

Giving eyesight to the blind

Penn Vet ophthalmologists' work in the lab restores vision to dogs and provides hope to human patients

BY KELLY STRATTON

You may remember a dog named Lancelot. In 2001, Dr. Gustavo D. Aguirre, professor of medical genetics and ophthalmology at Penn Vet, restored vision to the dog, who was born blind – and they both made national headlines.

Their next stop? Washington, DC.

“Because of publicity following Lancelot’s recovery of vision, he was invited to Capitol Hill to meet with members of Congress,” said Dr. Aguirre. “During the meeting, Lancelot laid down near the podium and *looked* carefully at all the members of the audience who asked questions. Unlike dogs that are blind but can hear, Lancelot fixed his stare on the people who asked questions.”

Dr. Aguirre’s goal in traveling to Washington with Lancelot was to get people to understand and appreciate the power of and need to continue to develop new therapy technologies for treating inherited blindness.

Their sojourn to Washington was a success and Dr. Aguirre’s point was made.

“They all wanted to adopt Lancelot,” said Dr. Aguirre, “so to me that meant they connected with the idea that this kind of work is important.”

Serving as lead researcher of the project at the time, Dr. Aguirre’s work began with Lancelot and a team that included researchers at Penn’s Scheie Eye Institute and University of Florida. Their goal was to restore vision to Lancelot and his littermates that were mixed-breed dogs of Briard origin. This is the first time that gene therapy was used to successfully restore vision to any living creature.

By injecting a genetically engineered virus carrying a healthy copy of the defective gene into the part of the dog’s retina that contained the retinal pigment epithelium, the cell

layer that is essential for the proper functioning of the light-sensing cells, Dr. Aguirre and his team were able to “infect” those cells with normal gene material. Within several weeks, cells with the healthy copy of the gene began to produce vitamin A in the proper form needed by the retina that, in combination with opsin, formed the visual pigment rhodopsin, allowing Lancelot to see.

This advance in veterinary medicine offers hope for curing a similar disease that blinds children at birth or in early childhood – Leber’s congenital amaurosis (LCA), an inherited retinal disease. And, because of the dog’s successful treatment, human clinical trials began in 2007 at several centers worldwide, including the University College of London, the Scheie Eye Institute and Children’s Hospital of Philadelphia.

But Dr. Aguirre’s and his colleagues’ passion for restoring sight to the blind using gene therapy didn’t stop with Lancelot.

A SECOND MAJOR BREAKTHROUGH

Working hand-in-hand with researchers at Temple University, University of Florida and Cornell University, Dr. András M. Komáromy, assistant professor of ophthalmology – one of Dr. Aguirre’s colleagues – has made another major breakthrough in vision recovery with developing a gene therapy to treat achromatopsia in dogs.

Otherwise known as day blindness, rod monochromacy or total color blindness, achromatopsia affects the cones in the eye and makes daytime vision impossible for affected dogs.



Lancelot on Capitol Hill

More Work in the Lab

Day blindness is rare in humans, affecting approximately one out of every 30,000 to 50,000, but because it affects the cones in the eye, Dr. Komáromy, lead author on the study, says it's a perfect model to study other diseases affecting the cones, like macular degeneration in humans. Cones, responsible for day and color vision, are essential for central visual acuity and most daily visual activities.

The gene therapy developed by Dr. Komáromy is delivered directly to the cone cells in the retina and targets mutations of the CNGB3 gene – a mutation discovered by Dr. Aguirre's research group several years ago. The CNGB3 gene codes for an ion channel that is crucial for normal cone function.

"We administer the virus carrying a normal copy of the CNGB3 gene underneath the retina," said Dr. Komáromy. "The treated part of the retina is temporarily detached following the injection. The eye is an enclosed organ so you can inject high doses exactly where you need it."

"It's the same gene responsible for 50 – 80 percent of human achromatopsia," said Dr. Aguirre. "This work can help researchers understand how to fight age-related macular degeneration in people, which is the leading cause of blindness in people over 60."

"Dogs don't have a well developed macula as in humans," said Dr. Komáromy, "but because achromatopsia is a model cone disease, it allows us to answer questions for human medicine and consider important factors like age and allows us to follow up over a long period of time."

Dr. Komáromy's treatment cured younger canines regardless of the mutation that caused their achromatopsia and was effective for the 33-month span of the study. The successful restoration of cone function was documented through a technique called electroretinography. Researchers also measured the dogs' ability to negotiate a short obstacle course in daylight.

"I remember the moment very well during the summer of 2006 when I recorded the recovered cone function for the first time by electroretinography," said Dr. Komáromy. "I became very excited, but I repeated the recordings multiple times and kept replacing the electrode wires to make sure that I was not dealing with a technical artifact...the cone signal persisted!"

While younger dogs had tremendous success with this kind of gene therapy, dogs older than 54 weeks of age had less success.

"Because of the success in younger dogs, it makes sense to keep going," said Dr. Komáromy. "We want to develop a treatment for older dogs."

The results represent the second successful cone-directed gene replacement therapy in achromatopsia animal models and the first outside of mouse models.

The work done by Drs. Komáromy and Aguirre holds promise for future human clinical trials of cone-directed gene therapy in achromatopsia and other cone-specific disorders. It holds great promise to treat dog-specific diseases like the Progressive Rod-Cone Degeneration (PRCD), a hereditary retinal disease that leads to blindness in dogs between the ages of three to seven years, and is the most common cause of inherited canine blindness. ■



Penn Vet's ophthalmologists' work in gene therapy has provided fundamental understanding of the multiple and specialized roles played by photoreceptors and the retinal pigment epithelium in vision, and how mutations in these genes result in disease.

This information, combined with development of gene transfer technologies, suggests that once-untreatable disorders may now be treatable with use of gene therapy.

One area of interest going forward for Dr. Aguirre is examining canine Progressive Rod-Cone Degeneration (PRCD), a hereditary retinal disease that leads to blindness in dogs between the ages of three to seven years.

"One of our major goals is to do something for dogs for dogs' sake," said Dr. Aguirre. "PRCD is the biggest cause of blindness that affects the retina, but it is rare in humans so we are hoping to get support to continue our work in this disease that is dog-specific."

In addition, Dr. William Beltran, assistant professor of ophthalmology and colleague of Drs. Aguirre and Komáromy, is busy working to develop new gene therapy approaches for two common forms of retinal degeneration in human patients: X-linked retinitis pigmentosa (XLRP) caused by mutations in the RPGR gene (RPGR-XLRP), and autosomal dominant retinitis pigmentosa (RHO-ADRP) due to Rhodopsin mutations.

RPGR-XLRP and RHO-ADRP include some of the most severe forms of retinal degeneration and together account for more than 18 percent of all RP cases in the US. RP is a group of genetic eye conditions that leads to progressive, incurable blindness in man and is the human counterpart of the group of dog diseases termed "progressive retinal atrophy" (PRA).

Dr. Beltran is testing in canine models that carry mutations in those same genes, whether corrective gene therapy prevents loss of rods (and cones) and rescues visual function. In collaboration with investigators at Penn's Scheie Eye Institute and the University of Florida, he has recently identified which viral vectors can carry the therapeutic genes to the appropriate photoreceptor cells of the canine retina.

As it is suspected that some mutations in the RPGR and RHO genes may cause the expression of a defective and toxic protein, his lab is now investigating whether gene augmentation (i.e., providing copies of the normal gene) is sufficient to protect rods and cones, or whether silencing the mutant gene and replacing it with the wild-type gene, will be necessary.

From left to right: Drs. András M. Komáromy, Gustavo Aguirre, William Beltran