FALL/WINTER 2024

Gillingham Lab

RESEARCH NEWSLETTER

CONGRATULATIONS, SHANNON!

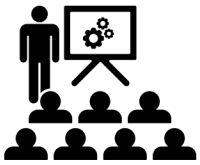
We are pleased to announce that Shannon Babcock graduated with her PhD in Molecular and Medical Genetics this past May. Shannon's contributions to our lab studying the molecular mechanisms and potential treatments of LCHADD chorioretinopathy in mice were extremely valuable. Shannon recently began a fellowship in laboratory genetics and genomics at the Henry Ford Hospital in Detroit, Michigan. Her career goal is to become a clinical lab geneticist. We will miss her and wish her the best in her career! You can download a copy of Shannon's recent publication "**The LCHADD Mouse Model Recapitulates Early-Stage Chorioretinopathy in LCHADD Patients"** (*https://iovs.arvojournals.org/article.aspx?articleid=2793780*)

WELCOME TO THE LAB, HAK!

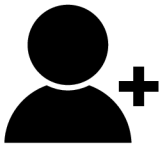
Hak Chung, PhD recently joined our lab as a postdoctoral researcher. Her educational background is in nutrition science, molecular genetics and immunology. She moved from Korea to the States for her doctoral studies at The Ohio State University and completed postdoctoral research at Cincinnati Children's Hospital Medical Center. She recently moved to Portland with her cat to join her husband. Hak describes herself as a yogi who enjoys all kinds of beverages, including coffee, tea, and wine, and she finds Oregon a beautiful place for outdoor activities like hiking. We are happy to have you with us, Hak!

PLEASE CHECK OUT THESE OTHER RECENT UPDATES ON OUR WEBSITE!

Dr. Gillingham was interviewed by the Journal of Inherited Metabolic Disease (JIMD) podcast about our research in LCHADD retinopathy and outcomes following early or late diagnosis. Find links to the episode on our **Publications and Presentations** page (*https://www.ohsu.edu/school-of-medicine/gillingham-lab/publications-and-presentations*).



Members of the Gillingham lab attended the 2024 Society for the Study of Inborn Errors of Metabolism (SSIEM) international conference in Porto, Portugal. See photos on our **Featured News** page (*https://www.ohsu.edu/school-of-medicine/gillingham-lab/featured-news*).



Ayah Asal joined our lab as an undergraduate researcher. Read more about her on our **People** page (*https://www.ohsu.edu/school-of-medicine/gillingham-lab/people*).

Gabriela Elizondo won an award at the Third Annual OHSU Department of Medicine Research Retreat for her poster "Acute Arrhythmias in a Long-chain-3-Hydroxyacyl-CoA Dehydrogenase Deficiency (LCHADD) Mouse Model". See the post on our **Featured News** page (*https://www.ohsu.edu/school-of-medicine/gillingham-lab/featured-news*).

Garen Gaston won 4th place for his poster "Stability of key fatty acid oxidation (FAO) enzymes vary by organ in human and mouse tissues with Long-chain hydroxy acyl-CoA dehydrogenase deficiency (LCHADD) caused by the common G1528C variant" at the 2024 INFORM meeting. See the post on our **Featured News** page (*https://www.ohsu.edu/school-of-medicine/gillingham-lab/featured-news*). You can also find a link to view his poster on the INFORM website (*https://informnetwork.org/annual-meeting/#posterawards2023*).

IN VITRO STUDY OF THE LCHADD RETINAL PIGMENT EPITHELIUM AND HADHA GENE ADDITION

We recently published a paper titled "**iPSC-Derived LCHADD Retinal Pigment Epithelial Cells Are Susceptible to Lipid Peroxidation and Rescued by Transfection of a Wildtype AAV-HADHA Vector**" (*https://iovs.arvojournals.org/article.aspx?articleid=2800802*) in the journal Investigative Ophthalmology and Visual Science (iovs). The paper describes our work characterizing the abnormal changes in LCHADD retinal pigment epithelial cells (RPE) (cells at the back of the eye) due to LCHADD and testing whether gene therapy (where we add a working copy of the LCHAD gene into LCHADD-RPE cells) corrects these abnormalities.

Retinal pigment epithelial cells are an important layer of cells between the blood vessels and the photoreceptors (light-detecting cells) (**Figure 1**). Our interest in studying RPE cells in treating LCHADD chorioretinopathy comes from our earlier research in which we have seen that the loss of RPE is greater than the loss of photoreceptors as the disease progresses. This has led us to hypothesize that RPE loss occurs before but ultimately leads to vision loss. The RPE also uses fat for energy and makes ketones to support the photoreceptors (cells that translate visual signals to the brain). A loss of enzyme function in the fat metabolism pathway may negatively affect RPE cells. We see that other fatty acid oxidation disorders do not develop chorioretinopathy, which suggests that the accumulation of hydroxy-acylcarnitines which are unique to LCHADD may be toxic to cells and contribute to RPE loss. Therefore, our goal is to develop a treatment for RPE cells to prevent LCHADD chorioretinopathy progression.

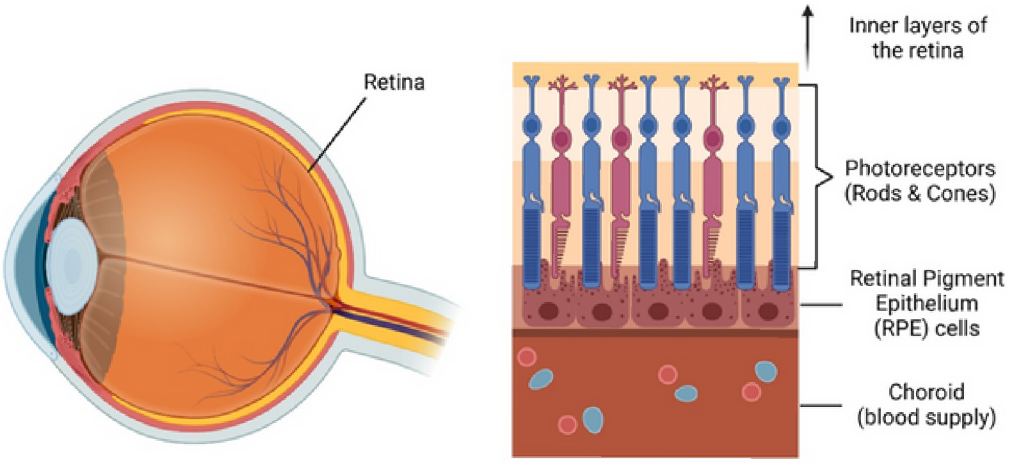


Figure 1. The retina is the layer of light-sensitive tissue at the back of the eyeball. Retinal pigment epithelium cells are an important layer of cells between the choroid, which has many blood vessels, and the photoreceptors, light-detecting cells. Damage and loss of the RPE layer may cause LCHADD chorioretinopathy.

LCHADD-RPE ARE SUSCEPTIBLE TO CELL DEATH UNDER HIGH-FAT CONDITIONS

We took fibroblast cells from previous skin biopsies of two LCHADD patients and reprogrammed them to induced pluripotent stem cells (iPSC), simple embryonic-like cells with the potential to develop into many kinds of cell types. We then added some genetic factors to push these cells to become RPE cells **(Figure 2).**

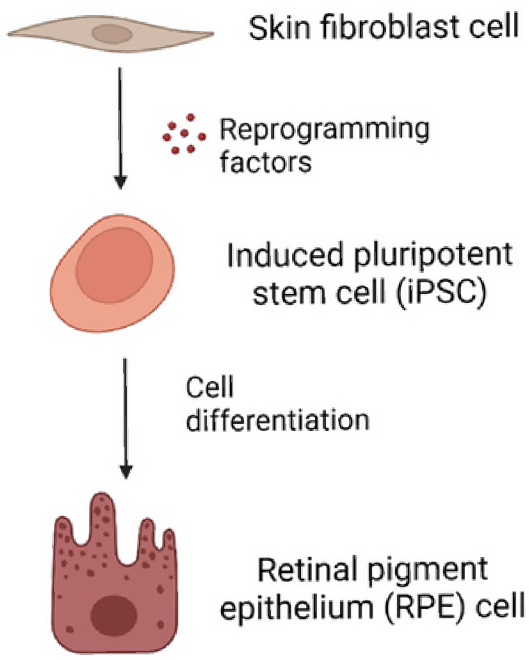


Figure 2. LCHADD skin fibroblast cells were reprogrammed into induced pluripotent stem cells (iPSC), simple embryonic-like cells with the potential to develop into many kinds of cell types, and then differentiated into retinal pigment epithelium to create an LCHADD-RPE cell.

To try and understand how LCHAD disease causes chorioretinopathy, we tested our LCHADD-RPE cells against RPE cells from healthy control persons (wild-type). In the presence of high levels of fatty acids and low glucose, the LCHADD RPE cells had impaired metabolism of fatty acids, lower ketones, and higher accumulation of partially metabolized fatty acids (3-hydroxy fatty acids and 3-hydroxyacylcarnitines). They also showed increased oxidative stress (a condition in which there is an imbalance between the number of unstable molecules called free radicals and the amount of antioxidants to get rid of them), oxidative damage, and cell death. We believe that the build-up of 3-hydroxy fatty acids and 3-hydroxyacylcarnitines in LCHADD RPE increases the cell's vulnerability to cell death caused by oxidative stress when circulating fatty acids are high, potentially explaining why RPE loss is uniquely seen in patients with LCHADD. In fact, when we exposed the cells to a potent antioxidant, N-acetyl cysteine (NAC), to reduce oxidative stress in the presence of high fatty acids, we found that it was able to rescue LCHADD RPE from cell death.

GENE ADDITION OF A NORMAL COPY OF HADHA RESCUES LCHADD-RPE

After confirming that our LCHADD-RPE displayed typical markers of LCHADD and were a good model of LCHADD chorioretinopathy, we tested whether gene addition of a normal LCHAD gene, *HADHA* DNA, could lead to a recovery of LCHADD-RPE function. To do this we incubated LCHADD-RPE with a recombinant adeno-associated virus (rAAV) containing the *HADHA* gene. An rAAV is a virus that can deliver genes to human cells without causing disease to the human. We tested and proved that the *HADHA* gene we introduced produced a stable LCHAD protein and that it was found in the mitochondria of the cell. We then tested whether the newly introduced *HADHA* gene would restore long-chain fat oxidation of the treated cells. We fed palmitate, a long-chain fatty acid, to RPE cells from 1) healthy controls (WT-RPE), 2) non-treated LCHADD-RPE, and 3) LCHADD-RPE treated with the *HADHA* gene. We then measured their oxygen consumption rate, a measure of fat metabolism. Like RPE from healthy controls, the addition of palmitate to LCHADD-RPE treated with the *HADHA* gene significantly increased their oxygen consumption rate, while non-treated LCHADD-RPE had no change in their oxygen consumption rate **(Figure 3).** This shows that long-chain fat oxidation was restored in the treated LCHADD-RPE.

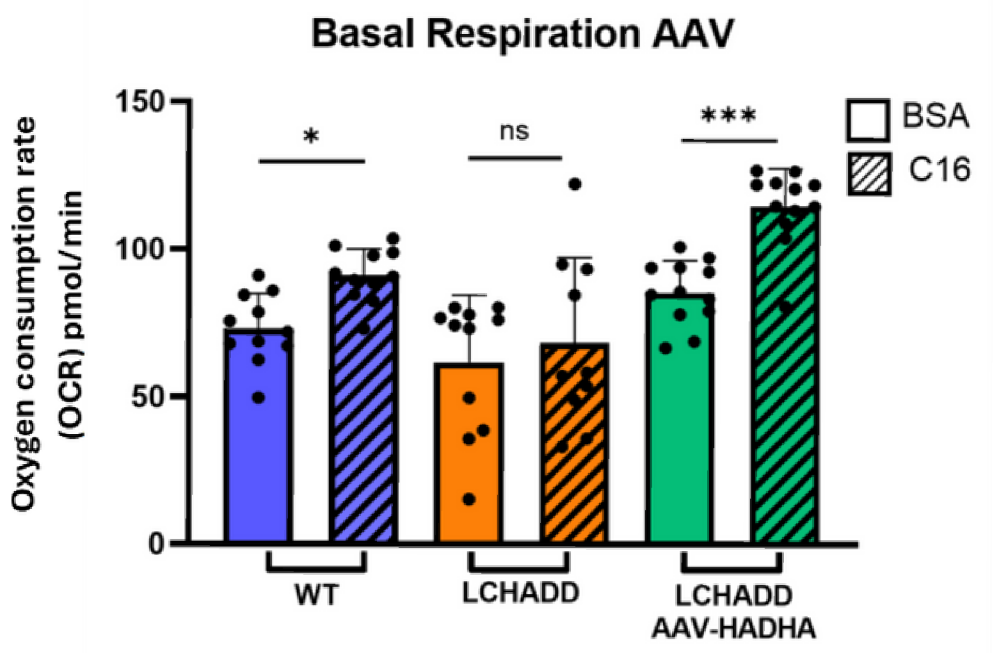


Figure 3. The oxygen consumption rate (OCR) of iPSC-derived RPE cells from healthy controls (WT, blue bars), cells without the addition of a normal *HADHA* gene (LCHADD, orange bars) and cells treated with addition of a normal copy of *HADHA* gene (LCHADD AAV-*HADHA*, green bars) were tested under baseline control (BSA, unshaded bars) and high fat (C16, shaded bars) conditions. Oxygen consumption significantly increased from baseline under high-fat conditions in both the healthy control RPE and LCHADD RPE treated with a normal copy of the *HADHA* gene, showing restoration of fat metabolism in the treated LCHADD-RPE.

Treated LCHADD-RPE also produced significantly more ketones in response to palmitate, experienced less oxidative damage and cell death after long-term exposure to fatty acids, and had significantly less long-chain 3-hydroxyacylcarnitines with high exposure to palmitate when compared to untreated LCHADD cells **(Figure 4).**

Although more preclinical research is needed and this study had limitations, the findings of this study suggest gene addition therapy may be a promising potential treatment for LCHADD chorioretinopathy.

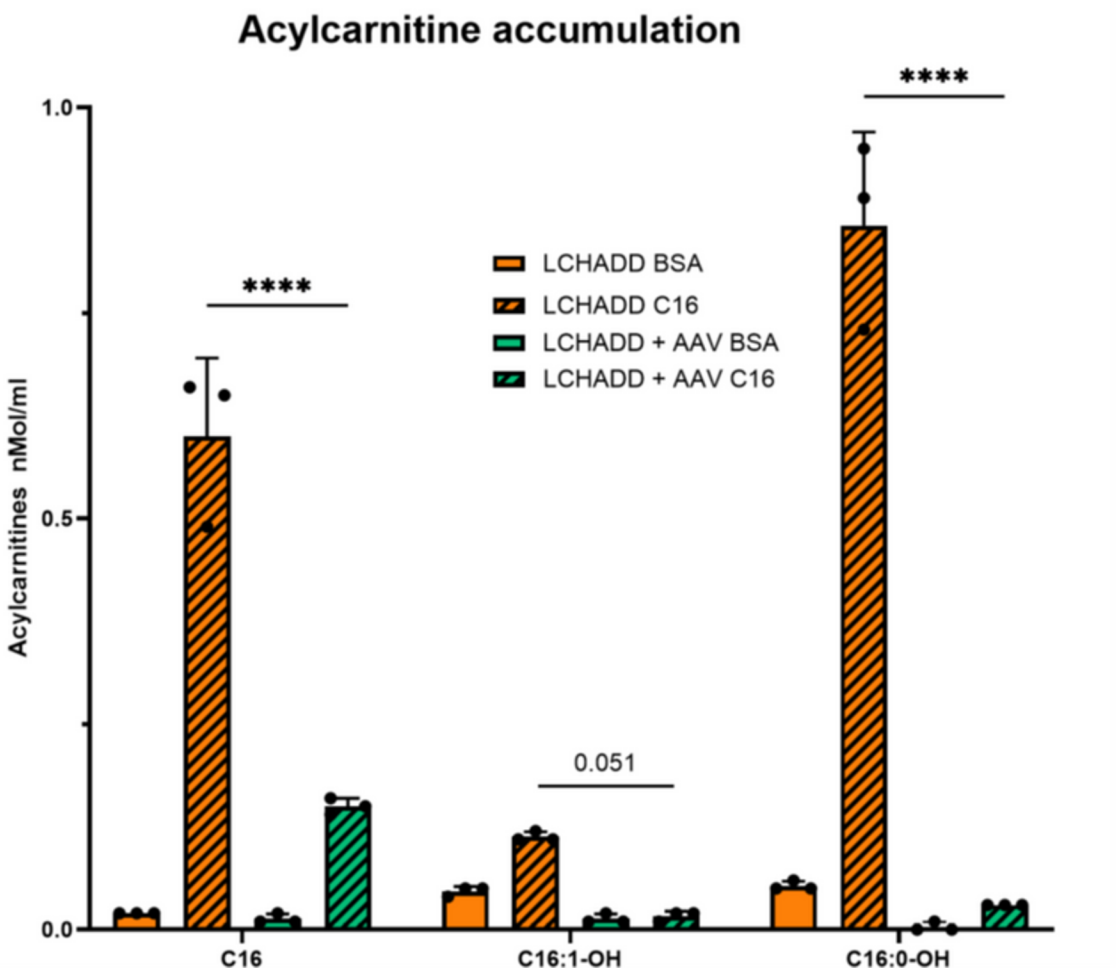


Figure 4. LCHADD-RPE cells without the addition of a normal copy of the *HADHA* gene (LCHADD, orange bars) and cells treated with a normal copy of *HADHA* gene (LCHADD + AAV, green bars) were tested under baseline control (BSA, unshaded bars) and high fat (C16, shaded bars) conditions. Treating the LCHADD cells with a normal copy of the *HADHA* gene significantly decreased 3-hydroxyacylcarnitine accumulation compared to the untreated cells.