



# Early results show promise for a potential retinoschisis therapy

American researchers have demonstrated successful use of gene-based medicine in an animal model of retinoschisis.

The research team, led by Dr Seok Min at the Department of Ophthalmology at the University of Florida in Gainesville, published the group's results in the journal *Molecular Therapy*.

The blinding disorder researched by the Florida team is a form of juvenile macular degeneration known as "X-linked juvenile retinoschisis" or "RS" for short. The disease is a recessive disorder of the central retina arising from a mutation in the RSL1 gene which resides on the X chromosome. The mutation in the gene gives rise to a schisis or splitting of the retina leading to functional degeneration of the photoreceptor cells. Because males have only one X chromosome, any detrimental mutation arising within a gene on the X chromosome may result in a disease.

Contrastingly, females with two X chromosomes are often heterozygous carriers of recessive X-linked traits; in most circumstances, they do not demonstrate any clinical abnormalities. Consequently, X-linked juvenile retinoschisis almost invariably manifests itself in males rather than females.

**"The disease is often detected in boys between the ages of five and ten years who have reading difficulties at school."**

The disorder is rare, with estimates of worldwide prevalence ranging from between 1 in 5,000 to 1 in 25,000. According to the researchers, the disease is often detected in boys between the ages of five and ten years who have reading difficulties at school.

## Gradual Blindness

Clinically, retinoschisis is characterised by a star-shaped or spoke-wheel like maculopathy, parafoveal intraretinal cysts, and an electroretinographic hallmark of a healthy rod a-wave but reduced b-wave with bright stimuli. Visual acuity is generally between 20/25 and 20/80 in teenagers and young adults but declines further to 20/200

during the sixth decade or so. This decline is mostly due to a secondary atrophy of the retinal pigment epithelium in the macula.

In its later stages, vision may be completely lost as a result of additional complications such as vitreoretinopathy, detached retina, or sclerosis of the choroid. Although studies have demonstrated a consistent reduced rod b-wave, patients do not usually report any incidence of night blindness and dark-adapted visual thresholds are close to normal.

The gene at fault, "RSL1," codes for a protein that is believed to be secreted from cone photoreceptors, rod photoreceptors, and bipolar cells in the retina; the gene's main function is in maintaining retinal cell layer organisation and synaptic structure.

RSL1 in normally healthy retinas is thought to act as the "glue" that assists in maintaining the membrane structure created from a diverse population of highly specialised cells. When this "glue" fails to function correctly, splitting or schisis of the delicate retinal membrane can occur.

In common with a large number of retinal degenerative disorders, there are currently no treatments available for retinoschisis.

## Gene therapy experiment

In order to develop a therapeutic strategy to correct this molecular defect, the Florida research team first generated a so-called "knockout" mouse model of the human disease by deleting the mouse equivalent of RSL1, known as "Rsl1h."

This Rsl1h deficient mouse shares a number of diagnostic features with human RS, including the negative ERG response, development of cystic structures within the inner retina, and the loss of photoreceptor cells, according to the researchers. Given the commonality of the genes, the researchers noted that the Rsl1h mouse is a "valuable model for developing potential therapeutic interventions for human RS."

To test one such therapeutic approach, a correct copy of the Rsl1h gene that contained no errors and that was coded for the correct functioning protein, was genetically engineered into a specialised vector. This vector, known as adeno-associated virus or AAV, is a naturally occurring virus that was modified in the laboratory to take advantage of its ability to efficiently "transduce" mammalian cells and tissues. The AAV was modified in a number of key functions, most important of which was the insertion of healthy Rsl1h gene for delivery to the photoreceptor cells of the mouse retina.

Mice aged 15 days received an injection of the therapy into the right eye and no

injection into the left eye. The researchers injected the therapy with a blunt 33-gauge needle through a corneal hole. Each injection contained approximately 1 microlitre of vector or about 4 X 10<sup>12</sup> AAV vectors with the correct copy of the Rsl1h gene.

**"Treated eyes showed a distinct separation of inner and outer nuclear layers similar to that observed in wild-type animals, an absence of gaps between bipolar cells, and an increase in the thickness of the outer nuclear layer."**

The researchers then compared the right eye, which had received the treatment, to the left eye of the same animal which had received no injection. In the first instance, the team was able to show that the RS protein was being made in the right eye but not in the left eye indicating that the gene had been delivered efficiently and was actively producing the required protein.

## Treated eyes show normal retinal structure

Analysis of both eyes six months after injections using a scanning laser ophthalmoscope demonstrated significant structural differences between treated and un-treated eyes. The treated eyes showed an inner retinal structure similar to what is observed in normal animals which provided encouragement that the therapy was functioning correctly.

To confirm such functional rescue, Dr. Min carried out electroretinographic analyses of the eyes at regular intervals over a three-month period. Electroretinographic data reported an improvement in b-wave amplitudes two- and three-months post-injection while there was also a similar improvement in the rod-mediated a-wave over a three-month period.

Finally, the researchers also looked to see what the distribution of retinoschisis in the retina looked like in the mouse model. They were again encouraged to observe that "treated eyes showed a distinct separation of inner and outer nuclear layers similar to that observed in wild-type animals, an absence of gaps between bipolar cells, and an increase in the thickness of the outer nuclear layer." The researchers also reported that "the outer nuclear layer of the injected eye had an average of 10.5 ± 1.0 photoreceptor nuclei per column of cell bodies, whereas the untreated contralateral retina had only 7.7 ± 1.5 nuclei."

This suggested a therapeutic benefit of approximately 36% over control eyes, the researchers calculated. Furthermore, preliminary studies have indicated that the structural improvements have lasted more than one year.

Prof. William Hauswirth, an author of the study and professor of ophthalmic molecular genetics at the University of Florida, predicted that such gene therapy could be introduced into clinical studies within two to five years depending on the outcome of preliminary safety studies.

## Glossary

**Heterozygous carrier:** refers to an individual who carries two different copies or "alleles" of the same gene.

**Knockout:** refers to genetic research technology that uses very specific gene targeting tools to remove a particular gene from an animal to observe the effects of that gene loss and thereby understand the pathology of a particular disorder.

**Recessive:** refers to a gene whose physical consequences are generally only observed when both copies of a gene are at fault. Cystic fibrosis is an example of a recessive gene disease in which both copies of the cystic fibrosis gene need to be damaged for the disease to occur. If you have one healthy copy of the cystic fibrosis allele then in general you do not suffer the disease.

**Synapse:** refers to the connection point – which may not necessarily create a physical contact – between two cells across which a variety of stimuli or signalling may occur. This allows for often rapid communication from cell to cell within the body.

**Transduce:** refers to the process of transferring one or more genes of vectors containing genes into foreign cells.