Team takes first step toward macular dystrophy gene therapy

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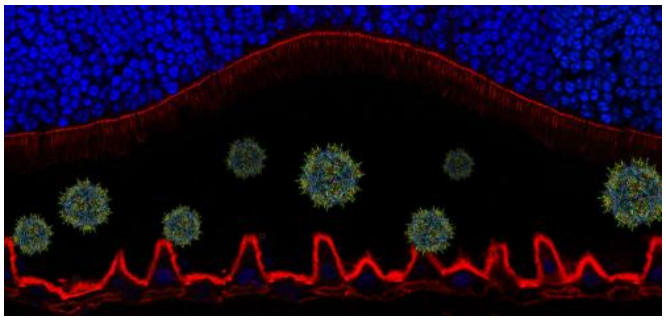

A photomicrograph collage shows the subretinal space of a vision-impaired dog with a gene therapy vector delivering a "healthy" copy of the affected gene. Credit: Confocal photomicrograph: Karina E. Guziewicz, Art: Mary Leonard

Vitelliform macular dystrophy, also known as Best disease, is one of a group of vision-robbing conditions called bestrophinopathies that affect children and young adults. Caused by inherited mutations in the BEST1 gene, these diseases cause severe declines in central vision as patients age.

With a new study, University of Pennsylvania researchers report "encouraging" findings that mark the first clear step in developing a gene therapy that could prevent vision loss or event restore vision in individuals suffering from these conditions.

The research, conducted in dogs, which can naturally develop a disease similar to Best disease, was led by Karina E. Guziewicz and Gustavo D. Aguirre of Penn's School of Veterinary Medicine. Penn Vet collaborators included Barbara Zangerl, Andras M. Komaromy, Simone Iwabe and William A. Beltran. The Penn team worked with University of Florida investigators Vincent A. Chiodo, Sanford L. Boye and William W. Hauswirth. They reported their findings in the journal *PLOS ONE*.

"Step one in designing a gene therapy for these conditions is to make sure that we can target the cells that are affected," Aguirre said. "That's what our study has done."

Aguirre's lab has previously designed successful therapeutics for other forms of blindness that strike both dogs and humans, including retinitis pigmentosa, Leber congenital amaurosis and achromatopsia.

The "dog version" of Best disease is called canine multifocal retinopathy (CMR), and shares many of the hallmarks of the human condition. Knowing that Best disease and CMR, like these other vision problems, is a heritable condition attributable to mutations in a single gene, the Penn Vet researchers sought to deliver a healthy copy of the BEST1 gene to a portion of retina to substitute for the malfunctioning copy.

As in previous work in Aguirre's lab, the scientists performed this delivery using a vector—a harmless virus genetically modified to carry specific genetic material. The cargo included either the human or canine version of BEST1.

The researchers injected the vector beneath 18 retinas in 12 dogs, all of which either had normal copies of BEST1 or had one normal copy and one mutated copy. To make sure the injected gene was going to the correct location in the retina, they tagged the vector with green fluorescent protein, which would "light up" where the healthy gene was introduced. They tested two different vectors, called rAAV2/1 and rAAV2/2, both of which are under consideration for use in human clinical trials for other types of vision-related gene therapy.

Injecting the vector under the retina, the researchers then tracked expression of the protein up to six months. They found that expression

peaked four to six weeks after injection, and remained stable for six months—a sign that the therapy would be lasting.

The researchers were surprised, however, to see that dogs that received injections from vector rAAV2/1 had some "funny green cells," according to Aguirre. When they investigated further, they found what appeared to be damage to cone cells, which give color vision and central visual acuity, as a result of the treatment.

"It was a serendipitous finding, because when we looked at the retina in sections it looked perfectly normal," Aguirre said. "But when we looked at these green cells we found that all the cones were dead. If this therapy were given to the entire retina, it could have significant implications for vision impairment, the ability to see colors and function in bright light."

The result raises questions about the potential usefulness of rAAV2/1 in future therapies.

"One of the goals of our study was to compare the two vectors," Guziewicz said. "We now see that the rAAV2/2 vector is the clear candidate for gene therapy, and can let other researchers know that rAAV2/1 needs further evaluation if it is going to be considered."

Both the human and canine versions of BEST1 behaved the same way, offering encouragement that such an approach could translate to humans.

Though Best disease is relatively uncommon, affecting approximately one in 10,000 people, the approaches being refined to treat it by Aguirre and Guziewicz team could be extended to still other macular diseases.

"Some of the hallmarks of disease are shared," said Guziewicz "If this works, we could apply our platform to benefit other types of macular degeneration that affects the retinal pigment epithelial cells."

"Our preliminary work leaves us feeling very optimistic," Aguirre said.

Provided by University of Pennsylvania