a safety policy against loss of critical genotypes. A strict regimen for monitoring the reproductive health of the colony is in place, and the breeding performance of the RDSF colony has been both outstanding and in stark contrast with the poor natural fertility and health of the foundation stock for most of the strains. Colony dogs now reach sexual maturity by 9 months old; conception rates average over 0.8; female dogs come into estrus every 7 months, routinely produce more than 1 litter/year and deliver an average of over 5 viable pups per litter.

Since this project was initiated in 1976, we have continued to adjust the breeding strategies in response to evolving research needs. When critical animals from these pedigrees are culled, either for research or because their useful reproductive life is past, tissue samples are preserved as an archived DNA resource for future molecular genetic/biochemical studies. These tissues are stored at -80°C until required by investigators.

Special Testing/Monitoring We perform clinical, functional and molecular monitoring to ascertain the consistency of the genotype and phenotype to permit comparisons across multiple studies by all investigators. Clinical and ERG testing is performed for disease confirmation or characterization. We also have well-established protocols for morphologic diagnosis by light microscopy, electron microscopy, and immunohistochemistry (IHC) for each strain (e.g. see[10,27,28,34]), and have developed molecular tests to establish genotype-based diagnoses[44].

Planned Productivity There are two specific issues that need to be emphasized: **a**) with increased demand, we could moderately increase colony production without incurring significant cost increases, as operating costs would not change significantly. Conversely, we can only achieve significant cost savings by dramatic changes in colony size and productivity. Our planned productivity is therefore critically dependent on the stability and level of funding, and reductions will have severe, disproportionate and long-lasting effects on our ability to meet our goals. This issue is detailed in *Budget Justification*; **b**) In this proposal, we request a 5-year renewal period because: **b.i** - the extended canine breeding cycle, lifetime, and the timeframes of the diseases make such a period necessary to plan breedings to schedule the availability of dogs at appropriate ages and stages of disease for studies; **b.ii** - investigators need a reasonable assurance that the resource will continue to be available before they can commit to undertaking prospective studies.

Model Production and Availability It is not possible to keep all models at maximal productivity/availability both from physical and budgetary standpoints. Based on interest expressed by outside investigators, or by the scientific needs of the PIs and collaborators, we have classified the available models into three categories:

a) *No production*: those with no current demand, e.g. macular coloboma (CEA[45]), vitreoretinal dysplasia[46], a new *PDE6B* mutant allele[47], *rcd2*[48] (RD3[49,50]), and S-antigen mutation/late-onset IRD[51] are maintained as a semen-banked resource. As needed, we can re-derive breeders to subsequently produce affected dogs for research within a 1.8- to 2-year time period.

b) *Limited production*: those models for which few investigators have requested dogs, e.g. *prcd*[52] (Kolandaivelu[53]), *crd3*[54] (*ADAM9*[55]), and *erd* (*STK38L*[56,57]) are maintained in 'limited production' status; this satisfies the investigators' needs and allows for the expansion or contraction of the breeding nucleus if needs change. An example of changing needs is exemplified by the *RPE65*-LCA model for which demand plummeted after the proof-of-concept gene therapy studies were done[1,19], and three independent clinical trials initiated (see[20] for review). Now there is an increased demand to assess combinatorial therapy with gene augmentation therapy and to correlate the PR structure at the time of treatment with the therapeutic outcomes[58].

c) *Full production*: These are models for which there is great demand, both for research studies proposed by the PIs in this application, by independent or collaborative scientists in academia or industry, or to maintain the breeding nucleus of dogs that supports other funded grants - ***i***) *NIH*: RO1 (-17549, -22975), R24 (-22023, -23937), U24 (-29890), and R44 (-025905); ***ii***) *foundations*: [redacted] (PI: Miyadera); ***iii***) *pharmaceutical industries*: sponsored research agreement with [redacted] These models have mutations in the following genes: *RPE65*[1,59], *PDE6B* (*rcd1*[14,60]), *CNGB3* (achromatopsia[3,61]), *NPHP5* (LCA[47]), *BEST1* (cmr[11,62]), *RPGR* (XLRP[3,63]), *RHO* (T4R[64,65]), and are used for testing IRD therapies or examining molecular mechanisms of disease to identify therapeutic targets.

Problems, Alternative Strategies, and Benchmarks. No problems requiring alternative strategies are anticipated. Benchmark for success will be to meet demands while maintaining the reproductive viability and genetic diversity of colonies that have a consistent genotype and disease phenotype.

Aim 2. Continue existing or initiate new independent or collaborative studies with outside investigators directed at **a)** understanding the molecular mechanisms of disease in the canine models; **b)** evaluating short- and long-term outcomes of potential new therapies.

Overview: This aim focuses on continuing pre-existing studies, or initiating new ones, that inform on molecular mechanisms of disease, identify therapeutic targets, or that evaluate potential therapies to establish critical proof-of-principle results in preparation for human clinical trials. These studies are initiated by independent (academia, industry) scientists outside the PIs' 'group', and utilize the animal/tissue resources or obtain tissues/models for use in their own facility [redacted]. Alternatively, investigators rely on our resources to carry out their own independent studies with the assistance of the PIs or associates (see below). So far, Pharma studies fall in the 'independent-assistance' category.

Ongoing studies: For 40+ yrs, this project has been actively involved in collaborative research with the laboratories of literally hundreds of different investigators (21 investigators and 3 companies in the past 5 years). In many cases, investigations began as pilot studies entirely within the scope of EY006855; in others, this project has functioned as a research resource for independently initiated projects. We anticipate that this pattern of research will continue in the proposed period. Selected projects exemplifying these collaborative research programs are described below, and listed at the end of the *Resource Sharing Plan*. A comprehensive description of investigational methods or needs for dogs are not detailed because of space limitations, and most have already been peer-reviewed by the NIH or [redacted] For NIH, selected examples of funded grants include R01EY017549-Aguirre, R01EY022975- Flannery, R24EY023937-Van Gelder, R44EY025905 - Nanoscope, and U24EY029890-Wolfe/Beltran/Gamm. Fo[redacted] two projects have been awarded for work led by [redacted] scientists [redacted] TRAP award; Guziewicz-Best disease gene therapy). As well, our collaborator [redacted] work has been awarded the *Restore Vision 20/20 Award* by the [redacted] in 2018. It needs to be stressed that there is no overlap between the programs or budget of EY006855, and the collaborative research programs described. The EY006855 project provides the canine models and breeding stock, the physical infrastructure for specific studies, a highly skilled staff of animal care personnel specifically experienced in planning and overseeing investigational canine studies, and the advice and assistance of the PIs and associates. The actual costs of the studies in the investigator's laboratory are the responsibility of each investigator.

Studies in support of Pharma present a comparable situation and are a mechanism that enables companies to carry out projects to assess potential products for human therapies. Overall, we provide intellectual and infrastructure support to carry out proof-of-principle studies, including assistance with

developing MTA's with the University, advising on model selection and developing outcome measures. During the current grant period, we have assisted [redacted] through the SRA mechanism to begin clinical trials for *RPGR*-XLRP[24]. For the coming 5-year period, we have already agreed to assist [redacted] with optogenetic studies and [redacted] to bring *RHO*-adRP to pre-IND submission and to eventual clinical trials.

Lastly, it needs to be stressed that although many of the studies undertaken as collaborative or independent projects facilitated by EY006855 involve varying teams of investigators, supported by different funding sources, they are all united in the common goal of accelerating progress towards safe and effective therapies for human IRDs. This applies to both the many established investigators with whom the EY006855 project has interacted previously, and to the newer, younger investigators that we collaborate with. Thus, progress in any one collaboration or independent effort is likely to have synergistic influences on others.

Collaboration or service? The canine models are made available for either collaborative or independent studies. The decision as to whether to use these models in a collaborative program or independently, is entirely up to the investigator making the request. If the request is to provide dogs or tissue samples or performing a purely technical function (e.g. enucleating eyes, injecting eyes with drugs, etc.), the work is service and **not** done on a collaborative basis, and under no circumstances is authorship expected. In several cases, however, the functions performed for projects are far and above a service role. This would include such topics as designing part of the research study, performing the research in a manner that goes beyond technical service, analyzing the data and/or writing the relevant parts of the paper, and reviewing the finished manuscript prior to submission. In such cases, the investigator usually will include one or more of the EY-006855 scientists as authors, but the decision to do so is theirs. These functions meet or exceed the generally accepted criteria for authorship ('must be limited to those who have contributed substantially to the work'). The following example clearly illustrates the service vs collaboration functions: [redacted] contacted us on 8/26/2016 following our discussion related to his presentation on PRCD at ARVO 2016. He inquired on the availability of canine tissues, *PRCD* mutants and wild type, for his studies on the role of PRCD in PR outer segment maintenance. Our response – "*I am very happy to help. For the moment, any tissues you want to use need to be archival tissues, as fresh tissues are not available. Once PRCD protein is better understood and its importance in photoreceptor function and viability established…we can expand the colony sufficiently to be able to provide you samples. In regards to serving as a collaborator in your grant, I would be happy to be a collaborator provided I can contribute sufficiently to your studies to warrant this.*"

Advertising the resource: Based on our past track record, we predict that established or new investigators, and Pharma companies, will solicit models or services to carry out additional studies as new findings arise. Such studies cannot be predicted and are therefore not included in the *Approach* section for *Aim 2*. However, the program has the flexibility to be responsive to emerging opportunities. Even though there has never been a line item for advertisement in the budget, the resource and models are extremely well known in the scientific community, and additional advertising would not warrant the expense.

In addition to requests that come from the vision research community, it is important for SRG members and NEI to be aware of the process in which we help and solicit outside investigators. In many cases, the [redacted] has been actively involved in encouraging Pharma companies to undertake proof-of-principle or validation studies in the dog models. This effort has resulted in work now being done with [redacted] and [redacted] As well, when we become aware of specific advances made by other investigators, we approach them, bring to their attention the resources available at the RDSF, and ask how we can assist in their work. One example is [redacted] and PRCD; selected other examples include [redacted] (Univ of Toronto) on neuroprotection in an anti-neogenin shRNA strategy to inhibit PR cell death in rcd1 dogs; and [redacted] collaboration with one of the [redacted] examining the spectral signature and distribution of RPE fluorophores in canine and human bestrophinopathies using Hyperspectral Autofluorescence Imaging (HAI) and a Non-Negative Tensor Decomposition (NNTD) algorithm for the nonlinear fluorescence spectra unmixing.

Problems, Alternative Strategies, and Benchmarks. We anticipate no problems requiring alternative strategies. There is an active pipeline of investigators that use the models and services. Provided appropriate funding levels are received and maintained, we anticipate production to satisfy demand. Benchmark for success will be to meet in a timely manner the needs of multiple research investigators.

Aim 3. PI directed studies to understand the genetic and molecular mechanisms of diseases and evaluate potential methods of disease prevention, therapy or amelioration. Special emphasis will be placed on studies to **a)** find new canine models of naturally-occurring IRD, and characterize the cell biological mechanisms underlying disease pathogenesis; **b)** examine the role of inflammation and microglia/macrophages in retinal

degenerative processes, and identify stages and therapeutic targets amenable to disease modulation; **c)** optimize gene augmentation therapies targeting ON-bipolar cells in stationary night blindness; and **d)** test the efficacy of ocriplasmin to increase vector entry into retina and facilitate intravitreal AAV therapies.

Overview: This work focuses on investigator-initiated research studies important to our understanding of IRD mechanisms and their treatment. Studies are conducted by investigators working with the PIs and associates (Beltran, Guziewicz), or with collaborators at UPenn (Drs. Jacobson/Cideciyan) and other institutions (Drs. Hauswirth-FL, [redacted] see letters of collaboration).

Aim 3a) find new canine models of naturally-occurring IRD and characterize the cell biological mechanisms underlying disease pathogenesis. The complete canine genome sequence and its variations have facilitated the genome-wide association study (GWAS) approach to map loci associated, in particular, with simple Mendelian traits. GWAS has been very effective in identifying chromosomal loci of association using a group of dogs of the same breed/strain where linkage disequilibrium (LD) is more extensive than the heterogeneous human populations. In parallel, next-generation sequencing (NGS) (e.g. WGS, RNA-seq) is carried out to obtain sequence and expression information. Coupled with the GWAS data that points to the loci to focus the effort for inspection, the gene/mutation is discovered. During the current grant period (see *Progress Report*), we used GWAS and NGS to identify mutations responsible for stationary night blindness and two early forms of IRD. The stationary night blindness model will be utilized in studies assessing AAV-gene therapy targeting of ON-bipolar cells (see **3c**). Additional diseases to be utilized in the proposed grant period are:

①**Retinal disease model discovery** – our program has become an 'unofficial' referral center for dogs with IRDs; these models have been characterized, mutations identified, and, if of sufficient relevance to human IRD, the disease models developed and added to the colony. Recent examples include mutations in *RHO*, *RPGR*, *BEST1*, and *NPHP5*; so far, all but *NPHP5* are now in Phase 1 clinical trials or IND-enabling studies (*BEST1*). We plan to devote a small %effort/funds to continue these activities in the coming grant cycle. While it may be considered a 'fishing expedition', the excellent outcomes of these activities, e.g. identification of canine *ABCA4* Stargardt model, in moving therapies to the clinic are proof positive of its importance.

②**Cone-rod dystrophy with foveomacular degeneration** is a disease identified in Std. Poodle and Poodle-crosses ('Doodles') and initially characterized as achromatopsia based on absolute day blindness. All 6 known achromatopsia candidate genes (*CNGA3*, *CNGB3*, *GNAT2*, *PDE6C*, *PDE6H*, and *ATF6*[66]) were excluded from causal association. Subsequently, detailed clinical, OCT and ERG examination indicated that the disease, instead, was a cone-rod dystrophy with foveomacular degeneration accompanied by absent cone-mediated and markedly attenuated rod ERG responses (**Fig 1**). The foveomacular region in the dog is a recently recognized structure that, like in humans, is affected first by inherited maculopathies[26]. GWAS performed in a small subset of available dogs identified suggestive peaks in the canine chromosome (CFA) 12 and 20 – however, the 6 positional and functional candidate genes in these intervals were excluded. Lastly, we used homozygosity mapping and found that the 4 affected dogs were homozygous for a ~2 Mb interval in CFA8 that contained 2 significant retinal ciliopathy genes: *TTC8* (mutations causing BBS8 and RP51[67-69]) and *SPATA7*, a gene causing LCA, cone-rod dystrophy and early-onset RP[70-72]. Identification and validation of the causative gene and mutation are now ongoing. This canine model will provide insight into foveomacular degeneration and early-onset cone-rod dystrophy and can potentially be utilized to identify therapeutic interventions for either one of two important human IRDs. We have collected semen from several well-characterized affected males and already have pregnant females for introducing the disease into the colony for the studies proposed.

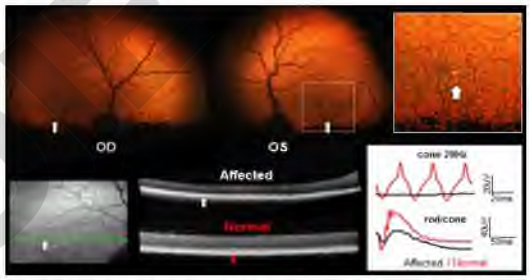

Fig 1. *Above*, Fundus images of a 12 m.o. affected dog with distinct bilateral foveomacular degeneration (white arrows). *Below*, ONL thinning is evident in sd-OCT (arrows pointing to the foveomacula). Undetectable cone ERG and markedly reduced rod/cone ERG support the cone-led etiology.

③**Oligogenic *RPGRIP1* cone-rod dystrophy (cord1)** – cord1 is an oligogenic ciliopathy and the only large animal IRD model where the concurrent involvement of more than one gene has been identified. A 44bp insertion in *RPGRIP1* C-terminus of exon 3 (previously termed exon 2) was initially thought to be the sole cause of cord1[73]. However, the Co-PI later identified *MAP9* as a modifier of disease age onset[29,74]. Both gene products localize to the connecting cilia of rods and cones[28,75]. In a canine research colony, we recently reported significant variability in cone ERG phenotype (normal or absent) among *RPGRIP1*$^{ins/ins}$ dogs that could not be explained by *MAP9* or environmental factors[28,76] (**Fig 2**). We then carried out a

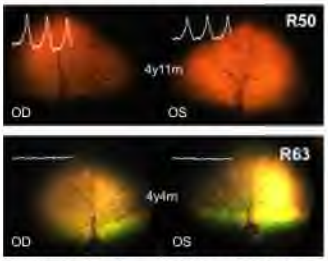

Fig 2. Both dogs R50 and R63 are *RPGRIP1*$^{ins/ins}$ *MAP9*$^{+/del}$, but the latter lacks cone ERG responses.

GWAS to search for additional loci underlying the cone ERG phenotype. Forty-two *RPGRIP1*$^{ins/ins}$ dogs differentiated by the cone ERG phenotype were genotyped using the 210K Canine chip (Illumina). We found a single peak strongly associated with the cone phenotype at CFA30 (-logP=7.46), and linkage analysis also showed association in the same region. Subsequent haplotype analysis confirmed a 4Mb interval of homozygosity in 18/21 (86%) of the absent cone ERG group, but only in 1/19 (5%) of the normal group (**Fig 3**)[77]. Retinal RNA-seq is now in progress using samples from selected *RPGRIP1* mutants with normal vs absent cone ERGs. In collaboration with [redacted] (retinal disease gene bioinformatics), we plan to examine cone-specific and cone-enriched genes for sequence and/or expression changes that inform on the cone ERG phenotype. Our results indicate that three recessive loci – *RPGRIP1*, *MAP9* and the third locus we mapped – appear to concurrently contribute to the variable expression of cord1. The complex hierarchical architecture e.g. epistatic vs modifier of these loci remains to be determined functionally. As the newly identified locus is directly associated with cone function, given the cone-led phenotype of cord1, a primary role in disease pathogenesis is indicated. If RNA-seq fails to identify expression changes in genes in the 4.1 Mb candidate region on CFA30, we will either carry out targeted or whole genome sequencing with a focus on this region to examine for sequence changes in candidate genes that are associated with the cone phenotype. We prefer WGS for this approach as it is more comprehensive and, surprisingly, **less** costly, at least for dogs, than targeted capture sequencing.

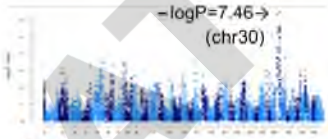

Fig 3. Mapping of a third locus associated with cone ERG to a 4.1Mb region on CFA 30.

④Canine *ABCA4*-Stargardt disease – As an effort to search for new disease gene/mutations in a population of dogs affected with new IRD phenotypes that have not been accounted for by the known mutations, we have carried out WGS on selected affected and carrier dogs. This led to the identification of a 'C' insertion in exon 28 of *ABCA4* that results in a frameshift/premature stop codon. Semen from 6 of the males has been obtained, and breedings to unrelated females from our colony initiated; 2 females are pregnant and puppies are expected in mid-Nov, 2018. The aim is to have by the -02 year of the grant a sufficient number of affected dogs to be used for independent and collaborative studies and then expand the colony for in-depth studies including medical and gene-based therapies. At the time that the breeding studies were being initiated, we became aware of an online publication describing the same loss-of-function mutation in dogs in Scandinavia resulting in cone-rod dystrophy with RPE hypertrophy secondary to cytoplasmic accumulation of fluorophores[78]. While that mutation was found among the pet population which poses challenges in developing a research colony, our work has the advantage in that we have already been able to initiate breeding given our established capacity.

Problems, Alternative and Strategies. As we have published extensively on all the methods used, the approach to be used is summarized with references. **i)** *Mapping*: GWAS with Illumina Canine HD BeadChip; association analysis with PLINK software, and population stratification by principal component analysis. **ii)** *Positional candidate analysis*: mapped interval genes examined for the role in disease; lack of 'obvious' candidate will direct use of retinal RNA-seq data from multiple normal dogs archived in our lab as well as publicly available resources (Expression Atlas[79], Tissues[80], iSyTE[81], mouse retina SAGE library[82,83], GeneNetwork database[84], murine retina transcriptome[85]) for genes expressed in the retina; expression data used to identify and prioritize positional candidates. **iii)** *Narrowing critical interval*: develop SNP-based haplotypes for recombination or LD mapping; additional SNPs used for fine mapping of haplotypes. We are familiar with this approach, and since 2006, have used it successfully to identify nine IRD models[45-48,51,52,54,56,86] four of which are caused by mutations in novel ocular or IRD genes[45,46,52,56]. Of these models, we have successfully developed gene-based therapies that are now commercialized[6] (*RPE65*-Luxterna™), are in Phase 1/2 clinical trials (*CNGB3* achromatopsia[25]; *RPGR*-XLRP[24]) or are in pre-IND enabling studies (*BEST1*-Best disease[10]; *NPHP5*-LCA[34,87]; and *RHO*-adRP[7,64]. **iv)** *Validation of candidate gene and mutation:* phenotype/genotype concordance, changes in mRNA expression, where appropriate, and testing a large population of non-affected dogs, of the same or other breeds, to exclude rare polymorphism. The techniques used to characterize validated candidate gene structure and function are standard in our laboratory[46,52,56,57,62,63,88]. In cases where the candidate interval is too large and/or gene-rich, or positional candidates are excluded, we will use WGS through an established [redacted] collaboration. This approach has been used in the current funding period (See *Progress Report*) to identify mutations in *LRIT3* causing stationary night blindness, *CCDC66* causing early-onset ciliopathy, and *PPT1* causing non-syndromic retinal degeneration.

Significance to retinal cell biology and disease: The diseases proposed for investigation are already in development in the colony. This allows for ready access to tissues to examine the molecular mechanisms of disease, and establish similarities to the human disease that facilitate translational studies of disease and

therapy. We have a proven track record in this area; e.g. studies on *RPGR*-XLRP[3,22], *CNGB3*-achromatopsia[2], *NPHP5*-LCA[34,87], *BEST1*-Best disease[10], and *RHO*-adRP[7,64] among others[1].

Cone-rod dystrophy with foveomacular degeneration is a form of LCA with selective targeting of the newly recognized fovea-like region in dogs[26]. Although there is severe visual impairment early in life, i.e. an LCA model, there is a relative structural preservation of the outer retina at least for a year (**Fig 1**). This dissociation of structure and function is similar to *NPHP5* and *CEP290* among a subset of ciliopathies[34], and, at least in *NPHP5*, amenable to gene augmentation therapy[87]. Such treatment will be investigated once gene and mutation are identified.

Oligogenic RPGRIP1 cone-rod dystrophy (cord1). There are 298 IRD genes/loci currently enlisted in RetNet[89] corresponding to monogenic etiologies. However, less straightforward genotype-phenotype correlations are being recognized even among patients harboring the same mutation across the IRD spectrum[90], indicating that modifier(s), or more crudely, the heterogeneous genetic background, plays a role in disease expression. Such emerging complexity has complicated the molecular diagnostics and targeted treatments. The complex interactome of the many molecular players in retinal physiology contributing to the finely orchestrated cascades leading to vision continues to be unveiled. It is possible that minor mutations or polymorphisms that are not pathogenic by themselves can have a cumulative effect affecting the phenotype observed[91]. Unfortunately, the human population has extensive genetic diversity, even among family members, complicating the assessment for the contribution of each molecular variant in disease expression. Mouse strains, on the other hand, typically have fixed genetic background leading to relatively uniform phenotypes that can be manipulated by changing the genomic backgrounds or 'adding' known allele(s)[92-97]. Hence there has been an unmet need for an animal model that can fill the gap between human (=extremely heterogeneous) and mouse (=uniform); canine strains are excellent models allowing sufficient degrees of background genetic variability to identify new modifiers that segregate and give rise to phenotypes that can readily be assessed. The 26+ naturally-occurring and molecularly characterized canine IRDs have been all found to be monogenic[44] with cord1 being the only model that is associations with more than one established mutation/locus. Our studies of *RPGRIP1*$^{ins/ins}$ dogs in outcrossed subpopulations have so far identified two additional alleles – *MAP9*[29,74] and a third locus on CFA30[77], affecting the variable disease expression; *MAP9* appear to modulate disease severity (onset and progression) while the CFA30 locus affect cone function. These two loci represent alleles previously unrelated to IRDs, and are potentially ubiquitously expressed. It is important to understand the retina-specific role of these gene products in normal and disease retinas. Future studies, not part of this proposal, will test whether gene augmentation of a normal copy of one of the three established alleles is sufficient to rescue the disease and allow determination of any epistatic relationship between these alleles to provide insights into the role(s) of each gene products in the retina.

Canine ABCA4-Stargardt disease. Until now, our lab and others have attempted to identify dogs with *ABCA4*-associated IRDs without success (e.g.[98,99]). The extant rodent models have been extensively studied and have been critical in developing medical and gene-based therapies. However, while the rodent disease recapitulates the biochemical and physiologic abnormalities[100,101], the retinas do not degenerate. Thus the association between the underlying molecular/biochemical abnormality and PR degeneration remains speculative and not clearly defined. In addition, the mouse retina lacks a foveomacular region which is the target site for one of the important phenotypes (Stargardt macular degeneration) associated with *ABCA4* mutations. The canine model to be developed in this proposal is characterized by retinal degeneration with cones more severely affected, and RPE hypertrophy secondary to A2E accumulation[78]. While foveomacular involvement was not described, our laboratory is not only experienced with the assessment of this region but also in establishing its involvement in inherited maculopathies[26]; these studies will be part of the proposal. This model will allow for in-depth studies of the molecular/biochemical aspects of the disease. As well, the canine model is a disease homolog of the human and will permit a variety of medical and gene-based therapies which, to date, have been translationally challenging.

Aim 3b) examine the role of inflammation and microglia/macrophages in retinal degenerative processes, and identify stages and therapeutic targets amenable to disease modulation. Microglia are the primary resident immune cells of the retina/CNS, and are phagocytic sentinels that share immunophenotypical and functional characteristics of macrophages/monocytes and antigen presenting cells; they play critical roles in the control of inflammation, innate immune defense, and retinal development and function. Microglia are particularly

sensitive to changes in the surrounding environment, becoming readily activated in response to injury (reviewed by[102,103]). Microglia respond to pathological events by progressing from a resting ramified state to an amoeboid active state[104]. In the retina, these phagocytically active sentinels have been implicated in the pathogenesis of various retinal diseases[105-107]. Our lab has characterized retinal microglia populations[108], and established their role in IRDs. In early-onset diseases, such as rcd1, there is a peak of cell death that is mutation-dependent and occurs ~4-7 wks postnatally[109], an age range that encompasses the *induction* and *execution* phases of disease[109]. Soon after, there is microglial activation and migration into the OPL and PR layers (**Fig 4**). Subsequently, the *execution* phase is followed by *chronic cell death* and a constant but lower level of apoptosis that continues until the outer retina atrophies[31,109]. Microglia in the OPL and PR layer are prominent during this phase as well[32].

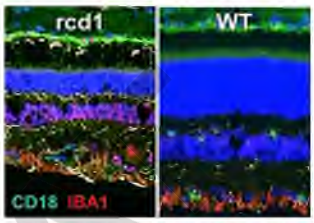

Fig 4. Reactive microglia marker IBA1 and microglia/macrophage marker CD18 indicating microglial activation and migration from inner to outer retinal layers in the early-onset IRD canine model (rcd1; 8 wks).

Similarly, age-related macular degeneration (AMD) is characterized by complement activation and inflammation. RPE and microglial cells play a central role in the initiation and perpetuation of inflammatory responses that lead to macular damage and functional loss. Moreover, our studies have shown that inflammatory mechanisms including activation of the inflammasome and complement pathways contribute to xlpra2 which is a canine homolog of human x-linked *RPGR*-RP in a manner similar to human AMD[32], suggesting that therapeutic intervention to suppress inflammatory changes that drive PR degeneration can translate to human IRDs such as atrophic AMD. We have initiated studies in collaboration with [redacted] examining the role of microglia and microglial activators in modulating IRD in dog models. His laboratory has shown that microglia regulate neuronal cell survival, and modulation of pathogenic microglial activation states and effector mechanisms has been linked to neuroprotection[110]. Among the agents used, [redacted] a nonsteroidal estrogen receptor modulator, has an inhibitory effect on inflammation[111-114] and exerts marked neuroprotective effect on PRs by modulation of microglia activation and production of inflammatory cytokines[111,114]. In this collaboration, [redacted] in canine rcd1, a model used extensively to test neuroprotection and cell survival[14]. Because of the potential for side effects from systemic administration and to increase retinal bioavailability, we also will employ an intravitreal sustained-release platform comprising [redacted] initially characterized at UPenn's Chemical and Nanoparticle Synthesis Core. All other reagents for this study are commercially available. As part of this study, we will first examine intraocular pharmacokinetics and distribution of [redacted] by *in vivo* fluorescence assays and compare these results with systemically administered [redacted] in equivalent dose. Thereafter, we will examine downstream effects of [redacted] on the retinal layers by evaluating retinal morphological changes in treated vs untreated retinas using IHC with a range of retinal cell markers, and using TUNEL assay to evaluate the extent and timing of apoptosis. If a positive therapeutic effect is obtained, we will examine the signaling pathways modulated by [redacted][15] by identifying molecules that act directly with [redacted] using immunoprecipitation and mass spectrometry approaches and comparing retinal gene expression in treated and untreated eyes using RNA-seq.

This study will provide data on pharmacokinetics and distribution of [redacted] in the canine eye, which is comparable in size to the human eye. Further, it will provide a model for intravitreal extended-release delivery system for other [redacted] utilized extensively in reducing inflammation and in cystoid macular edema. Currently, such poorly water-soluble drugs are generally administered in combination with solubility enhancers with limited efficacy, and data generated will inform on the intraocular drug delivery for future human clinical trials in patients with IRD and/or AMD.

Problems, Alternative Strategies, and Benchmarks. No problems in production and administration of [redacted] are anticipated. Likewise, toxicity from intravitreal administration of the product is not expected as it will be subjected to rigorous purification in a facility that makes these therapeutics for clinical applications in patients. It is possible, however, that the product tested has no microglial modulating effect in the dog, and positive outcomes are not observed. This can be a species-specific effect or a dose-effect. In the latter case, higher drug concentrations will be tested, either systemically, or by intraocular administration. Alternatively, we will use other microglial modulating agents being developed in [redacted] to see if we can obtain positive therapeutic outcomes.

Aim 3c) optimize gene augmentation therapies targeting ON-bipolar cells in stationary night blindness. Current IRD gene therapies target either the RPE or the rods and cones, all located in the outer retina. While animal models have played a major role in the proof-of-concept studies for gene augmentation in these cell populations[1-3,41], progress to develop therapies targeting other critical cells in the visual pathway localized in the mid- and inner-retinal layers has been hampered in part by the lack of appropriate large animal models with specific defects in these layers. ON-bipolar cells are the secondary retinal neurons in the mid-retina whose dysfunction leads to congenital stationary night blindness (CSNB). During the current grant period, we have characterized a naturally-occurring canine CSNB[27], and subsequently identified a mutation in *LRIT3*, which has previously been associated with CSNB in patients and mice[116]. LRIT3 stabilizes TRPM1, a channel that plays a major role in the mGluR6 signaling cascade occurring in ON-bipolar cells. A commercial antibody now used in our lab clearly localizes LRIT3 to the dendritic tips of canine ON-bipolar cells (**Fig 5**).

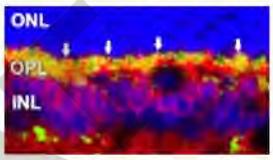

Fig 5. Normal canine retina labelled with LRIT3 (green) and Goα (red; ON-bipolar cell marker). Punctate signals (arrows) in the OPL indicate LRIT3 localization to the bipolar dendritic tips.

We now have the *LRIT3*-CSNB strain bred and maintained at the RDSF ready for pre-clinical studies with established outcome measures that can readily assess CSNB phenotypic recovery including full-field ERG, and a light-adjustable obstacle course for vision testing[22]. Using this canine CSNB model, we will determine if augmentation of ON-bipolar cells with wild type LRIT3 can lead to stable functional rescue and disease correction. One of the research scientists in our group (W. Beltran) has a collaboration with [redacted] aiming to identify vectors that target different cells/layers including ON-bipolar cells through a directed-evolution approach[117,118]. AAV vectors selected as optimal include those for subretinal ($AAV2^{K9\#4}$) or intravitreal ($AAV2^{K9\#12}$) injections. Further, modified mGluR6 promoters designed and tested in mouse models by Lu et al.[119] for efficient expression of transgenes in ON-bipolar cells have been adapted in our vector constructs to drive *LRIT3* expression in ON-bipolar cells. Preliminary studies using a GFP reporter with each vector/route showed robust and specific GFP expression among the mid-retinal cells (**Fig 6**), and ongoing work aims to characterize the classes of bipolar cells targeted and to examine transduction efficiency.

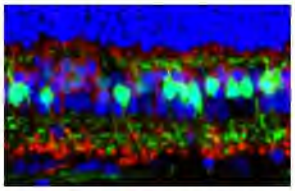

Fig 6. Subretinal injection of $AAV2^{K9\#4}$-GFP in wild type canine retina shows transduction of INL neurons. Counter-labelled with rod bipolar marker PKCα (red).

Of further interest, the ON-bipolar cells, along with the retinal ganglion cells in the inner retina, have a broader clinical implication as the potential target site of optogenetic therapies. In this emerging field, these mid- and inner-retinal cells are targeted to confer photosensitive properties in cases where extensive PR degeneration is already present due to end-stage IRDs[37,120-122]. Once the proof-of-concept for gene augmentation and phenotypic recovery is established in the canine *LRIT3*-CSNB model, it can then be made available as a platform to test new strategies targeting ON-bipolar cells, including optogenetics which is a disease gene-independent approach and has a relatively wide window of intervention. The CSNB model is particularly useful because the retinal architecture is minimally affected and the target cells (ON-bipolar cells) are intact for transduction, yet the functional defect is readily identifiable using outcome measures such as ERG and behavior testing, routinely carried out at our facility.

Problems, Alternative Strategies, and Benchmarks. We do not anticipate any problems with vector construction, and subretinal AAV delivery as our group and collaborators have extensive experience in this process. Should intravitreal AAV delivery pose difficulties such as requiring high vector doses leading to intraocular inflammation, or low penetration rate through the internal limiting membrane and into the retinal layers, we will plan to adopt the ocriplasmin reagent that will be tested in **Aim 3d**. By having animals of different ages available for treatment, we will establish if age is a factor in both the therapeutic outcome and the long-term stability of correction.

Aim 3d) test the efficacy of [redacted] to increase vector entry into retina and facilitate intravitreal AAV therapies. A clear goal for gene therapy in human IRDs is to effectively treat the mutant cells without secondary vector-associated or surgical trauma. Effective transduction of PRs by the intravitreal (IVit) route would be optimal, provided that treatment is effective, and transduction of non-diseased cells, if it occurs, is not detrimental. The *RPE65*-LCA clinical trials have highlighted central defects in some patients associated with treatment (macular hole[123], foveal thinning[20,124]), and loss of visual acuity[20,125]. Thus, developing therapies that target affected retinal cells/layers without surgically invading the subretinal space is important, and one such route is IVit delivery. Such a route has some limitations among which include very thick inner limiting membrane (INL) in non-rodent models, and enriched heparan sulfate domains in ILM and vitreous gel that can bind AAV vectors[126]. Moreover, inflammation secondary to IVit vector administration is common in animal studies[127] and a human clinical trial[128] and is dose-dependent. Thus a product that enhances retinal vector penetration via IVit route will permit using lower vector doses and prevent associated inflammatory sequela.

We have started a collaboration with Thrombogenics (now Oxurion) who will provide, free of charge, clinical grade Jetrea ([redacted]) for use in the proposed experimental studies. ([redacted] is a recombinant human serine protease that has proteolytic activity against protein components of the vitreous and vitreoretinal interface, e.g. collagen fibronectin, laminin, and is clinically indicated to dissolve the protein matrix responsible for vitreomacular adhesions[129]. The main complications resulting from this treatment are reversible reductions in full-field ERG amplitudes in some patients which are not associated with visual acuity decreases[130]. The product has been tested in minipigs (49 µgm/mL of vitreous) and cynomolgus monkeys (68 µgm/mL) with no adverse effects. In cats (9 µgm/mL) and farm pigs (29 µgm/mL), there is posterior vitreous detachment that is complete by 21 days in cats, and complete in most pigs by 8 weeks ([redacted] personal communication-3/27/18).

Prior to therapeutic use of [redacted], we have just initiated preliminary studies in normal control dogs to test the safety and efficacy of the product at the following doses: 9, 17, 35, 52 µgm/mL of vitreous. In all cases, the product was well tolerated with no ocular inflammation, and, short-term, no adverse effect on the full field ERG. At the highest dose tested, we found by OCT focal outer retinal atrophy at the site of injection. Treated dogs are now being followed to determine the time course of posterior vitreous detachment.

In the proposed studies, we will first determine the optimal dose and time of full posterior vitreous detachment in control dogs. Because such detachment is not rapid in dogs, unlike cats, we will limit its use, at least initially, to non-progressive diseases such as CSNB-*LRIT3*, or when we aim for treating more advanced disease stages *RPGR*-XLRP so that *Ocri* can be administered well ahead of the therapeutic vector. In regards to CSNB-*LRIT3*, we will assess inner retinal targeting by using a vector that has good penetration into the mid/inner retinas by the IVit route ([redacted] unpublished), and use a modified mGluR6 promoter and GFP reporter (AAV2$^{K9\#12}$-mod mGluR6-GFP) to establish efficiency of targeting ON-bipolars in eyes with/without prior IVit *Ocri*. For this, we will inject reporter vector at peak vitreous lysis, and use IHC to identify cell targeting. If there is enhanced ON-bipolar targeting with [redacted] in comparison to BSS controls, we will then use the same vector/promoter but with *LRIT3*. CSNB dogs will be injected with [redacted] or BSS at 10 wks, followed subsequently with the therapeutic vector, and we will compare functional rescue and structural recovery of the ON-bipolar dendrites. Successful rescue will be followed by repeat experiments in older dogs – 6-12 months – where a thicker vitreous cortex may limit functional/structural rescue but represent a more clinically relevant treatment age.

A similar experimental paradigm will be used in the *RPGR*-XLRP model (xlpra2)[22]. We will first use in normal control dogs a vector-promoter and GFP reporter combination (AAV2$^{k9\#16}$-GRK1-GFP; Byrne, Flannery, unpublished and[22]) that targets rods and cones by the IVit route, and ascertain enhanced targeting by *Ocri* vs BSS. If positive, we will then compare the targeting efficiency and distribution of IVit [redacted]+vector in one eye, vs the standard subretinal vector (AAV5-GRK1-GFP). If the results are comparable, we will then use the therapeutic vector subretinally (AAV5-GRK1-RPGR)[22] in one eye and IVit ([redacted]+ AAV2$^{k9\#16}$-GRK1-RPGR) in the fellow eye. If there is a comparable therapeutic outcome using our published structural and functional studies[3,22], we will then expand the studies to late-stage disease. We anticipate that these studies will be a first step towards improving IVit gene therapies for diseased PRs and mid/inner retinal neurons.

In summary, this application proposes the continuation of a centralized research resource that provides specific canine mutant models of IRD and supports collaborative studies to utilize them. The program focuses on the development and pre-clinical testing of new effective approaches for therapy of human IRDs. In parallel, investigator-initiated studies drive the research that identifies new IRD models; examines the role of inflammation and microglia to identify therapeutic targets, optimizes targeting of ON-bipolar cells for gene-specific as well as optogentic approaches, and tests the efficacy of a proteolytic product in facilitating intravitreal AAV injections – all using the appropriate canine IRD models.

TIMELINE: The table shows the timeline proposed for completion of the study aims.

Timelines for experimental tudies	Grant Year				
	01	02	03	04	05
Aim 1: Maintain models critical for development of novel therapies	X	X	X	X	X
Aim 2: Continue existing or initiate new independent or collaborative studies with outside investigators	X	X	X	X	X
Aim 3: PI-directed studies to understand the genetic and molecular mechanisms of diseases, and evaluate potential methods of disease prevention, therapy or amelioration					
a) identify new naturally-occurring canine forms of IRD, and investigate the cell biologic mechanisms critical to the pathogenesis of the diseases: ①Retinal disease model discovery, ②CRD with foveomacular degeneration, ③oligogenic *RPGRIP1*-cord1, ④canine *ABCA4* Stargardt disease	① ② ③	① ② ③ ④	① ② ③ ④	① ② ③ ④	① ④
b) examine the role of inflammation and microglia/macrophages in retinal degenerative processes, and identify stages and therapeutic targets amenable to disease modulation	X	X	X	X	X
c) optimize gene augmentation therapies targeting ON-bipolar cells in CSNB	X	X	X		
d) test the efficacy of [redacted] in vector permeability to facilitate intravit. AAV injections		X	X	X	X

4. Progress Report Publication List

PEER REVIEWED PUBLICATIONS SINCE LAST COMPETITIVE REVIEW

Last renewal was submitted on 2/14/2014

2/14/2014-11/30/2015

Beltran WA, Cideciyan AV, Guziewicz KE, Iwabe S, Swider M, Scott EM, Savina SV, Ruthel G, Stefano F, Zhang L, Zorger R, Sumaroka A, Jacobson SG, Aguirre GD. Canine retina has a primate fovea-like bouquet of cone photoreceptors which is affected by inherited macular degenerations. PLoS One. 2014;9(3):e90390 (PMC3944008).

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Tanaka N, Dutrow EV, Miyadera K, Delemotte L, MacDermaid CM, Reinstein SL, Crumley WR, Dixon CJ, Casal ML, Klein ML, Aguirre GD, Tanaka JC, Guziewicz KE. Canine CNGA3 gene mutations provide novel insights into human achromatopsia-associated channelopathies and treatment. PLoS One. 2015;10(9):e0138943 (PMC4583268).

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Appelbaum T, Becker D, Santana E, Aguirre GD. Molecular studies of phenotype variation in canine RPGR-XLPRA1. Molecular vision. 2016;22:319-31 (PMC4830396).

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Sudharsan R, Simone KM, Anderson NP, Aguirre GD, Beltran WA. Acute and protracted cell death in light-induced retinal degeneration in the canine model of rhodopsin autosomal dominant retinitis pigmentosa. Investigative Ophthalmology & Visual Science. 2017 January 1;58(1):270-281 (PMC5464465).

Ye, G.-j., Komaromy, A.M., Zeiss, C.J., Calcedo, R., Harman, C.D., Koehl, K.L., Stewart, G.A., Iwabe, S., Chiodo, V.A., Hauswirth, W.W., Aguirre, G.D., Chulay, J.D. Safety and efficacy of AAV5 vectors expressing human or canine CNGB3 in *CNGB3*-mutant dogs. Hum Gene Ther Clin Dev **28**(4): 197-207, 2017. (PMC5733651)

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ABSTRACTS PRESENTED AT SCIENTIFIC MEETINGS

Last renewal was submitted on 2/14/2014

2/14/2014-11/30/2015

Aguirre, G.D., Downs, L., Genini, S., Scott,E.,Iwabe, S., Beltran, W.A. Photoreceptor development, degeneration and retinal gene expression in the canine NPHP5 Leber congenital amaurosis model. 2015 Annual Meeting, ARVO, Denver, CO. #5420

Appelbaum, T., Beltran, W.A., Santana, E., Aguirre, G.D. Early disease phenotype in canine RPGRORF15 model is characterized by strong up-regulation of pro-inflammatory genes and down-regulation of neuroretinal maintenance genes. 2015 Annual Meeting, ARVO, Denver, CO. #4648

Becker, D., Milano, A., Pearce-Kelling, S., Aguirre, G.D. A genetic and candidate gene association study for optic nerve hypoplasia in dogs. 2015 Annual Meeting, ARVO, Denver, CO #2537

Beltran, W.A., Cideciyan, A.V., Iwabe, S., Lewin, A.S., Swider, M., Guzman, J., Boye, S.L,, Hauswirth, W.W., Jacobson, S.G., Aguirre, G.D. Gene therapy for RPGR-XLRP in a canine model results in retained photoreceptors and vision for at least 2.5 years. 2015 Annual Meeting, ARVO, Denver, CO. #2588

Das, G., Santana, E., Iwabe, S., Aguirre, G.D., Miyadera, K., Cone structure and function in dogs with RPGRIP1 mutation. 2015 Annual Meeting, ARVO, Denver, CO. #4653

Downs,L., Beltran, W.A., Aguirre, G.D. Expression of apoptosis inhibiting proteins, XIAP and Survivin, in the mammalian retina. 2015 Annual Meeting, ARVO, Denver, CO. #5523

Guziewicz, K.E., Tanaka, N. Dutrow, E.V, Miyadera, K., Delemotte, L., MacDermaid, C., Dixon, C., Tanaka, J., Aguirre, G.D.. Molecular mechanism of canine CNGA3-associated channelopathies. 2015 Annual Meeting, ARVO, Denver, CO.#4661

Iwabe, S., Sudharsan, R., Aguirre, G.D., Beltran, W.A.. Genetic ablation of 11-cis retinal accelerates retinal degeneration in the canine T4R-RHO model of adrp but prevents light-induced damage. 2015 Annual Meeting, ARVO, Denver, CO. #5418

Sudharsan, R., Iwabe, S., Appelbaum, T., Lewin, A.S., Hauswirth, W.W., Aguirre, G.D., Beltran, W.A. Evaluation of the efficacy of mouse opsin promoter for rhodopsin gene augmentation in dogs. 2015 Annual Meeting, ARVO, Denver, CO. #5408

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Aguirre, G.D., Cideciyan, A.V., Boye, S.L., Iwabe, S., Dufour, V.L., Marinho, F.P., Downs, L.M., Hauswirth, W.W., Jacobson, S.G., Beltran, W.A. AAV-mediated gene augmentation restores retinal function and vision in the canine model of NPHP5 Leber congenital amaurosis. 2016 Annual Meeting, ARVO, Seattle, WA #2293

Appelbaum, T., Becker, D., Santana,E., Aguirre, G.D., A quantitative trait locus on canine chromosome 31 influences disease severity phenotype of XLPRA1, 2016 Annual Meeting, ARVO, Seattle, WA #D0340

Beltran, W.A., Cideciyan, A.V., Iwabe, S., Dufour, V.L., Marinho, F.P., Ye, G-j, Shearman, M., Chulay, J.D., Hauswirth, W.W., Jacobson, S.G., Aguirre, G.D. Two AAV2/5-mediated RPGR gene augmentation constructs driven by the GRK1 promoter rescue photoreceptor structure and function in a canine model of RPGR X-linked retinitis pigmentosa, 2016 Annual Meeting, ARVO, Seattle, WA #C0074

Beltran, W.A., Cideciyan, A.V., Sudharsan,R., Dufour, V.L., Marinho,F.P., Kuznetsova,T. Swider,M., Kosyk,M., Iwabe, S., Hauswirth, W.W., Jacobson, S.G., Lewin, A., Aguirre, G.D. Gene therapy for RHO-ADRP: Efficient knockdown of rhodopsin expression in a canine model. The XVIIth International Symposium on Retinal Degeneration (RD2016) Kyoto, Japan

Byrne,L.C., Visel, M.,Dufour, V.L., Marinho, F.P., Aguirre, G.D., Beltran,W.A. Schaffer,D., Flannery,J.G. Directed evolution identifies novel AAV capsid variants for targeting the canine outer retina following intravitreal delivery, 2016 Annual Meeting, ARVO, Seattle, WA#C0065

Das, G., Santana,E., Iwabe, S., Aguirre, G.D., Miyadera,K., RPGRIP1 mutant dogs show variable cone ERG functions, 2016 Annual Meeting, ARVO, Seattle, WA #B0032

Downs, L.M., Scott, E.M., Genini, S., Beltran, W.A., Aguirre, G.D., Photoreceptor structure and gene expression in the canine NPHP5-LCA ciliopathy model, 2016 Annual Meeting, ARVO, Seattle, WA #B0288

Dufour, V.L., Pan, W., Ying, GS., Aguirre, G.D., Beltran, W.A. In-vivo assessment of changes in outer nuclear layer thickness with age in the young canine retina, ECVO Congress 2016, Budapest, Hungary

Gardiner,K.L., Downs, L.M., Berta-Antalics, A., Santana, E., Aguirre, G.D., Genini, S. Photoreceptor proliferation and dysregulation of cell cycle genes in early onset inherited retinal degenerations, AALAS 67th National Meeting, Charlotte, NC.#P241

Guziewicz, K.E., Beltran, W.A., Cideciyan, A.V., Komáromy, A.M., Iwabe, S., Dhingra, A., Boesze-Battaglia, K., Hauswirth, W.W., Jacobson, S.G., Aguirre, G.D., The first best thing: insights from a naturally occurring animal model of BEST1- associated maculopathies, XXII Biennial Meeting of the International Society for Eye Research 2016, Tokyo, Japan

Guziewicz,K.E., Beltran, W.A., Cideciyan, A.V., Komaromy,A.M., Iwabe, S., Dhingra,A., Boesze-Battaglia,K., Hauswirth, W., Jacobson,S.G., Aguirre, G.D. Molecular pathology of BEST1-Associated Maculopathies. The XVIIth International Symposium on Retinal Degeneration (RD2016) Kyoto, Japan

Guziewicz,K.E., Beltran,W.A., Iwabe, S., Cideciyan, A.V., Jacobson, S.G., Dhingra, A., Battaglia K., Aguirre, G.D., Bestrophinopathy is an RPE-photoreceptor interface disease, 2016 Annual Meeting, ARVO, Seattle, WA #4330

Miyadera, K., Das, R., Marinho, F.P., McDaid, K., Iwabe, S., Aguirre G.D. Variable cone ERG in a multigenic canine model of cone-rod dystrophy with RPGRIP1 and MAP9 mutations, XXII Biennial Meeting of the International Society for Eye Research 2016, Tokyo, Japan

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Nikonov,S., Dolgova, N., Tochitsky,I., Van Gelder, R.N., Kramer, R., Aguirre, G.D., Beltran, W.A., Azobenzene-based DENAQ photoswitch confers light sensitivity to degenerated but not to healthy canine retina, 2016 Annual Meeting, ARVO, Seattle, WA #B0075

Sudharsan,R., Aguirre, G.D., Beltran, W.A., Implication of inflammation and innate immune responses in photoreceptor survival and death in late stages of early onset photoreceptor degenerative diseases, The XVIIth International Symposium on Retinal Degeneration (RD2016) Kyoto, Japan

Sudharsan,R., Aguirre, G.D., Beltran, W.A., Study of pro-apoptotic and pro-survival pathways at late stage disease in two non-allelic forms of photoreceptor degeneration, 2016 Annual Meeting, ARVO, Seattle, WA #B0031

12/1/2016-11/30/2017

Aguirre, G.D., From the cage to the bedside-concepts and strategies in retinal gene therapy, 2017 Annual Meeting, ARVO, Baltimore, MD #2436

Appelbaum, T., Santana, E., Aguirre, G.D., Upregulation of pro-inflammatory genes accompanies photoreceptors demise in canine models of retinal degeneration, 2017 Annual Meeting, ARVO, Baltimore, MD #3587

Beltran, W.A., Cideciyan, A.V., Sudharsan, R., Massengill, M., Rossmiller, B., Dufour, V.L., Pompeo Marinho, F., Appelbaum, T., Swider, M., Kosyk, M.S., Iwabe, S., Hauswirth, W.W., Jacobson, S.G., Lewin, A.S., Aguirre, G.A., AAV-mediated knockdown and replacement of rhodopsin protects rods in a canine model of RHO-ADRP, 2017 Annual Meeting, ARVO, Baltimore, MD #4483

Byrne,L.C., Visel, M., Dufour, V.L., Marinho, F.P., Aguirre, G.D., Beltran,W.A., Flannery, J.G., Schaffer,D., Directed evolution of AAV vectors guided by deep sequencing creates variants that bypass structural barriers in canine retina. 2017 Annual Meeting, ASGCT, Washington, DC.

Dufour, V.L., Iwabe, S., Aguirre, G.D., Beltran, W.A., Influence of ambient illumination on the course of retinal degeneration in a canine model of RHO-ADRP, 2017 Annual Meeting, ARVO, Baltimore, MD #278

Gardiner,K.L., Downs, L.M., Berta-Antalics, A., Santana, E., Aguirre, G.D., Genini, S. Photoreceptor proliferation and dysregulation of cell cycle genes in early onset inherited retinal degenerations, AALAS 67th National Meeting, Charlotte, NC.#P241

Guziewicz, K.E., Beltran, W.A., Cideciyan, A.V., Dufour, V.L. , Komaromy , A.M., McTish, E., Lewin, A.S., Hauswirth, W.W., Jacobson, S.G., Aguirre, G.D., Outcome measures for BEST1 gene augmentation therapy: restoration of RPE-photoreceptor interface homeostasis, 2017 Annual Meeting, ARVO, Baltimore, MD #2273

Guziewicz, K.E., Gamm, D.M., Cideciyan, A.V., Lewin, A.S., Sinha, D., Hauswirth, W.W., Jacobson, S.G., Aguirre, G.D. AAV-mediated therapy for best vitelliform macular dystrophy. 2017 Feb TRAP Meeting, Atlanta, GA.

Léger, H.M., Beiting, D.P., Beltran, W.A., Aguirre, G.D., Luca, F., NDR kinases regulate retinal development, homeostasis and gene expression, 2017 Annual Meeting, ARVO, Baltimore, MD #353

Liu, J., Beltran, W.A., Dufour, V.L., Marinho, F.P., Martynyuk, I., Ye, G-j., Song, C., Knop, D.R., Aguirre, G.D., Chulay, J.D., Shearman, M.S. Evaluation of AAV2tYF-GRK1-RPGR vectors in a canine model of RPGR-XLRP. 2017 Annual Meeting, ASGCT, Washington, DC.

Massengill, M., Patel, D., Beltran, W.A., Lewin, A.S., Tandem Delivery of Short Hairpin RNAs and Rhodopsin cDNA to Combat Retinal Degeneration in Autosomal Dominant Retinitis Pigmentosa, 2017 Annual Meeting, ARVO, Baltimore, MD #4082

Miyadera, K., Marinho, F.P., McDaid , K., Dufour, V.L. , Iwabe, S., Das, G., Aguirre, G.D., Variable cone dysfunction and progressive ophthalmoscopic changes in a canine model of RPGRIP1 and MAP9 multigenic cone-rod dystrophy, 2017 Annual Meeting, ARVO, Baltimore, MD #269

Sudharsan, R., Simone, K.M., Anderson, N.P., Aguirre, G.D., Beltran, W.A., Development of an optimized light damage paradigm to assess efficacy of gene therapy in a canine model of RHO-ADRP, 2017 Annual Meeting, ARVO, Baltimore, MD #282

Ye, G., Beltran, W.A. , Dufour, V.L., Pompeo Marinho, F., Martynyuk, I., Song, C., Knop, D., Aguirre, G.D., Chulay, J.D., Shearman, M.S., Evaluation of AAV2tYF-GRK1-RPGR vectors in a canine model of RPGR-XLRP, 2017 Annual Meeting, ARVO, Baltimore, MD #4486

12/1/2017-PRESENT

Aguirre, G.D., Cideciyan, A.V., Boye, S.L., Iwabe, S., Dufour, V., Swider, M., Roszak, K., Hauswirth, W.W., Jacobson, S.G., Beltran, W.A., Long-term preservation of photoreceptor function and structure following early-stage treatment by AAV-mediated gene augmentation in canine model of *NPHP5* Leber congenital amaurosis, 2018 Annual Meeting, ARVO, Honolulu, HI #6006

Aguirre, G.D., Gardiner, K.L., Swider, M., Jacobson, S.G., Beltran, W.A., Cideciyan, A.V. Long-term assessment of RPE65 expression and photoreceptor preservation in dogs after RPE65 gene therapy. RD2018 meeting, Killarney, Ireland.

Aguirre, G.D., Pre-clinical gene therapy development for Leber congenital amaurosis caused by NPHP5 mutations, 2017 Translational Retinal Research and Therapies Symposium, PennVet, Philadelphia, PA

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Beltran, W.A., Cideciyan, A.V., Iwabe, S., Dufour, V., Charng, J., Lisi, B., Guzman, J., Badiei, A., Tsilfidis, C., Hauswirt, W.W., Jacobson, S.G., Aguirre, G.D., AAV-mediated expression of X-linked Inhibitor of Apoptosis protects photoreceptors in two canine models of early onset, 2018 Annual Meeting, ARVO, Honolulu, HI #4527

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Sudharsan, R. Identification of photoreceptor-specific promoters for use in gene therapy at advanced stages of retinal degeneration. RD2018 meeting, Killarney, Ireland.

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PHS Human Subjects and Clinical Trials Information

OMB Number: 0925-0001 and 0925-0002
Expiration Date: 03/31/2020

Are Human Subjects Involved	○ Yes ● No
Is the Project Exempt from Federal regulations?	○ Yes ○ No
Exemption Number	❑ 1 ❑ 2 ❑ 3 ❑ 4 ❑ 5 ❑ 6 ❑ 7 ❑ 8
Does the proposed research involve human specimens and/or data	○ Yes ● No
Other Requested information	

Performance site

The live animal studies are carried out at the [redacted] located on the [redacted] of the School of Veterinary Medicine, University of Pennsylvania.

1. Description of Procedures

A central function of this proposal is to maintain a breeding colony comprising specific mutant strains of dogs, and normal dogs with comparable genetic/genomic background for control purposes. Animals produced in this colony are made available for independent and collaborative research investigations, or used by the PI for research studies that are part of this proposal. All dogs to be used in this project will be produced in the colony described within this application. Additional information relative to the breeding, housing, and care of this colony is described in the *Budget Justification* section. The dogs to be used comprise both the breeding colony itself, and the progeny to be derived from them.

The numbers and specific identification for the breeding colony dogs are readily definable. However, the numbers and specific identification for their progeny represent projections only, and are subject to factors of varying predictability.

• *Specific identification and estimates of numbers of dogs*

The breeding colony (Includes both breeding stock (>12 months old), and replacement breeders (aged 2-12 months), but not pre-weaned litters (< 2 months) or transient dogs assigned to specific investigational studies) is detailed below:

[redacted]ensus: Core Breeders, Study and Potential Breeders, and Potential Replacement Breeders as of September 30, 2018

Strain	Mutant Gene	Core Breeders	Study dogs/ potential breeders	Potential Replacement Breeders	Comments on future plans
Achromatopsia*	*CNGB3*	2	3		To expand slightly
Cea*	*NHEJ1*	0			Frozen semen stock
Cngb3/rcd1	*CNGB3/PDE6B*	2			In development to produce double homozygous dogs
Cngb3/rpe65*	*CNGB3/RPE65*	1			To expand slightly
Cnga3*	*CNGA3*	2	2		To expand slightly
Cmr*	*BEST1*	9	14	3	Stable
crd1*	*PDE6B*	0			Frozen semen stock
crd1/rcd1*	*PDE6B²/PDE6B¹*	0			Frozen semen stock
crd1/crd2	*PDE6B²/NPHP5*	3	2		In development to produce double homozygous dogs
crd2*	*NPHP5*	8	12		Stable
crd3*	*ADAM9*	0			Frozen semen stock
drd1*	*COL9A2*	0			Frozen semen stock
drd2*	*COL9A3*	1			Decrease to frozen semen stock
CSNB	*LRIT3*	4	12		Stable
Erd*	*STK38L*	4	1		Stable
Foveo-macular degeneration	In progress	2			To expand
IG-PRA*	*DFNB31* or *AKNO*	0			Frozen semen stock
Normal*	None/NA	10	6	4	Stable

Prcd*	*PRCD*	1			To expand
Prcd/crd2	*PRCD/NPHP5*	2			To expand
rcd1*	*PDE6B*	10	4		Stable
rcd2*	*RD3*	4			To expand slightly
rcd1/crd2	*PDE6B^{1}/NPHP5*	4	6		Stable
rpe65*	*RPE65*	2	3		Stable/To expand slightly
Rpe65/erd	*RPE65/STK38L*	1			To expand slightly
rpe65/T4R*	*RPE65/RHO*	0			Frozen semen stock
RPGRIP	*RPGRIP1*	9	5		Stable
Stargardt*	*ABCA4*	0			To expand
T4R*	*RHO*	7	15	25	Stable
Xlpra*	*RPGROrf15*	8	12		Stable
PWD*	*CCDC66*	2			To expand
Total =		**98**	**97**	**32**	

*These models have had semen frozen, and can be developed and distributed upon request, or stored semen is being used in order to expand or develop a study colony (e.g. Foveo-macular degeneration and Stargardt disease).

Note: some crosses, e.g. Cngb3/rpe65, Cngb3/rcd1, crd1/crd2, rcd1/crd2, are aimed at producing dogs with severe and complete phototransduction defects that can be used for optochemical and optogenetic studies, or to dissociate the RPE visual cycle from the RHO mutation (rpe65/T4R). Other crosses, e.g. Rpe65/erd, Prcd/crd2, are made to increase the genetic diversity of the erd and PRCD lines, and increase reproductive performance. The progeny then will be backcrossed to the parental lines.

The numbers in this table represent the current makeup of the breeding colony. Over the entire proposed period of the grant, some of these dogs will be retired and replaced. Approximately 10-20% of the breeding colony will turnover each year; this will be strain-dependent. Replacement animals will be the progeny of the breeding colony, however, and are therefore included in the estimates.

• *Colony-produced dogs*

Colony production serves two main purposes. The first is to produce animals for research investigations; the second is to generate replacement stock for the breeding colony. From the total number of pups produced, the most suitable are retained for breeding replacement. Both the total number produced, and the breakdown into the various strains, are adjusted according to demand from investigators. Where appropriate, for each such investigator, the request must represent a peer-reviewed protocol; in all cases, the protocol must be approved by the animal care committee (Institutional Animal Care and Use Committee [IACUC] or equivalent) from the investigator's institution. For testing experimental drugs from Pharmaceutical companies, or for providing tissues from the archival resource, or prospective matings for pilot studies, the IACUC's are generated at the University of Pennsylvania as approved amendments to our existing protocols. The actual numbers that will be produced therefore represent a variable controlled by the breeding capacity of the colony and demand from investigators.

Based on the structure of the colony and strains available, we have the capacity to produce ~35-45 litters per year, although in practice fewer breedings will be undertaken because production has to meet space limitations and demand. For example, many more breedings can be performed if the progeny produced is used within the first 2-3 months of age. The number of pups per litter ranges from 1-12 and averages 5-6. This is strain dependent as some strains, specifically in the early stages of development may have poor reproductive vigor and fertility. The actual number to be produced will vary according to demand from investigators, and the needs to increase numbers of specific strains for breeding. The minimum likely to be produced is of the order of 20-25 litters, or about 75-100 pups; and the most likely number is around 30 litters (~125 pups or more) based on current productivity data. This data has been detailed as part of the Resource Sharing component in Progress Reports from 2015-2018.

In addition to serving as a resource for independent and collaborative studies by other investigators, this proposal also includes PI-directed studies to understand the genetic and molecular mechanisms of diseases, and evaluate potential methods of disease prevention, therapy or amelioration (**Aim 3**). This will necessitate that a subset of the dogs produced over the 5 year funding period be used for this work. The *Research Strategy* section details these studies, and the animal numbers are summarized in the table below:

Aim 3a Cone-rod dystrophy with foveomacular degeneration and Canine *ABCA4*-Stargardt disease. Both colonies are at the same stage of development with semen banked from affected or carrier animals, respectively, as well as with natural matings in the former. In both cases, the procedures used to develop the colony are the same. In progress are breedings to non-affected females-2 for each line currently are expected to have puppies in November, 2018. Additionally, 2-3 additional matings for each line will be done in Q1 of 2019. In parallel we will direct our efforts to obtain females for each disease from outside the colony, either affected or carrier, for breeding. By Q1 of the new grant (Dec, 2019-Feb 2020) we expect to have sufficient number of carrier dogs for intercrosses/backcrosses within each disease strain; if we are able to obtain affected breeding females, this will be a 'short-cut' to producing homozygous affecteds. The genotype of all the progeny produced will be determined by haplotype analysis for cone-rod dystrophy, and SNP genotyping for "C" insertion in *ABCA4*. The aim is to have by Q2-3 of the -02 grant year a sufficient number of affected dogs to be used for independent and collaborative studies, and then expand the colony for in-depth studies including medical and gene-based therapies. *Based on the above, we aim to have for breeding 6 carrier dogs of each strain by Q1 of the new grant; these females are then backcrossed to affected or carrier males, and progeny DNA tested (see above) to identify genotypes*. This will establish a small breeding nucleus of dogs for each strain that will permit initial disease characterization and development of preliminary outcome measures and therapy strategies as the colony is expanded for more extensive studies towards the end of the grant cycle. While this timeline may seem extremely prolonged for those working on rodent models that have a much shorter breeding cycle, it is important to emphasize that this is the same strategy that has been used successfully to develop colonies of dogs with *RPE65*-LCA, *CNGB3*-achromatopsia, *RPGR*-XLRP, *RHO*-adRP, *BEST1*-Best macular dystrophy, and *NPHP5*-LCA. The first 3 diseases are now commercialized (*RPE65*) or in Phase 1 Clinical trials, and the remaining 3 are in pre-IND enabling studies. This high level of success emphasizes the 'speed' is not necessarily the fastest way to get to the clinic.

In regards to **Aim 3a (***Oligogenic RPGRIP1***)**, the initial RNA-seq and WGS will aim to identify the gene(s) and sequence changes that direct the cone function phenotype. The breeding nucleus of dogs is already available to produce dogs to carry out the molecular, biochemical and structural studies to define the function of the gene(s) involved. Breeding studies that will follow will aim to 'separate' this gene locus from two other involved genes, *RPGRIP1* and *MAP9*. The process has been initiated already, and both *RPGRIP1* and *MAP9* mutations already are present independently. Given that the 3rd locus is in a different chromosome (CFA30) and will segregate independently of *RPGRIP1* and *MAP9*, this can be accomplished in a 1-1.5 yr timeline.

Therapy studies

The Table below summarizes the experimental therapy studies and list the planned dog use.

Aim 3b: Retinal inflammation and microglia/macrophages activation

	rcd1 model	**control**
[redacted]		
a) intraocular pharmacokinetics and distribution of intravitreal (IVit)		8 dogs
[redacted]		6 dogs
c) Retinal rescue by [redacted]	6 dogs	
d) Downstream effects of [redacted] at 35 day; sample 60 or 90 days. RNA-seq and other studies	6 dogs.	6 dogs

Aim 3c: LRIT augmentation in CSNB	**CSNB LRIT model**	**control**
Vector validation, dose and route		6 dogs
a) AAV2$^{K9\#4}$-mod mGluR6-GFP (subretinal)		
b) AAV2 $^{K9\#12}$-mod mGluR6-GFP (IVit)		
c) Optimal vector/route selected	4 dogs	

d) If functional rescue, do older ages	6 dogs
If no rescue, younger ages	6 dogs

Aim 3d: Ocriplasmin (Ocri) to enhance intravitreal vector gene delivery.

	CSNB LRIT model	**control**
a) establishing optimal dose/safety and efficacy of vitreo-retinal lysis		8 dogs
b) validate optimal dose and targeting. Efficiency with/without Ocri: AAV2 $^{K9\#12}$-mod mGluR6-GFP		3 dogs
c) Ivit gene therapy with/without Ocri	4 dogs	
d) Test older ages	4 dogs	

	***RPGR*-XLPRA2 model**	**control**
a) Optimize photoreceptor targeting by IVit route		6 dogs
b) Compare IVit (+ Ocri) vs subretinal at optimal dose		4 dogs
AAV2$^{k9\#16}$-GRK1-GFP (IVit)		
AAV5-GRK1-GFP (subretinal)		
c) mid-stage (12 wks) treatment	4 dogs	
c) Late stage (26 wks) treatment	4 dogs	

Sample size selection: Based on the statistical advice from [redacted] (Director of the Biostatistics core of the Vision Research Center, Univ of Pennsylvania; P30 EY001583) our experiments are designed so that a minimal number of dogs are used, but allows us to achieve statistical significance. Experimental groups comprise a minimal number of biological replicates of N = 3 (eyes or dogs depending on experimental design) as it has been our experience that this provides sufficient power to detect differences in parameters, e.g. ONL thickness, ERG amplitudes, that are both statistically and biologically significant. In some case, we have selected sample sizes of 4 if variations in outcomes may be present as in case for [redacted] studies of vitreous lysis. For all studies, there will be equal gender distribution when sample size of 4 is used. With sample sizes of 3, we will use males or females in a 2 to 1 ratio, and achieve gender parity when studies are repeated. Our approach is based on the assumption that a statistically significant effect in a valid experimental animal model has to be robust and biologically relevant. For example we do not consider that preserving 1 row of photoreceptor nuclei will be sufficient to support further pre-clinical and clinical development. Consequently, if ONL thickness is used as an outcome measure of structural rescue, with an N = 3 we achieve the following power in a paired (one-sided) situation:

Power	N	Alpha	Beta	Mean ONL Difference	SD
0.99058	3	0.05	0.00942	2	0.5
0.88140	3	0.05	0.11860	2	0.75
0.71216	3	0.05	0.28784	2	1.0
0.99997	3	0.05	0.00003	3	0.5
0.99058	3	0.05	0.00942	3	0.75
0.93077	3	0.05	0.06923	3	1.0

Duplication: To the best of our knowledge these studies have not been previously performed by ourselves or others.

References

- Machin, D., Campbell, M., Fayers, P., and Pinol, A. 1997. Sample Size Tables for Clinical Studies, 2nd Edition. Blackwell Science. Malden, MA.

- Zar, Jerrold H. 1984. Biostatistical Analysis (Second Edition). Prentice-Hall. Englewood Cliffs, New Jersey.

For the molecular profiling and gene expression studies, a group size of 3 dogs enables detection of differentially expressed genes having a 2-fold increase/decrease in expression at $p<0.05$. This strategy has been used in several of our published studies, for example:

- Genini S, Zangerl B, Slavik J, Acland GM, Beltran WA, Aguirre GD. Transcriptional profile analysis of RPGRORF15 frameshift mutation identifies novel genes associated with retinal degeneration. *Invest Ophthalmol Vis Sci* 2010;51:6038-6050. (PMC3061521)

- Genini S, Beltran WA, Aguirre GD. Up-regulation of tumor necrosis factor superfamily genes in early phases of photoreceptor degeneration. *PLoS One* 2013;8:e85408. (PMC3868615)

- Sudharsan,R. Beiting, D., Aguirre, G.D., Beltran, W.A.. Involvement of innate immune system in late stages of inherited photoreceptor degeneration. *Scientific Reports*, 2017; Sci Rep **7**(1): 17897 (PMC5738376)

2. Justification of species

The dog was selected as a species for these studies for several reasons:

a) The naturally-occurring forms of retinal degeneration that occur in dog show close similarity with the phenotypes of patients. The close phenotypic similarity is evident from several of our studies in the *RPE65*-LCA, *CNGB3*-achromatopsia, *RPGR*-XLRP, *RHO*-adRP, *BEST1*-Best macular dystrophy, and *NPHP5*-LCA models, and these have been used for past and current translational studies.

b) The large size of the canine eye, and the anatomy of the retina that comprises a cone-rich region in the center of the area centralis called "fovea-like area" are close to that of the human and has the same cone density. The size similarity between the canine and human eyes allows procedures such as injection of vector or drug using the same surgical approaches and dose volumes, or implantation of devices (e.g. retinal prostheses or for delivery of therapeutic agents) that are identical to those intended for human trials. In addition, the instruments and methods for *in vivo* outcome assessments are the same.

c) The response of the immune system to the delivery of AAV vectors can be monitored and controlled via therapies that are much more informative in the dog than that in rodents.

d) Surgical interventions, doses of AAV, volume injected, methods of delivery to specific rod-enriched or cone-enriched areas, and non-invasive *in vivo* imaging techniques that are used/developed in the dog can be translated easily to the human.

This project serves as a unique, centralized national and international research resource for investigators of inherited retinal diseases (IRDs). Specific strains of dogs, affected with well-characterized IRDs together with appropriate non-affected control dogs, are bred and maintained. The diseases in these dogs are studied in collaboration with investigators from many centers throughout the United States and abroad, not only to understand the specific diseases themselves, but also as models of retinitis pigmentosa, a major heritable cause of blindness and other forms of IRDs in man.

Dogs bred in the colony, other than those required for colony maintenance, are committed to such studies either directly within the EY06855 project, or by collection, processing and distribution of requested tissues. The numbers of dogs represent estimates of both projected colony productivity and projected requirements of research investigators. The actual number of dogs used each year will vary. To some extent this will be beyond our control, depending on variations in reproductive performance and demand from investigators for the various strains, from year to year. To the extent possible, colony productivity will be adjusted to match the level of demand from research investigators. The choice of species, breed and strain of animal is dictated by the naturally-occurring genetic defects that are unique to these specific strains of dog.

As noted in the *Research Strategy* section and summarized in the Justification part of the introduction to this section, the dog and the canine eye offer advantages for a broad range of translational studies. It is critical to emphasize, however, that regardless of their translational value, the canine models are *not alternatives* to

other laboratory model systems such as rodents. Rather they are a complementary and synergistic model, serving as an intermediate between rodents and man that provides an excellent test bed to develop or test new therapies. Lastly, although *in vitro* systems to develop pluripotent stem cell (PSC)-derived photoreceptor precursor cells and model disease(s) have been developed, these do not allow addressing any of the current challenges at targeting subretinally or intravitreally diseased photoreceptors; which is precisely one of the goals of this proposal. Nor are *in vitro* systems available to optimize surgical approaches, optimize immunosuppression (if needed), and circumvent physical barriers in the retina. Similarly, no computer models have been developed that mimic the process of photoreceptor disease/death and that could be used for assessment of gene therapy intervention.

3. Minimization of Pain and Distress

Drs. Aguirre and Miyadera, as veterinarians, provide consultative veterinary care, and develop and institute planned preventative health care for colony animals. They ensure that appropriate vaccination schedules, routine diagnostic procedures, and similar strategic health programs are in place. If a health problem arises, they form the first professional level of decision making, diagnosis and implementation of care. The University of Pennsylvania's Unit for Laboratory Animal Research (ULAR) has a veterinary staff that provides supervision of colony health status, and serves as the attending veterinarian of record for regulatory purposes. They are available to assist with any medical problems that may arise, for consultation, for direct diagnostic aid, and for implementation of treatment. For veterinary health problems that require specialized diagnostic procedures or intensive care on an emergency or non- emergency basis, dogs are transferred to the small animal Veterinary Hospital of the University of Pennsylvania, in Philadelphia, PA. The veterinary staff at the [redacted] consists of 2 post-doctoral clinical fellows who, in addition to carrying out the ophthalmic research studies, performed routine medical care including C-sections, and care of bite wounds. The goal in all of these medical/surgical procedures is to minimize pain and distress.

All procedures and protocols performed as part of this project are reviewed and approved by the IACUC of the University of Pennsylvania and by the veterinary staff of the ULAR.

Occasionally, as part of the routine handling for the breeding and health maintenance programs, procedures may require medication to prevent or alleviate pain or distress. In such circumstances one or more of the following drugs may be appropriately used:

Drug	Dose (mg/kgm)	Route	Frequency
Acetylpromazine	0.01-0.2	IM or SQ	As required
Morphine	0.5	IM, SQ, IV	As required
Butorphanol	0.5	IM, SQ, IV	As required
Carprofen	2.2	Oral	As required

Experimental procedures performed as part of this project include collection of tissues for investigators, and are undertaken either postmortem or preterminally under pentobarbitone at a surgical plane of anesthesia (and followed immediately by euthanasia, without intervening recovery from anesthesia). Electroretinography (ERG), non-invasive imaging (cSLO/sd-OCT), subretinal injections of viral vector solutions are routine procedures that we perform in dogs at our research facility under general anesthesia. For dogs, procedures such as ERG and non-invasive imaging (cSLO/sd-OCT) requires anesthesia for restraint rather than for pain relief (the identical procedure is used in fully awake human patients).

Any procedures that could cause pain or distress are performed under general anesthesia as follows:

Procedure	Anesthetic	Aftercare
1) ERG, non-invasive imaging (cSLO/sd-OCT), subretinal injections, vitreo-retinal surgery.	IV Propofol (4-6 mg/kg) or Ketamine (10-15 mg/kg)/Valium (0.5 mg/kg); Isoflurane or Ketamine for maintenance	Not applicable for ERG or non-invasive imaging. This is a clinical procedure with no post-anesthetic pain or discomfort. For subretinal injections, and vitreo-retinal surgery post-surgical analgesia is provided as noted above.
2) Non survival enucleation and tissue collection	IV Pentobarbital	Enucleation is performed immediately following euthanasia by barbiturate overdose.

Qualification and training: Members of the Aguirre and Miyadera labs have extensive experience with dogs and all of the proposed procedures.

4. Euthanasia

At the end of the experiment, euthanasia is performed by administration of sodium pentobarbitone by intravenous or (in already fully anesthetized animals) intracardiac injections. Highly excitable animals may first be tranquillized or anesthetized by one or more of the drugs listed above. Commercially formulated euthanasia solution (usual concentration = 390 mg/mL) is used, at a dose rate of at least 1 mL per 5 kg. This rate, equal to 80 mg pentobarbital/kg, is a minimum dose only; in practice a larger dose is often routinely used, and in all cases additional solution is administered if total cardiac and respiratory arrest does not rapidly ensue. This method is consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association and is approved by IACUC at University of Pennsylvania.

MULTIPLE PD/PI LEADERSHIP PLAN

Grant EY006855 (Models for Therapy of Hereditary Retinal Degenerations) has been funded from December 1, 2014-November 30, 2019. This is a project that was initiated at the University of Pennsylvania as an NIH contract in 1976, and then changed to a cooperative agreement (UO1-EY06855); for the past 33 years it has been an RO1.

Following the last competing renewal, Dr. Keiko Miyadera was appointed as Key Personnel in the -29 grant year associated with her successfully becoming a Diplomate, American College of Veterinary Ophthalmologists and her expanded roles in the project. Since that time, Dr. Gustavo Aguirre has served as the PI and Dr. Keiko Miyadera has been Key Personnel although her role and responsibilities have expanded and she has transitioned to be, for all practical purposes, the de facto CoPI. In this renewal, we request formal recognition of Dr. Miyadera as PI in the multiple PI project. We believe that this change will benefit the program, and, at the same time, recognize Dr. Miyadera's leadership role and contributions to the project. This change will be implemented without an increase in effort or cost to the project. The following points address the change in structure and operations in terms of governance of the leadership team:

• Communications: Drs. Aguirre and Miyadera have adjoining offices and share common laboratory space both [redacted] that houses the research dogs. On a daily basis, both meet face to face and discuss relevant aspects of the project, or by phone when one of the investigators is [redacted] As well, on a 1-2 week basis, or more often if necessary, we both meet with collaborators, either in person (Drs. Cideciyan and Jacobson at PENN's Scheie Eye Institute) or by phone (Dr. Hauswirth, Univ. of Florida) for the part of the project dealing with PI-directed studies. We also meet jointly with investigators in academia or Pharma to discuss specifics of projects that use the research dogs and/or facilities, and set timelines and resources needed to address requests.

• Decision making process on scientific direction: Although Dr. Miyadera does not currently serve as PI of the project, she has been involved in this process since becoming Key Personnel in -29 grant year. All decisions are made jointly, and with extensive input from collaborators or independent investigators depending on the project. Drs. Aguirre and Miyadera have had a long and stable working relationship since May, 2011, and Dr. Miyadera has progressed from a post doc fellowship to ophthalmology residency, and now to a tenure track assistant professor position with an independent research program.

During the tenure of this grant, Dr. Miyadera will serve as the contact PI, and will assume fiscal and administrative management including maintaining communication with outside independent investigators, project scientists and other personnel. Dr. Aguirre will be responsible for communication with NIH and submission of annual reports. Both PIs will be responsible for the implementation of the scientific agenda, the Leadership Plan and the specific aims. They will ensure that systems are in place to guarantee institutional compliance with US laws, DHHS and NIH policies including biosafety, animal welfare, data management and facilities.

Specifically for this project, Dr. Miyadera takes the lead when making decisions based on surgical techniques, post operative treatments, objective vision testing and morphologic evaluation of tissues, e.g. using confocal microscopy and immunohistochemistry. Dr. Aguirre will take the lead in planning the breeding and production of research dogs, clinical assessment of treatment outcomes, and non-invasive imaging and ERG studies. However, it is essential to stress that, although one or the other PI's takes the lead in these decisions, in general they are made jointly after discussions and due consideration. Also important to emphasize that both PIs are equally able to take over, as needed, some of the responsibilities of the other as their training and expertise overlap.

• Procedures for resolving conflict: Based on >8 years of working closely together, it is fair to say that we do not anticipate that any conflict could arise that would jeopardize our scientific and personal interactions or adversely affect the research project. In the unlikely event that such would arise, we will ask one of our close collaborators, e.g. Dr. William Beltran or Samuel Jacobson, to arbitrate the dispute. If this were to fail, we would ask the Department Chair at our Institution to intercede and resolve the conflict, and collectively, we have agreed to abide by the Chair's decision.

• Delineation of the administrative, technical and scientific responsibilities: See above, and details in Budget Justification.

• Delineation of the Budget and resource distribution: We have provided a detailed Budget Justification to

facilitate the review process. On a monthly basis, the PIs will meet (continuing a process that has been in effect for 4 years), and assess current and projected expenditures and determine the need to re-allocate resources and/or effort to maximize continued success of the project while complying with the principal goals of the project and all the NEI/NIH guidelines.

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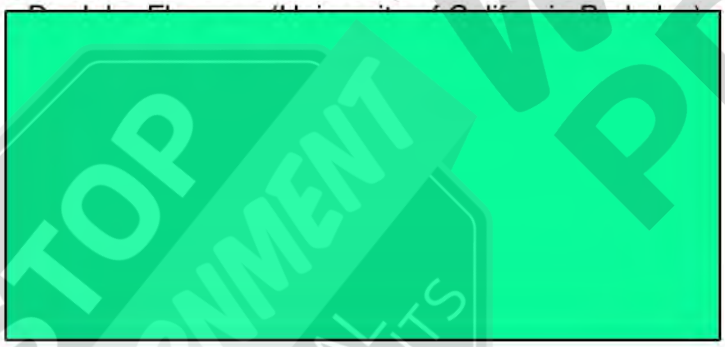
Letters of Support

Four different groups of investigators/collaborators are considered in this section:
a - Other Significant Contributors who collaborate on independent, investigator driven research studies;
b - Pharmaceutical Companies for whom we provide intellectual, physical and/or animal resources that allow them to test and validate proprietary agents in the canine models for potential translation to patient trials;
c - Collaborators, scientists with whom we collaborate on other NEI or FFB funded projects, but which require the use of infrastructure resources (OCT, ERG, objective vision testing, and/or surgery) available at the facility to carry out this work. This includes scientists whose NEI funded projects use the facility to maintain and support the breeding nucleus of dogs required for their studies;
d - Independent scientists to whom we provide models or tissues, but do not participate in the research.

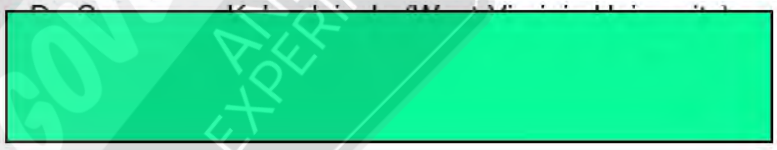
a - Other Significant Contributors who collaborate on independent, investigator driven research studies (studies detailed in Research Strategy)
- Dr. William Beltran
- Dr. Karina Guziewicz
- Drs. Artur Cideciyan and Samuel Jacobson
- Drs. Bill Hauswirth and Al Lewin

b - Industry scientists and companies to test and validate potential therapies and capsule summary of projects.

c - Academic scientists directly involved in studies with Facility scientists and capsule summary of projects.



d - Independent scientists that receive models or tissue samples.



a - Other Significant Contributors who collaborate on independent, investigator driven research studies (studies detailed in Research Strategy)

- Dr. William Beltran
- Dr. Karina Guziewicz
- Drs. Artur Cideciyan and Samuel Jacobson
- Drs. Bill Hauswirth and Al Lewin

Penn
Veterinary Medicine

University of
Pennsylvania School of
Veterinary Medicine
Department of Clinical Studies-Philadelphia
3900 Delancey Street, VHUP room #2050
Philadelphia, PA 19104-6010
Phone: 215-898-4284 (Lab)
215-898-4692 (Office)
Email: wbeltran@vet.upenn.edu

William A. Beltran: DVM, PhD, Dip. ECVO
Assistant Professor of Ophthalmology

July 5, 2018

Dear Drs. Aguirre and Miyadera:

I hereby provide a letter of unconditional support for the renewal of your RO1 grant EY- 06855 entitled "*Models for Therapy of Hereditary Retinal Degenerations*". During the past funding period this grant was critical to the successful translation into clinical trial of a gene therapy for the *RPGR* form of X-linked RP, and to the development of a corrective gene therapy for *RHO*- autosomal dominant RP and its testing in a canine model. All *in vivo* experimental studies were conducted at the Retinal Disease Studies Facility. While project specific grants covered all costs associated with experimental dogs, per diem costs for breeders were supported by R01 EY-06855. We will soon be submitting a one year no-cost extension to the *RHO*-adRP gene therapy grant (R24- EY022012), and in addition will be initiating IND-enabling studies through a sponsored research agreement with a biotech company (Ophthotech). This project has and will continue to benefit considerably from the excellent animal technical support and the unique access to *in vivo* retinal imaging service and visual behavior testing that are partially funded by this RO1EY-06855 grant.

A critical role of this RO1EY-06855 grant is also to carry the cost of identifying and characterizing new large animal models of retinal degeneration that can then be provided to investigators for their own funded projects. Over the past funding period we have created two new models that each carry mutations in two genes (*PDE6B* and *NPHP5*). Dogs that are homozygous mutant at both loci have an early-onset impairment of both rod and cone-mediated vision. These two models will be used to establish proof of concept of optogenetic and optochemical strategies for vision restoration through ongoing collaborations that I have established with UC Berkeley investigators (John Flannery, Ehud Isacoff, Richard Kramer) and at Univ. of Washington (Russell van Gelder).

In summary, RO1EY-06855 has over the past funding period continued to provide the scientific community with clinically-relevant large animal models, and has served as a launch pad for novel therapies that are (or soon to be) in the clinic. The return on investment of RO1EY-06855 is simply unparalleled, and thus I strongly support its continued funding.

Sincerely,

Penn Veterinary Medicine

Gustavo D. Aguirre, VMD, PhD
Prof. of Medical Genetics & Ophthalmology
Ophthalmology School of Veterinary Medicine
University of Pennsylvania
Philadelphia, PA 19104

Keiko Miyadera, DVM, PhD
Assist. Professor of
School of Veterinary Medicine
University of Pennsylvania
Philadelphia, PA 19104

October 18, 2018

Dear Gus and Keiko,

This letter is to express my most enthusiastic support for the renewal of your NEI/NIH EY06855 grant entitled '*Models for Therapy of Hereditary Retinal Degenerations*'. Over the past award period, this grant proved pivotal in the development of new large animal models of inherited retinal diseases (IRDs) and in accelerating our translational studies toward clinical applications.

Specifically, our recent research efforts led to the identification of a first clinically-relevant model for Best vitelliform macular dystrophy, one of the most prevalent forms of juvenile maculopathies in man, thus far considered untreatable. This new canine model was established and maintained at the Retinal Disease Studies Facility (RDSF), and served as a primary translational platform for the successful development of a safe and effective AAV-based gene therapy for *BEST1*-associated maculopathies. These proof-of-concept studies now enter preclinical phase (through SRA) toward initiation of the first gene therapy trial for bestrophinopathies in 2020. Without a doubt, the ready access to the core clinical resources at RDSF supported by this grant played a major role in maximizing the momentum for translation of this project to the clinic.

Most importantly, the exceptional RDSF resources, such as modern non-invasive imaging modalities, greatly facilitated our comparative studies conducted in close collaboration with Drs. Cideciyan and Jacobson (Scheie Eye Institute), including preliminary assessment of two recently discovered disease models, canine ACHM2 and a new form of LCA-ciliopathy. These two new canine models of pediatric IRDs are currently under development, and continuation of this NEI/NIH grant support is instrumental in the rapid translation of this research into clinical practice. To that end, I strongly support the EY06855 grant renewal as the role of this funding in promoting new therapy models for a wide range of inherited retinal diseases cannot be overstated.

Sincerely,

Karina E. Guziewicz, PhD
Research Assistant Professor of Ophthalmology

UNIVERSITY of PENNSYLVANIA
School of Veterinary Medicine Department of Clinical Sciences & Advanced Medicine
3900 Delancey St., Philadelphia, PA 19104-6010
Tel: 215-898-7479
Email: karinag@vet.upenn.edu

UNIVERSITY OF PENNSYLVANIA HEALTH SYSTEM
Department of Ophthalmology
Scheie Eye Institute

July 5, 2018

Re: EY-06855; Models for Therapy of Hereditary Retinal

Degenerations. Keiko Miyadera, DVM PhD Gustavo Aguirre,

VMD, PhD
Assist. Prof. of Ophthalmology Prof. of Medical Genetics &
Ophthalmology School of Veterinary Medicine School of Veterinary
Medicine
University of Pennsylvania University of
Pennsylvania Philadelphia, PA 19104 Philadelphia, PA
19104

Dear Drs. Miyadera and Aguirre:

The decades of human clinical work we have performed to understand and now treat blinding retinal diseases has hinged on collaborating with you to perform key experiments in the canine animal models of the disorders. The stepwise pursuit of treatment strategies for these otherwise incurable retinal degenerations has depended on testing hypotheses and then performing proof-of concept experiments in the relevant canine models. Some experiments can and should be performed in rodent models, but the large animal canine models make the ultimate case for translation to human patients.

We remain enthusiastic about continuing to collaborate with you on this translational research. As users of the resource you have created and as collaborators, we bring expertise in quantitative non-invasive assessment of outcomes of treatment in your established canine models with retinal degeneration. Also, we have techniques to characterize new models as you identify them. Our clinical grounding permits the models to be compared with the human diseases and decisions made about how best to approach treatment. We look forward to continuing this longstanding and productive collaboration with you that aims to bring therapy to our many patients with incurable retinal blindness.

Sincerely,

Samuel G. Jacobson, MD PhD Professor of

Artur V. Cideciyan, PhD
Research Professor of Ophthalmology

UF UNIVERSITY of FLORIDA

College of Medicine
Department of Ophthalmology

1600 SW Archer Road
PO Box 100284
Gainesville, FL 32610-0284
352-392-3318

August 21, 2018

Gustavo Aguirre, VMD, PhD
Professor of Medical Genetics
Ophthalmology and Ophthalmology

Keiko Miyadera, DVM PhD
Assistant Professor of

School of Veterinary Medicine, University of Pennsylvania 3900 Delancey St., Philadelphia, PA 19104-6010

Dear Gus and Keiko,

It is our pleasure to continue our collaboration with you and to support your NEI/NIH research grant: EY-06855, Models for Therapy of Hereditary Retinal Degeneration. This will continue our long-standing relationship in which you have been identifying new RD models in dogs, and we have collaboratively
been developing new gene-based strategies for treating them. Clearly, your facility has been in the lead in bringing these therapeutic paradigms to human clinical trials.

Specifically, we will provide you with Adeno-associated virus (AAV) vectors for transduction of photoreceptor and other cells of the retina, both to test improvements in gene transfer to the retina and to develop promoters and novel vector capsids for late stage RP, RPGR-XLRP as well as for your NPHP5 dog model of LCA. In particular, we have been designing vectors with specific small promoter sequences in order to express genes either in rod and cone photoreceptors, so that gene expression can be targeted to a specific subset of photoreceptors, depending on the disease to be treated.

As our part of the collaboration, we will test these vectors in tissue culture and when possible in mice before making large scale preparations for your use in dogs. The continuation of this NEI/NIH proposal is critical to help ensure that novel and specific therapies are being validated in your important canine models before initiating Phase I studies in humans.

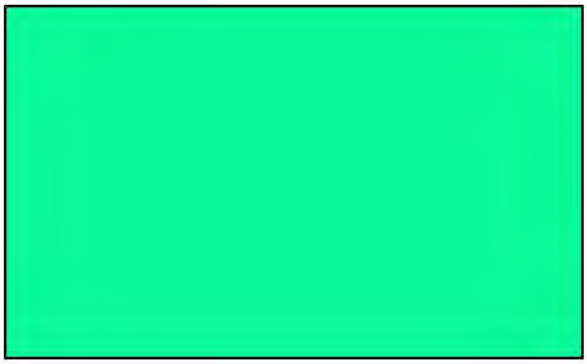
We have had a productive working relationship for nearly two decades, and we look forward to continuing it.

Yours truly,

William W. Hauswirth
Maida and Morris Rybaczki Professor

Alfred S. Lewin
Shaler-Richardson Professor

b - Industry scientists and companies to test and validate potential therapies and capsule summary of projects.



[redacted]

Gustavo Aguirre, VMD PhD
Professor of Medical Genetics and Ophthalmology

Keiko Miyadera, DVM PhD
Assistant Professor of Ophthalmology

School of Veterinary Medicine
University of Pennsylvania
3900 Delancey St, Ryan Veterinary Hospital
Philadelphia, PA 19104

October 24, 2018

Dear Gus and Keiko:

We are writing in support of you competing renewal of NIH grant EY-06855; Models for Therapy of Hereditary Retinal Degenerations.

We have collaborated with you and other UPenn investigators in studies of gene therapy for [redacted] This canine colony is a valuable resource that has allowed our joint research teams to generate proof-of-concept data that supports development of treatments for inherited retinal diseases in humans, which have now proceeded to Phase I/II trials. These data were also instrumental in the ability of [redacted] o obtain private venture capital investors to support advanced development, which demonstrates a significant return on investment from previous grant support for your canine colonies.

The resources and animal models at Penn are important not just for [redacted] but for translating basic research towards clinical trials for other inherited retinal degenerative diseases in which canine models offer unique opportunities to evaluate safety and efficacy of product candidates.

We enthusiastically support your grant application, and we look forward to collaborating with you in the future.

Sincerely,

[redacted]

[redacted]

Gustavo Aguirre, VMD, PhD, PhD (*hc*)
Professor of Medical Genetics and Ophthalmology

Keiko Miyadera, DVM, PhD
Assistant Professor of Ophthalmology

School of Veterinary Medicine
University of Pennsylvania
3900 Delancey Street, Ryan-VHUP 2050
Philadelphia PA 19104

October 18, 2018

Dear Gus and Keiko,

[redacted] is pleased to write a letter of our full support for the competing renewal of your R01 grant EY006855 "Models for Therapy of Hereditary Retinal Degeneration" from NEI/NIH.

In collaboration with you and your associate Dr. William Beltran, we are committed to investigate the safety and efficacy of A[redacted] gene augmentation therapy in the canine [redacted] disease model with the goal of bringing this [redacted] product candidate into the clinic. Your research contributions with your canine disease models are instrumental for us to bring forth the [redacted] gene therapy product candidate to a potential IND. We strongly believe that the IND-enabling preclinical studies utilizing your canine models have great promise in helping us to advance our novel [redacted] gene therapy to a potential Phase I clinical trial in order to treat patients with this inherited retinal disease for which there is no current treatment.

The canine [redacted] disease strain that has been bred and maintained at your research facility is important in carrying out our IND-enabling preclinical studies. As well, the established infrastructure at your [redacted] of UPenn with the excellent track-record of successful preclinical studies is paramount. The continued funding of EY006855 to support these resources will therefore be valuable to our work as well as for many others who benefit from the platform for testing new therapies to obtain proof-of-concept in relevant animal models.

Please feel free to contact me with any questions, and we look forward to continue to collaborate with you.

Sincerely,

[redacted]

[redacted]

re: [redacted] collaboration
in proposed NEI/NIH grant

Dr. Gustavo Aguirre
Dr. Keiko Miyadera
Section of Ophthalmology
School of Veterinary Medicine
University of Pennsylvania
Philadelphia, PA 19104-6010

Dear Drs. Aguirre and Miyadera,

It is my pleasure to write and affirm our interest in the continuing studies that are proposed in your NIH grant application (EY-06855: Models for Therapy of Hereditary Retinal Degeneration).

It is clear from your published work that, to date, the most efficient and effective delivery of viral vectors to the outer retina, RPE and/or photoreceptors has been by direct injection into the subretinal space. Considering that many of the treated eyes, both in experimental animals and human patients, have advanced degeneration of the fragile photoreceptor cells, it is surprising that the retina tolerates so well this invasive procedure. However, complications do occur. Bypassing the subretinal injection route, e.g. by delivering vectors using the intravitreal (IVit) route of administration, would be a decided advantage as it would eliminate surgical associated trauma, and provide wide distribution of viral vectors to both the foveo-macular regions and periphery. Your plan to develop the IVit route of administration to deliver viral vectors to the retina of canine models affected with several inherited retinal degenerations is an excellent and sound approach. As we have discussed previously, this route of delivery should be greatly facilitated by the use of [redacted] prior to vector administration. This [redacted]

[redacted]

In regards to your proposed studies, [redacted] prepared to continue this important collaboration, and will provide the regents that now are in clinical use for use by your research group at no cost.

We look forward to continuing this fruitful collaboration. Good luck,

[redacted]

[redacted]

Gustavo Aguirre, VMD PhD
Professor of Medical Genetics and Ophthalmology

Keiko Miyadera, DVM PhD

Assistant Professor of Ophthalmology
School of Veterinary Medicine University of Pennsylvania
3900 Delancey St, Ryan Veterinary Hospital Philadelphia, PA 19104

October 18, 2018

Re: EY-06855; Models for Therapy of Hereditary Retinal Degenerations.

Dear Gus and Keiko:

I am writing with great enthusiasm in support of your competing renewal of grant EY-06855 "Models for Therapy of Hereditary Retinal Degenerations" for funding of innovative works utilizing your unique research colony of canine strains affected with inherited retinal diseases.

As you know, our collaborative work with you and your associate Dr. William Beltran has been awarded NIH funding (2R44EY025905-02A1: "Ambient light activatable opsin based therapy for age related macular degeneration"). We are excited to move forward in testing the new technologies we have developed with your well-established canine model of retinitis pigmentosa. We value highly your expertise in the study to assess safety and efficacy using the canine model and look forward to collaborating with you.

The support by NEI through the grant EY-06855 for the canine colony in which these inherited retinal diseases are maintained is greatly appreciated by [redacted] These canine colonies are one of the only few opportunities for us to evaluate our technologies for ophthalmic applications in large animal models of retinal degenerative diseases. Without these colonies, our development of therapeutics based on ambient light activatable opsin for human eye diseases would be very difficult.

Sincerely,

[redacted]

[redacted]

October 12, 2018

Gustavo Aguirre, DVM, PhD
Professor of Medical Genetics and Ophthalmology
Keiko Miyaderal, DVM, PhD
Assistant Professor, Ophthalmology
Section of Ophthalmology
School of Veterinary Medicine University of Pennsylvania
Ryan-VHUP, Room 2050
3900 Delancey St.
Philadelphia, PA 19104-6010

Dear Gus and Keiko,

I am writing to affirm our enthusiasm and support for your project "EY-06855; Models for Therapy of Hereditary Retinal Degeneration" – a long term collaboration that I think has been immensely successful for all of us, and most importantly for vision research. Specifically we commit to providing you with molecules developed at [redacted] that we (you, [redacted] have shown to be efficacious in protection against loss of vision in various animal models of retinitis pigmentosa (RP). Most importantly, as described in brief below, these molecules match to several aims of your proposal in that the molecules were designed to protect mitochondrial homeostasis independent of the RP-related genomic mutations and, thus, the [redacted] could be well suited as adjuvants to gene-therapy approaches.

As you know, [redacted] and I hypothesized that many of the mutations in widely disparate genes that cause RP might do so partly indirectly via induction of bioenergetics stress that would cause a gradual loss of mitochondrial homeostasis. The resulting mitochondrial dysfunction could lead to irreparable loss of photoreceptor function. We screened a large, chemically diverse library of molecules for protection of mitochondrial function in cell lines exposed to bioenergetics poisons and from the protective compounds we arrived at a single structural class that was then synthetically elaborated into more than 3000 analogues. One series, represented by the [redacted]s, has proven to be our lead molecule.

The [redacted] is formulated for topical delivery via eye-drops, and it has been tested in RP models of mouse, and rat. More importantly, our collaborative stidies have shown that the topically delivered compounds are safe, and efficacious in the rcd1 and RPE65 canine models of RP. The compound has been shown to be very safe with a therapeutic index of about 1 million, it exhibits no ocular toxicity over months, and it is stable to liver microsomes. The systemic exposure following eye-drop delivery is not detectable in serum above 0.5 ng/mL and it is well tolerated when delivered once to twice every. Your observations

that the dogs have no real aversion the eye-drops is a result that we take pride in.

In vitro assays indicate that [redacted] protects against mitochondrial stressors, and in those cases in which mitochondrial function is impaired, [redacted] improves function. Thus, we would anticipate that [redacted] can improve the function of retinal cells that have otherwise been stressed, even when nuclear genetic defects have been corrected and, thus, we anticipate synergy with your current excellent gene-therapy approach.

Best wishes,

[redacted]

c – Academic scientists directly involved in studies with Facility scientists and capsule summary of projects.

[redacted]

Monday, February 26, 2018

NIH study section reviewers:

It is my pleasure to write an enthusiastic letter of support for the continued funding of NIH grant EY- 06855: *Models for Therapy of Hereditary Retinal Degenerations.*

The U. Penn canine facility is essential for several of the viral mediated gene therapy projects in my lab, projects that are currently funded under other NIH grants, specifically NIH EY024958: *Converting Bipolar cells into red shifted optogenetic sensors for retinal therapy*, and EY022975 *Directed Evolution of Adeno Associated Virus for retinal gene therapy*. Going forward, this grant and facility are critical to our recently reviewed (February, 2018) application Optogenetic Vision Restoration: R01 EY028240-01A1 which received a 19% percentile, which is hopefully within the funding range.

In these projects we are developing new optogenetic tools, which sensitize to light the activity of signaling proteins and the cells in which they are expressed. We have made significant progress our major preclinical work, the restoration of vision in retinal pathologies that lead to loss of photoreceptors and blindness. In the initial 5 years of this work, we were primarily focused upon developing and characterizing the light activated sensor proteins themselves and the viral gene transfer tools with which to deliver them. In the initial studies, we were able to show restoration of retinal sensitivity, cortical VEPs, and very simple behavioural tasks. Now, in preparation for IND filing, we are focused on demonstrating efficacy and non- toxicity in a human sized eye in an animal in which we can demonstrate visually guided behaviors. These experiments are in response to our pre-IND meetings with the FDA, where it was stated that we must demonstrate a 'meaningful behavioural change' in a large animal model of blindness before moving forward to clinical application. These studies can only be done in collaboration with the U. Penn facility and Drs. Aguirre Beltran and Miyadera and using their canine models of blindness. We have begun the initial phases of these studies in collaboration with Penn.

In the other grant, "*Directed Evolution of Adeno-Associated Virus for Retinal Gene Therapy*", the purpose is to develop adeno-associated virus-based vectors to target gene delivery to photoreceptor neurons in large animals after a non-invasive injection into the vitreous humor and to examine whether delivery of specific nucleases can be used to block gene expression in the retina. The significance of this application was assessed as exceptionally high with the potential to develop agents that could be used to treat autosomal dominant retinal disorders, such as retinitis pigmentosa, cause by expression of mutant proteins. We will use the canine models developed by Dr Aguirre, Miyadera and Beltran to attempt to deal with a major problem with photoreceptor gene therapy- in that many retinal degenerations are autosomal dominant. While RNAi can yield a partial knockdown of pathological alleles, a full

[redacted]

ablation of such genes would be desirable. There have been recent advances in the development of site-specific DNA nucleases that can knock out target genes, and we will build upon these advances to knock out dominant alleles that underlie retinal degeneration using AAV delivered CrispR. These gene therapies for dominant retinal degenerations will be tested for efficacy and potential toxicity in Drs. Beltran, Aguirre and Miyadera's canine models for dominantly inherited retinal degeneration.

Clearly, the specific aims of my two ongoing NIH grants listed above and the one pending could not be accomplished without the continued support of EY-06855, the canine models for inherited retinal degeneration.

Kind regards,

[redacted]

[redacted]

March 11, 2018

Gustavo Aguirre, VMD, PhD, PhD (*hc*)
Professor of Medical Genetics and Ophthalmology
School of Veterinary Medicine
University of Pennsylvania Ryan-VHUP, Room 2050
3900 Delancey St.
Philadelphia, PA 19104-6010

re: EY-06855 competing renewal

Dear Gus and Keiko,

I am pleased to provide this letter expressing my enthusiasm to collaborate on projects supported by your NIH grant (EY-06855; Models for Therapy of Hereditary Retinal Degenerations) that combine our iPS cell technology and your extraordinary canine models to study retinal disease mechanisms and explore potential avenues for treatment.

As we have discussed on multiple occasions, your well-characterized canine models offer unique and powerful opportunities to test cell- and gene-based approaches to the treatment of retinal degenerative diseases. In addition, we are interested in reprogramming canine fibroblasts into iPS cells to evaluate the benefits of auto- vs. allografts of iPS cell-derived retinal progeny.

Thanks again for your interest in making these dog models available to the vision research community. I look forward to many more interactions with your research team in the future.

Sincerely Yours,

[redacted]

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service

National Institutes of Health
National Eye Institute
Bethesda, Maryland 20892

March 12, 2018

Gustavo Aguirre, VMD, PhD, PhD (hc)
Professor of Medical Genetics and Ophthalmology Section of Ophthalmology
School of Veterinary Medicine
University of Pennsylvania

Keiko Miyadera, DVM, PhD
Assistant Professor, Section of Ophthalmology
School of Veterinary Medicine
University of Pennsylvania

Dear Gus and Keiko,

I am very pleased to have the opportunity to describe our collaborative studies in support of the renewal of your NIH grant, **EY-06855; Models for Therapy of Hereditary Retinal Degeneration.**

Our research group here at the National Eye Institute (NEI) has had a long-term interest in examining the nature of neuron-glia interactions in photoreceptor degeneration, particularly those involving innate immune cells of the retina, such as microglial cells. In recent work performed in rodent models of hereditary retinal degeneration, we have discovered that microglial activation is an early event in photoreceptor degeneration (**Zhang et al., EMBO Mol Med, 1179-79, 2015**), and modulations of microglial physiology can result alterations in the rate of degeneration (**Zabel et al., Glia, 1479-91, 2016; Wang et al., J. Neuroscience, 3294-3310, 2017**). Work by us and others have shown that cellular events in the milieu of degenerating photoreceptors can non-cell autonomously contribute to degeneration, thus presenting therapeutic possibilities for slowing down degeneration by exploiting the mechanisms involved. This has led to our preliminary foray into microglial modulation as a therapeutic strategy in clinical trials of retinitis pigmentosa (**NCT02140164**) in our NEI eye clinic.

Importantly, work performed in your labs has corroborated the involvement of microglial activation and inflammatory changes in canine models of retinitis pigmentosa (**Appelbaum et al., PLoS One, e0177224, 2017; Sudharsan et al., Sci Rep., 17897, 2017**) which have previously been used in translational studies that have led to successful clinical intervention. As a result, we have embarked on collaborations with Drs. Aguirre's and Miyadera's research groups to examine cellular mechanisms involving microglia in the pathophysiology of photoreceptor degeneration in canine models. We are also interested in

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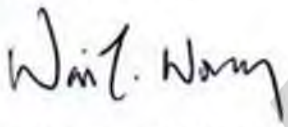
testing microglial modulation strategies found to be efficacious in rodent models in these canine models to provide justification for further translation into clinical studies.

This exciting collaboration is made possible by the animal facility at PENN, which is supported by the NIH grant EY-06855. The capabilities of this facility, as well as the experienced scientists that work there, constitute a unique and necessary resource to clinician-scientists as myself, who aim to translate significant findings found at the bench and in rodent models, to proof-of-concept clinical studies in humans. Verification, experimental scaling, and further biological discovery, employing canine degeneration models involving the larger dog eye will be instrumental in this translational step.

As we look forward to a productive and insightful collaboration with your research groups, I would like to express my enthusiasm for the continued support for the animal facility at PENN, as a vital resource to our work and the work of my fellow vision scientists.

With best wishes,



Wai T. Wong, MD PhD
Chief, Unit on Neuronal-Glia Interactions in Retinal Disease Senior Investigator
National Eye Institute
National Institutes of Health
wongw@nei.nih.gov

National Eye Institute, National Institutes of Health
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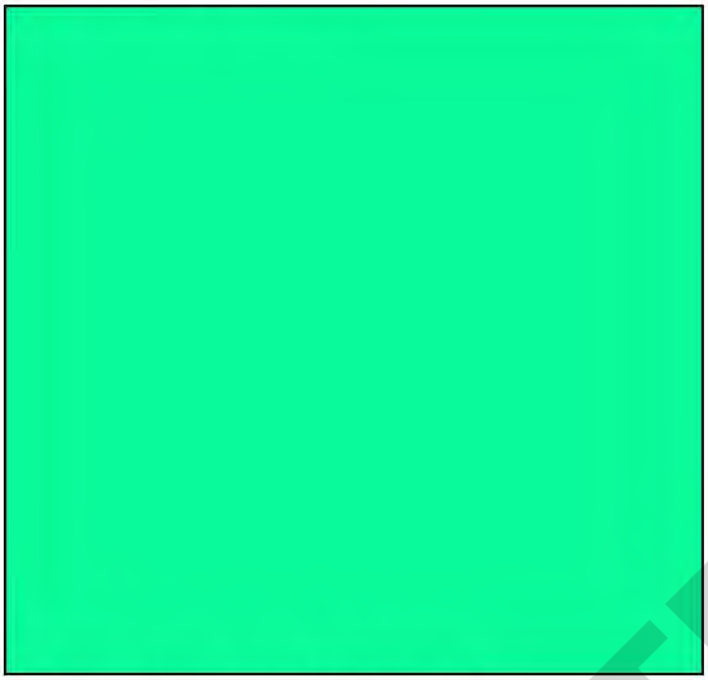


Gustavo Aguirre, VMD, PhD, PhD (*hc*)
Professor of Medical Genetics and Ophthalmology

Keiko Miyadera, DVM, PhD
Assistant Professor of Ophthalmology

School of Veterinary Medicine
University of Pennsylvania
3900 Delancey St. Ryan #2050
Philadelphia, PA 19104-6010

July 10, 2018

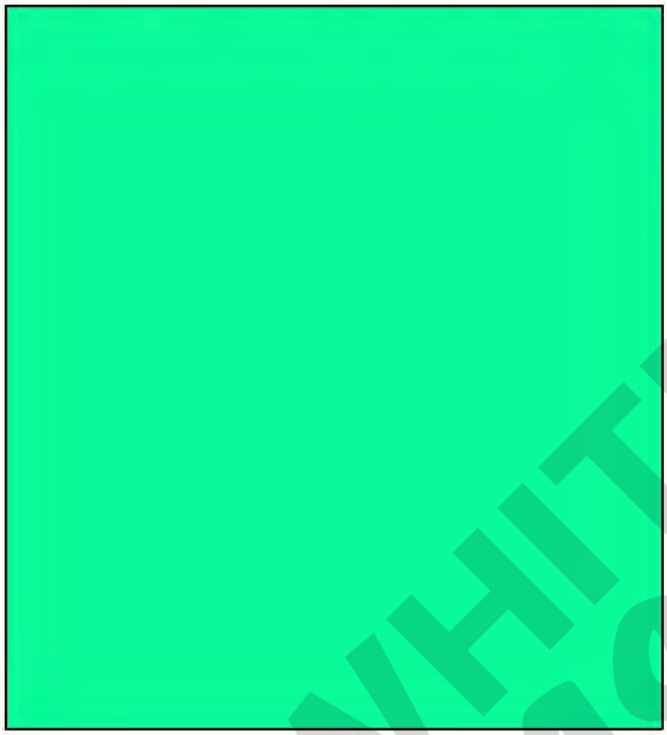
Re: Support for Grant EY-06855; Models for Therapy of Hereditary Retinal Degeneration

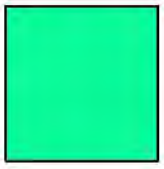
Dear Drs. Aguirre and Miyadera,

I am very excited to continue collaborating with Dr. William Beltran, a member of your group, studying the effect of neuroprotection using your canine model resources. As you know, I am investigating the anti-neogenin shRNA approach as a strategy to inhibit photoreceptor cell death. I would like to pursue this using your *rcd1* canine model by studying dogs produced in prospective matings as well as tissue samples of affected, carrier and normal dogs.

This work will be done through the canine model resource facility supported by your NEI/NIH grant (EY-06855; Models for Therapy of Hereditary Retinal Degenerations). The resources provided by the NIH-funded facility will be critical for accomplishing this work.

I wish you the best of luck with your application and hope that the reviewers will share my enthusiasm about this emerging research direction.





DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

National Institutes of Health
National Eye Institute
Bethesda, Maryland 20892

October 5, 2018

Gustavo Aguirre, VMD PhD
Professor of Medical Genetics and Ophthalmology
Keiko Miyadera, DVM PhD
Assistant Professor of Ophthalmology
School of Veterinary Medicine University of Pennsylvania
3900 Delancey St, Ryan Veterinary Hospital Philadelphia, PA 19104

Re: EY-06855; Models for Therapy of Hereditary Retinal Degenerations.

Dear Gus and Keiko:

This letter is in support of your competing renewal application for funding to study the naturally-occurring canine models of retinal degeneration at the University of Pennsylvania, Retinal Disease Studies Facility. Among our recent collaborative efforts is the investigation into the multigenic molecular basis of a form of cone-rod dystrophy in dogs. Your purpose-bred canine colony segregating the naturally-occurring retinal degeneration has created a unique opportunity to effectively isolate the different genetic contributors such as modifiers. We have previously assisted in your effort to generate retinal transcriptomics data from dogs representing the variable retinal phenotypes. The complex nature of the disease requires extensive bioinformatics and our lab has the resources and the expertise to help you further along the process. I believe that the identification of modifiers that play a role in disease expression could have an impact as therapeutic targets to potentially attenuate retinal disease phenotypes. The genes *RPGRIP1* and *MAP9* that have already been associated with this disease model are cilia genes with roles in photoreceptor integrity. The canine model could further inform on their critical role in ciliary structures and trafficking.
I look forward to continuing the collaboration with you in the areas of transcriptomics as well as others using your valuable canine retinal disease models.

Sincerely,

Swaroop, Anand (NIH/NEI) [E]
Digitally signed by Swaroop, Anand (NIH/NEI) [E]
Date: 2018.10.05 17:17:49-04'00'

Anand Swaroop, Ph.D.
Senior Investigator & Chief, NNRL/NEI

Schneeweis, David (NIH/NEI)
Digitally signed by Schneeweis, David (NIH/NEI)
Date: 2018.10.06 07:26:28 -04'00'

Sheldon Miller, Ph.D.
Scientific Director, NEI

Anand Swaroop, Ph.D., Senior Investigator and Chief,
Neurobiology Neurodegeneration and Repair Laboratory (N-NRL)
Bldg 6, Room 338, MSC 0610, 6 Center Drive, Bethesda, MD 20892-0610
Phone: 301-435-5754 Fax: 301-480-9917 Email: swaroopa@nei.nih.gov
http://www.nei.nih.gov/intramural/nnrl.asp

[redacted]

October 5, 2018

Gustavo Aguirre, VMD, PhD, PhD (hc)
Professor of Medical Genetics and Ophthalmology

Keiko Miyadera, DVM, PhD
Assistant Professor of Ophthalmology

Section of Ophthalmology School of Veterinary
Medicine University of Pennsylvania

Re: R01-EY08655 "Models for Therapy of Hereditary Retinal

Degenerations" Dear Gus and Keiko,

I am writing to support your competing renewal of R01-EY08655 "Models for Therapy of Hereditary Retinal Degenerations". Upon my visit to UPenn early this year, we discussed the values of my integrated approach to decipher regulatory networks in facilitating ocular gene discovery using your canine models. The global transcriptomics profiling made available by your canine resources brings challenges parsing through the large amounts of sequence and expression data. I am happy to collaborate with your group in providing my expertise in "in silico subtraction" using the tools such as iSyTE we have developed and other bioinformatics tools for large data analysis.

For example, in your studies searching for modifiers in the dog model of multigenic cone-rod dystrophy, I have provided initial bioinformatics assistance in extracting genes that are enriched in cone photoreceptors from a given canine chromosomal locus. I look forward to continuing our effort in studies of other valuable canine models supported by this grant.

[redacted]

[redacted]

October 15, 2018

Gustavo Aguirre, VMD PhD PhD (*hc*)
Professor of Medical Genetics and Ophthalmology

Keiko Miyadera, DVM PhD
Assistant Professor of Ophthalmology

School of Veterinary Medicine
University of Pennsylvania
3900 Delancey St, Ryan Veterinary Hospital
Philadelphia, PA 19104

Re: "Models for Therapy of Hereditary Retinal Degenerations" (EY-06855)

Dear Gus and Keiko

It is my pleasure to provide a letter of support for your competing renewal application of the NIH grant EY-06855 (Models for Therapy of Hereditary Retinal Degenerations)

[redacted] and I have developed a polymer based vector that has shown promising durability in ocular gene transfer. We have determined with a GFP expression construct that there is good GFP expression over a wide area of retina, RPE, and choroid after suprachoroidal injection Once we optimize the dose and treatment strategy using the S334ter-3 rat model of RP, it is critical to perform testing in a large eye animal model of RP. This portion of the work will be directed by you, Drs Gustavo Aguirre and Keiko Miyadera at the University of Pennsylvania, School of Veterinary Medicine. We will use the [redacted] carrying the therapeutic transgene or GFP as control. Scotopic and photopic ERGs will be done to test functional recovery with the therapeutic transgene, and autofluorescence images will be taken to assess expression of GFP.

We have applied for the [redacted] for this collaboration. If the early results of our collaboration provide promise,we plan to transition to a NIH grant to further expand this work with you. I wish you the best of luck with the competing renewal, and look forward to working with you

Sincerely

[redacted]

[redacted]

October 19, 2018

Gustavo Aguirre, VMD, PhD
Professor of Medical Genetics and Ophthalmology School of Veterinary Medicine
University of Pennsylvania
Ryan-VHUP,
Room 2050
3900 Delancey St.
Philadelphia, PA 19104-6010

Keiko Miyadera, DVM PhD
Assistant Professor of Ophthalmology

Re: EY-06855: Models for therapy of hereditary retinal degenerations

Dear Gus and Keiko:

I am delighted that you have been able to provide the canine animal models of hereditary retinal degeneration through the ongoing NIH-sponsored grant EY-06855 aiming at restoring vision in the model for the translation into patients.

As you know, our group has utilized your unique and outstanding canine resources for the past years in studies on the [redacted] potassium channel blockers (via our NEI grant, ("Photoswitchable channel blockers for treatment of blindness", Lead PI: Van Gelder. 5R24EY023937-05). These compounds are capable of conferring light- dependent firing to neurons. We have recently shown that these compounds can confer light responses to genetically blind mice. We are excited for the opportunity to continue to test these compounds in a larger animal model of hereditary retinal degeneration.

We look forward to continued work with your group on this most important

project. Sincerely,

[redacted]

Penn Medicine
Scheie Eye Institute

Department of Ophthalmology
Penn Dry Eye & Ocular Surface Center

Mina Massaro-Giordano, M.D.
Associate Professor,
Comprehensive Ophthalmology
Co-director,
Penn Dry Eye & Ocular Surface Center

Vatinee Y. Bunya, M.D.
Assistant Professor,
Cornea & External Disease
Co-director,
Penn Dry Eye & Ocular Surface Center

July 9, 2018

Re: Letter of Support for Dr. Gustavo Aguirre and Dr. Keiko Miyadera
Grant: R01 (EY-06855; Models for Therapy of Hereditary Retinal Degeneration)

Dear Gus and Keiko,

It is my pleasure to continue our collaboration with you and to support your dog facility. We are grateful for this collaboration that has allowed us to study novel topical medications for the treatment of dry eye disease that later could potentially be used in both canines and humans. Your dog facility is a critical and invaluable resource for the vision research community and for our continued work.

We remain enthusiastic about continuing to collaborate with you on testing new topical therapies for the treatment of dry eye disease.

I look forward to many more productive interactions with your research team in the future.

Sincerely,

Vatinee Y. Bunya, MD
Co-Director, Penn Dry Eye & Ocular Surface Center

51 North 39th Street | Philadelphia, PA 19104-4283 | 215.662.9791 | Fax: 215.243.4695

[redacted]

October 16, 2018

Gustavo Aguirre, DVM, PhD
Professor of Medical Genetics and Ophthalmology
Keiko Miyadera, DVM, PhD
Assistant Professor, Ophthalmology
Section of Ophthalmology
School of Veterinary Medicine
University of Pennsylvania
Ryan-VHUP, Room 2050
3900 Delancey St.
Philadelphia, PA 19104-6010

RE: EY-06855; Aguirre and Miyadera; Models for Therapy of Hereditary Retinal Degeneration.

Dear Gus and Keiko,

I am writing to affirm our enthusiasm and support for your project "EY-06855; Models for Therapy of Hereditary Retinal Degeneration" – a long term collaboration that I think has been immensely successful for all of us, and most importantly for vision research. Specifically we commit to providing you with resources and molecules developed in collaboration with my lab that you and I have shown to be efficacious in protection against loss of vision in various animal models of retinitis pigmentosa (RP). Most importantly, as described in brief below, these molecules match to several aims of your proposal in that the molecules were designed to protect mitochondrial homeostasis independent of the RP-related genomic mutations and, thus, the compounds could be well suited as adjuvants to gene-therapy approaches.

As you know, Dr. Beeson and I hypothesized that many of the mutations in widely disparate genes that cause RP might do so partly indirectly via induction of bioenergetics stress that would cause a gradual loss of mitochondrial homeostasis. The resulting mitochondrial dysfunction could lead to irreparable loss of photoreceptor function. We screened a large, chemically diverse library of molecules for protection of mitochondrial function in cell lines exposed to bioenergetics poisons and from the protective compounds we arrived at a single structural class that was then synthetically elaborated into more than 3000 analogues.

The molecules are formulated for topical delivery via eye-drops, and have been tested in RP models of mouse, and rat. More importantly, our collaborative studies have shown that the topically delivered compounds are safe, and efficacious in the rcd1 and RPE65 canine models of RP. The compounds have been shown to be very safe with no ocular toxicity over months, and they are stable to liver microsomes. The systemic exposure following eye-drop delivery is not detectable in serum above 0.5 ng/mL and it is well tolerated when delivered once to twice every day. Your observations that the dogs have no real aversion the eye-drops is a result that we take pride in.

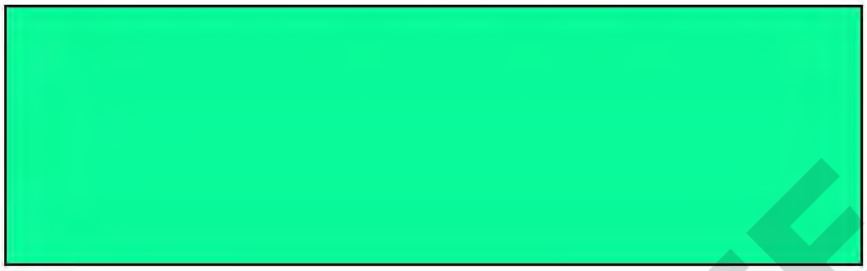
In vitro assays indicate that our compounds protect against mitochondrial stressors, and in those cases in which mitochondrial function is impaired, they improve function. Thus, we would anticipate that they can improve the function of retinal cells that have otherwise been stressed, even when nuclear genetic defects have been corrected and, thus, we anticipate synergy with your current excellent gene-therapy approach.

I am looking forward to our continued collaboration,

Best wishes,

[redacted]

d - Independent scientists that receive models or tissue samples.



[redacted]

Drs. Gustavo Aguirre and Keiko Miyandera
School of Veterinary Medicine | University of Pennsylvania
3900 Delancey St, Ryan-VHUP 2017 | Philadelphia, PA 19104 USA

Re: EY-06855 competing renewal

Dear Gus & Keiko,

I am very excited to support and collaborate with you on studying the role of "Progressive Rod Cone Degeneration" (PRCD) in retinal diseases in dogs. As you know, PRCD belongs to a very small group of proteins localized to photoreceptor discs membranes. There are several mutations in PRCD associated with "retinitis pigmentosa" in humans and dogs. Among these mutations, most common mutation in PRCD is C2Y identified in humans and multiple dog breeds. I would like to pursue this further by studying tissue samples of affected, carrier and normal dogs, as well as dogs produced in prospective matings. To understand the importance of PRCD, I would like to study using canine model resource facility supported by your NEI/NIH grant (EY-06855; Models for Therapy of Hereditary Retinal Degenerations).

As you know, I have already produced several mouse lines with targeted mutations in the *PRCD* gene; this work is supported by a newly funded grant (NEI/NIH 1R01EY028959-01). Having access to these samples (mouse lines) and canine models will be an excellent opportunity to study more in details as well as advancing our work.

I strongly believe that the resources provided by the NIH-funded facility will be critical for accomplishing this work. I wish you the best of luck with your application and hope that the reviewers will share my enthusiasm about this critical research direction.

[redacted]

[redacted]

October 19, 2018

Gustavo Aguirre, VMD, PhD, PhD (hc)
Professor of Medical Genetics and
Ophthalmology

Keiko Miyadera, DVM, PhD
Assistant Professor of Ophthalmology

Section of Ophthalmology
School of Veterinary
Medicine University of
Pennsylvania

Re: R01-EY08655 "Models for Therapy of Hereditary Retinal Degenerations"

Dear Gus and Keiko,

I am delighted to provide this letter in enthusiastic support of your competing renewal of R01 EY08655 "Models for Therapy of Hereditary Retinal Degenerations".

My lab investigates the molecular and cell biological bases of severe photoreceptor degenerative disorders, such as Retinitis Pigmentosa and Leber Congenital Amaurosis. We have identified key ciliary proteins that are involved in protein trafficking to the sensory cilium of photoreceptors. Among them are RPGR (Retinitis Pigmentosa GTPase Regulator) where naturally-occurring mutant canine models (xlpra1 and xlpra2) have been characterized, developed, and maintained by your group. The retina and other tissues from the *RPGR* mutant dogs provided by your facility have been particularly advantageous for my work in complementing the studies that use zebrafish, mouse and mouse embryonic fibroblasts model systems.

Further, as per our conversation at ARVO 2018, I am happy to work together with you in studying the protein-protein interaction of cilia proteins that have been associated with the *RPGRIP1* canine model of multigenic inherited retinal degeneration. We have generated and validated several reagents such as constructs encoding retinal ciliary proteins and antibodies against RPGR, RPGRIP1, CEP290 and their known interacting partners. In addition, protein-protein interaction and cilia growth and signaling assays that will be useful for validating functional interactions are routinely being carried out in my lab. I am happy to share our reagents and expertise with your group and look forward to continuing our effort in advancing the understanding of disease mechanisms by utilizing your valuable canine models.

[redacted]

[redacted]

October 22, 2018

Gustavo Aguirre, VMD, PhD, PhD (hc)
Professor of Medical Genetics and Ophthalmology

Keiko Miyadera, DVM, PhD
Assistant Professor of Ophthalmology

School of Veterinary Medicine
University of Pennsylvania
3900 Delancey St, Ryan-VHUP 2050
Philadelphia PA 19104

Re: R01-EY008655 "Models for Therapy of Hereditary Retinal Degenerations"

Dear Gus and Keiko,

It is my pleasure to provide this letter in enthusiastic support of your competing renewal of R01-EY008655 "Models for Therapy of Hereditary Retinal Degenerations". As you know, my lab is interested in understanding the basic mechanisms of cilia biogenesis and we have been characterizing several proteins, including NPHP5, whose dysfunction leads to retinal degeneration and vision loss. As most of our characterization studies are carried out on immortalized mammalian cell lines, it is becoming increasingly important to test our findings in a more physiologically relevant context. Your NPHP5 canine mutant is well described in the literature and provides an excellent resource for our studies. Since our collaboration started several years ago, we had performed a number of experiments using primary fibroblasts derived from dog skin biopsies you provided us. Recently, my lab has identified a molecular pathway involving NPHP5 and several proteins that is critical for cilia biogenesis, and I have written a grant proposal to study this pathway *in vivo* using your wild type and NPHP5 mutant dogs. Thus, the canine breeding colony you have developed and maintained at your research facility is an invaluable resource for us and many other researchers in the field. Without your support, we will not be able to advance our research. I appreciate you sharing your resources with us. I wish you all the best with your renewal and am looking forward to our continued collaboration.

Sincerely,

[redacted]

[redacted]

Gustavo Aguirre, VMD, PhD, PhD (hc)
Professor of Medical Genetics and Ophthalmology

Keiko Miyadera, DVM, PhD
Assistant Professor of Ophthalmology

School of Veterinary Medicine
University of Pennsylvania
3900 Delancey St, Ryan-VHUP 2050
Philadelphia PA 19104
October 23, 2018

Re: R01-EY008655 "Models for Therapy of Hereditary Retinal Degenerations"

Dear Gus and Keiko,

It is my pleasure to provide this letter in enthusiastic support of your competing renewal of R01-EY008655 "Models for Therapy of Hereditary Retinal Degenerations". Your research program using large animals is essential to the scientific community. The resource you provide to the vision community is an invaluable and necessary bridge to bring therapeutics from bench to bedside. You have created and maintained a tremendous resource that moves our research from theoretical discovery science to substantiate the development of clinically relevant therapies. Your genetic models that recapitulate human disease have been used by many in the vision community, and proposed in several of our grant applications to determine the safety of the therapeutic, the effectiveness of delivery mechanisms, distribution, and rescue of retinal disease. While we use rodent models to identify and develop our therapies, your work is an essential next step to examining critical components of the therapy that will be more relevant studied in a higher mammal such as dog than in the rodent. I echo the sentiments of the vision community that we appreciate your commitment and diligence in being an outstanding collaborator for many of us to help substantiate our studies and bring our therapies further along the translational pipeline.

I wish you the best of luck in your grant application. Your research program is an essential part of our vision community's and NIH's mission to develop therapies to treat vision loss.

[redacted]

Resource Sharing Plan(s)

1. Data Sharing Plan:

All project related data will be presented at scientific meetings and published in peer reviewed scientific journals as expeditiously as possible. The extensive track record of the PIs and associates in this area is well documented by their collaborations and publication output over many years. Because the EY006855 project acts as a resource for multiple independently funded investigators, this condition is made a prerequisite for such collaborations. The extensive track record of this project in this arena is well documented in the publication output of the past 40+ years, and briefly described in the *Significance* and *Progress Report* sections of the Research Strategy. The *Progress Report Publication List* includes all manuscripts and abstracts published since the last competitive review.

We have consulted with the IT Department at the School of Veterinary Medicine, University of Pennsylvania (UPenn) about setting up a "private" web resource for use of the investigators to deposit primary research data, as well as a "public" resource in which we can place selected study results that can be available to the general public. The IT Department strongly recommended against both web resources for the following reasons. In regards to the "private" web resource, UPenn can not guarantee the privacy of such a resource as there have been multiple situations where such 'secure' sites have been hacked into from the outside. As for a "public" web resource, the IT Department as well as other offices at UPenn emphasized that publicizing the use of dogs in this type of research would bring it to the attention of PETA and other animal rights groups, and compromise the security of the RDS Facility. In fact, on the instruction of the NIH grants office many years ago, the title of the project was changed from 'Canine Models for Therapy of Hereditary Retinal Degeneration' to 'Models for Therapy of Hereditary Retinal Degeneration'. Given that all abstracts and published manuscripts are readily accessible through web resources, we believe that such an approach is preferable as it would not compromise the security of the research facility from PETA and other animal rights groups.

2. Sharing Model Organisms:

We follow all rules and regulations set forth in NIH guidelines regarding the sharing of model organisms. The EY006855 project shares the specific canine mutant models maintained in this project as broadly, practicable and as effectively as possible with the community of research scientists investigating potential therapies for human retinal diseases. The extent of such sharing is only restricted by the finite limits of the resource itself. The PIs actively seek out and respond to requests for access to these model animals from potential collaborative and independent investigators, nationally and internationally, from academic institutions and industry (see Research Strategy, and *Collaboration or service?* and *Advertising the resource* sections. The extensive track record of this project in this arena is well documented in the publication output of the past 40+ years. Since the start of the current project in December 1, 2014 to the present, we have distributed dogs or tissues, or made planned breedings for investigators in industry (3 times to 2 different companies) and academia (36 times to 21 different investigators); these are listed at the end of the *Resource Sharing Plan(s)*. The number of academic investigators noted above **excludes** those associated with the PIs and associates (Drs. Beltran, Guziewicz) or collaborators at UPenn (Drs. Jacobson, Cideciyan) or Univ. of Florida (Drs. Hauswirth, Lewin). However, under the resource sharing information provided, these investigators are listed to give a complete picture of the animals used and the projects carried out.

The canine mutant models are made available to research investigators for either collaborative or independent studies. The decision as to whether to use these models in a collaborative program with the PI's of grant EY00685, or as a completely independent study, is entirely up to the research investigator making the application, but will often be influenced by several practical concerns.

Distribution of canine mutant models to independent investigators

A critical issue is whether the investigator requires delivery of live dogs, or requests tissue or other samples. In the latter case, the request can be fulfilled more expeditiously, depending on specific aspects of the investigators protocol. In particular, as this form of distribution is broadly covered by preexisting IACUC protocols at UPenn, such a request can be fulfilled with either a minor, or no amendment to such preexisting protocols.

The investigator is, however, required to provide a detailed protocol for such sample collection and delivery, completely specifying the samples requested by model, sample source (e.g. retina, blood, fixed or frozen eyes or other tissues), genotype, age, gender, method of sample collection, preparation and shipment, etc. The PIs of EY006855 will consult with such investigators to advise on, and help with,

preparation of an optimized protocol. Projected delivery schedules will be discussed with each research investigator applying; these schedules will be highly dependent on the details of the investigator's protocol, and the model availability. Requests that can be fulfilled from previously collected tissues (e.g. from frozen ocular or other tissues) can and will be fulfilled most readily.

Requests that require unique collection protocols, or collection at specific (especially advanced) ages, may take longer to fulfill. Such issues will be fully discussed with the applicant research investigator. If the request specifies samples from larger numbers of dogs, or from dogs at advanced ages, then a projected schedule for such deliveries will be discussed in consultation with the investigator. Investigators are advised that such schedules are, to a large extent, determined by the limitations of the canine colony size, and the natural length of the canine reproductive cycle, and thus require significant forward planning. To facilitate studies on older aged animals, e.g. for subretinal or epiretinal chip implantation which requires older animals with advanced retinal degeneration, we encourage the investigators to take the dogs at the youngest age possible in order to not occupy kennel space useful for other shorter-term projects, or to take older animals once they are retired from the breeding program.

If the investigator requests delivery of live dogs, then several additional issues need to be addressed. In consultation with the IACUC at UPenn, and as previously discussed with Program Officers at NEI/NIH, certain minimum requirements have been established. A formal request must be submitted, including a complete copy of the investigator's IACUC protocol application at the investigator's institution, and a copy of the approval letter by the investigator's IACUC. This must specify that the investigator is approved to receive dogs from the EY006855 supported colony at the RDSF, and define the number, age, gender and strain(s) approved. A letter from the Department or Office of Laboratory Animal Medicine (or equivalent) at the investigator's institution must also accompany such application, stating that the institution is prepared to receive, house, and care for the number of dogs applied for.

Such formal request has to be approved by IACUC at UPenn. This can usually be achieved by a minor amendment to our preexisting IACUC protocols. However, in certain cases it is often required by The UPenn IACUC that the applying investigator has demonstrated that the proposed study using the canine mutant models is supported by preliminary studies in either rodent models, or non-mutant dogs, or both, to establish baseline evidence of safety and or efficacy, thus justifying the use of these canine mutant models, and the numbers thereof. These are requirements mandated by Federal regulations, implemented by the institutional IACUC committee, and out of our immediate control.

The PIs will consult with investigators making such a request to advise and assist with preparation of such application, and define projected delivery schedules and potential costs. In general, if small numbers of young dogs are requested, then delivery F.O.B. can be expected within a few to several months following approval of the formal request; this is influenced by the longer gestation and breeding cycle in dogs in comparison to smaller rodents. Although applying investigators will be responsible for arranging shipment of dogs from [redacted] to their institution, and for the associated costs, the PIs of EY006855 and staff at [redacted] will assist with such arrangements. If larger numbers of dogs, or dogs at advanced ages are requested, then delivery will be contingent upon breeding schedules in the [redacted] colony, and dog availability. If, to fulfill such a request, specific breedings are required or dogs need to be held at [redacted] for prolonged periods post weaning, then a more extended delivery schedule will result. In some cases, investigators have requested adult animals for non-invasive and non-terminal procedures (e.g. nystagmography studies where topical medications were evaluated to control or reverse nystagmus). In such cases, dogs are transferred from the [redacted] to the investigator's institution, and returned when the studies are completed.

Please **note** that a request to provide dogs or tissue samples, or to perform a purely technical function (e.g. enucleating eyes, injecting eyes with drugs, etc), is considered a service function, and not done on a collaborative basis, and under no circumstances is authorship expected.

Availability of canine mutant models for collaborative investigations.

As an alternative to providing the canine mutant models directly to investigators for independent research programs, the possibility of undertaking collaborative studies is also available. This can be more practical and desirable for research investigators when their laboratory or institution is not experienced with or equipped to undertake research studies using dogs, or when those investigators have mutual interest in the research study. For example, an investigator interested in testing a novel means of therapy in canine mutant models may not have in-house ability to undertake certain studies for evaluation of therapeutic results in dogs. Such studies might include, as examples, electroretinography or visual testing, both of which the PIs of EY006855 have available at RDSF. Similarly, the applying investigator might prefer to have the

post therapeutic clinical course of the dog's health observed by animal caretakers, and by board certified veterinary ophthalmologists, who are particularly experienced with these dogs. If the work involves purely a service, and the PIs of EY006855 do not have an active, scientifically justifiable involvement in the project, the work can be done at the RDS facility purely as a service without any expectation of authorship. However, in true collaborative studies where the PIs or associates are actively involved in all aspects of the research, i.e. from conception, design, execution and data analysis, and/or writing the relevant parts of the paper, and reviewing the finished manuscript prior to submission, then authorship in the resulting publication appropriately reflects that involvement. These functions meet or exceed the generally accepted criteria for authorship ('must be limited to those who have contributed substantially to the work').

The PIs of EY006855 and staff will discuss this option with research investigators interested in undertaking studies utilizing the canine mutant models, and assist with preparation of a fully specified protocol for such studies. The protocol will then be submitted to IACUC at UPenn as an amendment to preexisting protocols, or as a new protocol application. As described above, it is often required, by IACUC, that the applying investigator has demonstrated that the proposed study using the canine mutant models is supported by preliminary studies in either rodent models, or non-mutant dogs, or both, to establish baseline evidence of safety and or efficacy, thus justifying the use of these canine mutant models, and the numbers thereof.

3. Genomic Data Sharing (GDS)

The PIs and University will share genomic and genotype data from canine models affected with retinal diseases as well as their controls by depositing these data in GenBank (repository funded by National Center for Biotechnology Information). In addition, whole genome sequencing data from dogs will be archived at the Dog Biomedical Variant Database Consortium (University of Bern, Zurich) and will be made available to participating geneticists. The genomic and genotype data will be made publicly available no later than the date of initial publication or six months after the receipt of final sequencing data, whichever comes first.

Investigators receiving animal or tissue resources since March 5, 2014

As EY006855 serves many different projects and functions, the term "animals that were distributed" has one of several meanings, and this is specifically based on the investigator's preference, and our ability to fulfill the request in a timely manner. Among these includes dogs that were physically transferred to investigators at other sites, dogs that were euthanatized at the facility and tissues were collected and transferred to other investigators for specific studies, studies done on the dogs at the facility for other investigators, and the tissues were provided at the end of study. Another category that has been available, but infrequently used, is the temporary transfer of dogs from the facility to another investigator for survival procedures that are non-invasive. Once completed, the dogs are "returned" to the facility for either breeding or other studies. Lastly, when an investigator requires dogs of a specific genotype, we assign a group of female animals specifically to produce the requested number of dogs; in such a situation, we consider these temporarily assigned dogs as being "distributed" as they are not available for other studies.

I. DISTRIBUTION OF TISSUES/ORGANS FOR OCULAR STUDIES:

2014 and 2015: For these years, this report covers two time periods: **a)** March 5, 2014 (last Progress Report provided at the time the competing renewal to EY006855 was submitted - November 30, 2014, **b)** December 1, 2014 - November 30, 2015 (which is the first year after the competing renewal was approved and funded)

MARCH 5, 2014-NOVEMBER 30, 2014:

[redacted]

DECEMBER 1, 2014-NOVEMBER 30, 2015:

[redacted]

[redacted]

DECEMBER 1, 2015-NOVEMBER 30, 2016:

[redacted]

[redacted]

DECEMBER 1, 2016-NOVEMBER 30, 2017:

[redacted]

[redacted]

DECEMBER 1, 2017-PRESENT:

[redacted]

Dogs produced for specific investigators but declined:

II. DISTRIBUTION OF TISSUES/ORGANS FOR NON-OCULAR STUDIES:

In an effort to maximize the utilization of as many research dogs as possible, the University of Pennsylvania IACUC has promoted the distribution of tissues and other organs to investigators who need canine tissues for non-vision related studies. These tissues are distributed after the dogs are euthanatized for the specific studies covered by EY006855 as well as other NIH funded projects. We have made investigators at other institutions aware of this resource and will be able to provide these tissues upon request. Details on this effort are presented below:

Table 1. F[redacted]Census: Core Breeders, Study and Potential Breeders, and Potential Replacement Breeders October 2017 - September 30, 2018

Strain	Mutant Gene	Core Breeders	Study dogs/ potential breeders	Potential Replacement Breeders	Comments on future plans
Achromatopsia*	CNGB3	2	3		To expand slightly
Cea*	NHEJ1	0			Frozen semen stock
Cngb3/rcd1	CNGB3/PDE6B	2			In development to produce double homozygous dogs
Cngb3/rpe65*	CNGB3/RPE65	1			To expand slightly
Cnga3*	CNGA3	2	2		To expand slightly
Cmr*	BEST1	9	14	3	
crd1*	PDE6B	0			Frozen semen stock
crd1/rcd1*	$PDE6B^{2}/PDE6B^{1}$	0			Frozen semen stock
crd1/crd2	$PDE6B^{2}$/NPHP5	3	2		In development to produce double homozygous dogs
crd2*	NPHP5	8	12		Stable
crd3*	ADAM9	0			Frozen semen stock
drd1*	COL9A2	0			Frozen semen stock
drd2*	COL9A3	1			Decrease to frozen semen stock
CSNB	LRIT	4	12		Stable
Erd*	STK38L	4	1		Stable
Foveo-macular degeneration	In progress	2			To expand
IG-PRA*	DFNB31 or AKNO	0			Frozen semen stock
Normal*	None/NA	10	6	4	Stable
Prcd*	PRCD	1			To expand
Prcd/crd2	PRCD/NPHP5	2			To expand
rcd1*	PDE6B	10	4		Stable
rcd2*	RD3	4			To expand slightly
rcd1/crd2	$PDE6B^{1}$/NPHP5	4	6		Stable
rpe65*	RPE65	2	3		Stable/To expand slightly
Rpe65/erd	RPE65/STK38L	1			To expand slightly
rpe65/T4R*	RPE65/RHO	0			Frozen semen stock
RPGRIP	RPGRIP1	9	5		Stable
Stargardt*	ABCA4	0			To expand
T4R*	RHO	7	15	25	Stable
Xlpra*	RPGROrf15	8	12		Stable
PWD*	CCDC66	2			To expand
Total =		**94**	**97**	**32**	

* These models have had semen frozen, and can be developed and distributed upon request, or stored semen is being used in order to develop a study colony (e.g. Foveo-macular degeneration and Stargardt disease).
Note: some crosses, e.g. Cngb3/rpe65, Cngb3/rcd1, crd1/crd2, rcd1/crd2, are aimed at producing dogs with severe and complete phototransduction defects that can be used for optochemical and optogenetic studies, or to dissociate the RPE visual cycle from the *RHO* mutation (rpe65/T4R). Other crosses, e.g. Rpe65/erd,

Prcd/crd2, are made to increase the genetic diversity of the erd and PRCD lines, and increase reproductive performance. The progeny then will be backcrossed to the parental lines.

Authentication of Key Biological and/or Chemical Resources

Antibodies: The antibodies we will use in this proposal are either commercially available antibodies purchased from established manufacturer or custom antibodies that were kind gifts obtained from other investigators.

[redacted]

manufacturer will authenticate these antibodies and detailed specification sheets will be provided upon receipt or available online. Examples of custom antibodies include anti-canine RPGRIP1 antibodies (C- and N-terminal epitopes) which were gifts from [redacted] We have affinity-purified these antibodies to increase specificity and reliability. For all types of antibodies, upon receipt and when we use them after a long period of storage (>6 months), we will authenticate the antibodies by Western blot using pooled control canine retinal lysates or COS1 cell lysates overexpressing the corresponding gene product.

Cell lines: The use of cell lines is not anticipated in this proposal. However, if they are used in cases for antibody verification as described above, we will use COS-1 monkey kidney cell lines for overexpression of proteins of interest. We will confirm that the cells are free of mycoplasma contaminations and will continuously monitor every 10th passages or 12-month of use, whichever comes first. The cells will be purchased from ATCC who will have authenticated the cells and provided specification sheets upon receipt.

Plasmids: Cloning plasmids for AAV vector construction will be purchased from commercial suppliers (Addgene). The manufacturer will authenticate them and detailed specification sheets will be provided upon receipt. We will authenticate cDNA constructs and expression plasmids to be generated in this proposal by direct sequencing. In subsequent amplifications, we will verify the identity of each construct by appropriate restriction enzyme digest, followed by gel electrophoresis, or direct DNA sequencing.

[redacted]