

Gene identified for key enzyme in the visual cycle

Nadja Geipert

A KEY gene discovery should help unlock some of the secrets of photoreception and retinal disease. After nearly twenty years of research, a group of scientists have discovered that the RPE65 gene product is the long-sought isomerase responsible for regenerating the photoreceptor molecule rhodopsin during light perception.

Isomerases are catalytic enzymes which convert one isomer into another. The isomerase in retinal pigment epithelium (RPE) catalyses the interconversion of vitamin A enzymes. By identifying RPE65 gene product as the isomerase involved in photoreception, the researchers solved two mysteries in vision science: first, the enzyme's identity and second, RPE65's concrete function in the visual cycle.

"It's been said that this isomerase is the holy grail of the visual cycle," said Gabriel Travis MD, professor of ophthalmology and biological chemistry at the Jules Stein Eye Institute at the University of California, Los Angeles and one of the study's investigators.

Variants of gene implicated in hereditary blindness

While different labs were searching for the enzyme's identity, RPE65 gene mutations were identified as causing at least 20% of the retinal degenerative disease, Leber's Congenital Amaurosis (LCA). In 2001, a group of scientists stunned the field of visual science when they cured blindness in three Briard dogs afflicted with a canine version of LCA by injecting a healthy version of RPE65 into the dogs' retina (Nature Genetics 2000 May; 28, 92-95).

Encouraged by the experiment's success, the National Institute of Health awarded a \$9.8 million grant to scientists seeking to use the gene therapy in humans with LCA. The first safety trial involving the treatment of LCA patients by injecting a virus carrying a normal version of RPE65 has been scheduled to begin in the fall of 2005.

Despite these massive financial investments, until now, no one understood the gene's actual function in the visual cycle and a widely regarded experiment had already ruled RPE65 out as the isomerase.

"People considered RPE65 as the enzyme and rejected it," Dr Travis said.

Process of elimination links gene to enzyme

To unravel the mystery of the isomerase's identity, Dr Travis and his colleagues performed a so-called unbiased expression screen. First, they generated cultured embryonic kidney cells that expressed most enzymes of the visual cycle, but not the isomerase. Then, they added to these cultured cells all of the genes that are expressed in RPE cells in 42 pools of 5000 genes each and tested each pool for isomerase activity.

When they identified a pool that expressed more retinoid isomerase activity than the other pools, the researchers split this pool down into successively smaller sub-pools, re-screening for isomerase activity each time. Finally they identified a single gene that conferred a very high isomerase activity to the cultured cells. When they sequenced this gene they discovered that it was identical to the RPE65 gene.

"This observation makes a bridge between two actually very different areas of investigation in vision science," said Dr Travis.

"I was a little bit surprised," said Rafael Caruso MD, an ophthalmologist, staff clinician and clinical researcher at the National

Eye Institute (NEI) in Bethesda, Maryland, USA about the findings. Prior experiments showed that removing RPE65 did not alter cells' ability to make isomerase, but in hindsight, it turns out that these experiments did not remove the gene completely, according to Dr Caruso.

Underlying mechanism of gene therapy better understood

While the discovery does not change the planned safety trials, it provides the involved researchers with important information on what their treatment actually does for the patient.

"This is a better insight of what the treatment is doing," said Dr Caruso.

While the success in the dog experiments justified the planning of human trials, it is safer to understand a gene's function when using it for therapy, added Dr Travis who will not be one of the researchers in the human trials.

"There was a slight uneasiness about putting a gene into people without knowing the gene's function. I think, we're in a more comfortable position to go ahead with the study that was planned," he said.

Dr Caruso agrees with this observation,

"In gene therapy, knowing the underlying mechanism is even more relevant than in the use of other conventional pharmaceuticals," added Dr Caruso.

In addition, the new understanding of RPE65's function might provide the researchers with more control during the trials, if they can test the virus for isomerase activity before injecting it into the subjects' eyes.

"We may be able to increase the activity by making changes to the isomerase, maybe we can tweak it," Dr Travis said.

In addition, identifying the isomerase might also propel the treatment and understanding of other retinal degenerative diseases, according to Dr Caruso.

"Since there are many disorders that are diseases of the visual cycle, we can think of approaches to increase or decrease the function of the enzyme. These pieces of information may be useful in the future even if they have no immediate use," he added.

The research appeared in the journal Cell, 2005 August; 122, 449-459.

travis@jsei.ucla.edu
carusor@nei.nih.gov