PRECLINICAL SCREENING OF A POTENTIALLY THERAPEUTIC COMPOUND TO TEST RESCUE OF RETINAL DEGENERATION IN A ZEBRAFISH MODEL OF USHER TYPE 1F

by

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A THESIS

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Title: Preclinical Screening of a Potentially Therapeutic Compound to Test Rescue of Retinal Degeneration in a Zebrafish Model of Usher Type 1F

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Primary Thesis Advisor

Usher syndrome (USH) is a genetic disorder that affects 4 to 17 people per 100,000 worldwide. Usher syndrome type 1 (USH1), the most severe form of USH, is characterized by hearing and balance disorders at birth, and a progressive loss of vision beginning in childhood. Usher syndrome type 1F (USH1F), a subcategory of USH1, is caused by mutations in the *PCDH15* gene. Mutations in *PCDH15* that prematurely terminate the information encoded in the gene lead to truncated versions of the protocadherin-15 protein (PCDH15). This reduced or absent PCDH15 protein function leads to structural abnormalities in the hair cells of the inner ear and photoreceptors in the eye. We used a zebrafish model of USH1F to investigate whether hexafluoro, an antioxidant compound, can improve vision or retinal pathology in fish with impaired Pcdh15 protein function. We used an optokinetic response (OKR) assay to test whether hexafluoro-treated mutants showed improved vision compared to control fish, and examined photoreceptor cell integrity and survival in treated and untreated mutants. We found that hexafluoro improved visual function in USH1F mutant fish, and showed potential for reducing photoreceptor cell death. Our data indicate that hexafluoro has a stabilizing effect on retinal defects in zebrafish USH1F models, which are comparable to the early stages of human USH1F pathology, and provide proof of principle for expanding the effects of this and other drugs on zebrafish models of USH.

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1 Introduction

1.1 Usher Syndrome (USH)

Usher syndrome (USH) is the most common cause of inherited deaf-blindness, accounting for 50% of the deaf-blind population. Individuals with USH experience hearing loss, progressive vision loss that occurs over the course of decades, and in some cases, balance problems (Millán et al., 2010). USH worldwide prevalence is estimated to be between 1 in 25,000 and 1 in 6,000 people (Toms et al., 2020).

The vision loss characteristic of USH is due to the dysfunction and ultimate degeneration of the light-sensing cells in the retina called photoreceptors. The photoreceptors in the peripheral retina are the first the be affected, and the dysfunction progresses toward the center of the visual field over time, causing night blindness initially, followed by progressive tunnel vision and loss of sight (Millán et al., 2010). Similarly, the hearing and (when present) balance deficits in USH are caused by the abnormal structure of the sensory cells in the inner ear that mediate sound perception and spatial orientation (Yan et al., 2010). In most USH cases, these inner ear cells develop abnormally *in utero,* resulting in dysfunction present at birth (Millán et al., 2010).

Clinical diagnosis of USH falls into three subtypes defined by the severity of hearing loss, presence or absence of balance issues, and the age of onset of the retinal degeneration (Yan et al., 2010). This thesis will focus on the most severe of the three clinical subtypes: Usher syndrome type I (USH1). USH1 accounts for 30-40% of USH cases, and is characterized by severe hearing and balance problems at birth, along with the deterioration of vision beginning in the first decade of life (Yan et al., 2010). The

retinal degeneration experienced by individuals with USH1 occurs over the course of several decades, which provides a wide timeframe for potential therapeutic intervention (Toms et al., 2020).

1.2 Genetic Inheritance

Humans are diploid organisms, meaning their DNA is composed of paired sets of chromosomes, in which one chromosome of each pair is inherited from each parent. In the 1860s Gregor Mendel used pea plants to demonstrate that inheritance of a trait is dependent on information passed from parents to offspring. Each gene, or inherited unit of DNA, serves as a template for the production of specific molecules, each with unique roles in biological development and function.

Changes to the DNA code are called mutations. Not all mutations are harmful, however, all genetic diseases result from mutations in the DNA. A dominant disease occurs when a mutation in one of the two copies of an inherited gene results in disease symptoms, even if a normally coded copy is inherited from the other parent. Huntington's disease is an example of a dominant disorder. Symptom onset is typically during middle-age, so affected individuals have often already passed the disease gene on to their children before they are aware that they have the condition (Walker, 2007).

In the case of recessive disorders (Fig. 1), disease symptoms only manifest in individuals who inherit mutated copies of a gene from both parents. An individual with only one mutated copy of the gene would not show symptoms of the disorder, but is a carrier of the mutation and can pass it on to his or her children. When two carriers with recessive mutations in the same gene reproduce, there is a 50% chance that each parent

will pass down their mutated gene. Therefore, there is a 25% chance that their child will inherit the defective copy from each parent, and be affected with the genetic disorder.

USH is categorized as a recessive disorder, and its prevalence varies based on the population being studied. Because people within the same ethnic group share certain versions of genes that have been passed down from common ancestors, recessive disorders have increased incidence within subsets of the global population due to geographical and cultural isolation. Some of the groups that exceed the upper estimates of USH worldwide prevalence of about 1 in 6,000 include Jewish populations in Israel, Berlin, and Germany, French Canadians in Louisiana, Argentineans, and Nigerian Americans (National Organization for Rare Disorders, 2018). Cultural groups that share the ancestry of these populations are also disproportionally affected. The populations with higher incidents rates of USH increase the motivation for investigating potential therapies.

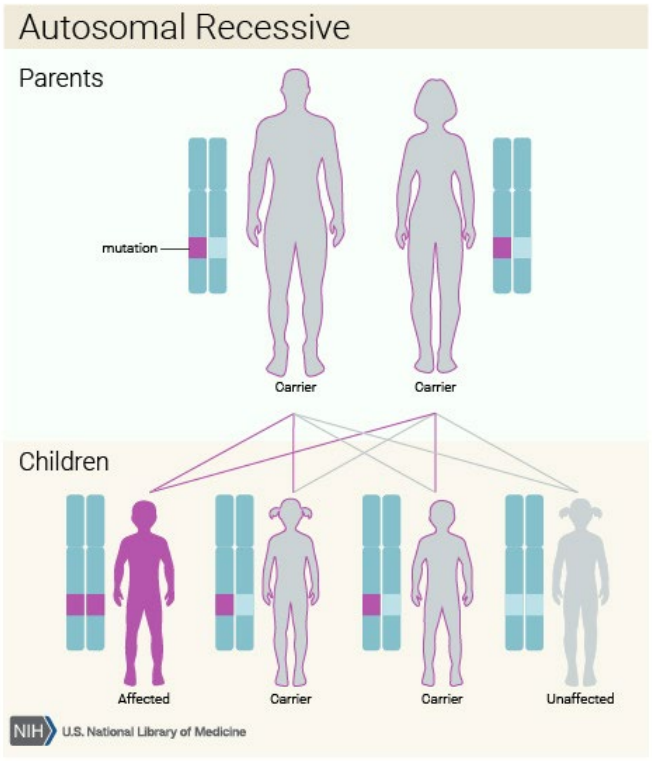

Figure 1: Recessive inheritance

This figure shows the mode of inheritance for a recessive mutation. The dark purple segments of DNA signify the disease-causing mutation, whereas the light blue segments of DNA signify DNA without the mutation. Carrier parents have a 50% chance of having a carrier child, a 25% chance of having a child without the mutation, and a 25% chance of having a child affected with the disease. Source: NIH U.S. National Library of Medicine.

1.3 Zebrafish as a Model Organism for Human Disease

Animal models are essential to understand better the molecular basis of human disease and for testing the safety and efficacy of various pharmaceutical interventions that may be useful in treating these diseases. Although numerous animal models are used to study human disease, zebrafish have many advantages that have enabled important contributions to human health research.

Zebrafish are vertebrate animals like humans. Using a vertebrate model rather than an invertebrate, such as fruit flies or nematodes, allows for more direct comparison between the model and human conditions. 71.4% of human genes have at least one zebrafish orthologue, which is a gene derived from a common ancestor that has retained the same function (Howe et al., 2013). In addition, zebrafish organs and body systems are highly similar to those of humans, which results in similar presentation of disease symptoms that can be used as a comparison for understanding human disease. Gene editing techniques targeted to zebrafish orthologs of human disease genes can precisely model known or suspected disease-causing genetic variants in humans. This method has allowed zebrafish researchers to recreate the molecular conditions that underlie many human genetic disorders, enabling greater understanding of the disease process and identifying new molecular targets for potential treatments (Phillips & Westerfield, 2014).

Zebrafish produce a large number of offspring that are fertilized and develop externally, providing observation of early developmental processes without disruption. Mammalian gestation, by contrast, occurs *in utero,* making observation less accessible (Dooley et al., 2000). In addition, zebrafish develop quickly, so observable abnormalities in development and behavior become apparent much sooner than they would in organisms that are slower to mature (Vona et al., 2020). From fertilization through the first week of development, many zebrafish tissues are transparent, enabling the study of cellular and molecular processes in living, growing animals (Phillips & Westerfield, 2014). Of relevance to this thesis, the structure of the zebrafish eye and ear are comparable to those in humans, and both organs are well developed and functional

within the first week of life, making zebrafish a good model for the study of USH (Blanco et al., 2017).

1.4 The Retina and Photoreceptor Cells

The vertebrate eye contains 6 distinct retinal layers: the retinal pigmented epithelium (RPE), the outer nuclear layer (ONL), the outer plexiform layer (OPL), the inner nuclear layer (INL), the inner plexiform layer (IPL), and the ganglion cell layer (GCL) (Fig. 2). Light enters through the lens, which focuses the light rays onto the retina. The RPE is a layer of tissue at the back of the eye that is responsible for delivering nutrients to the photoreceptor cells, recycling shed photoreceptor membranes, and preventing entering light from scattering. Photoreceptors form the ONL, the outermost portion of the retina. Photoreceptors communicate with horizontal and bipolar cells through synaptic connections, which form the OPL. The cell bodies of horizontal and bipolar cells, along with amacrine cells and neuronal support cells called glia, make up the INL. Bipolar cells synapse with amacrine cells and ganglion cells at the IPL. The GCL contains the cell bodies of ganglion cells, and the axons of ganglion cells bundle together to become the optic nerve (ON), which transports visual signals from the eye to the brain (Chhetri et al., 2014).

One difference between human and zebrafish eye anatomy is that the zebrafish eye continuously generates new retinal cells for the duration of its life. A region around the perimeter of the zebrafish retina, called the ciliary marginal zone (CMZ), contains stem cells that differentiate into the various types of retinal cells that populate the retina as the fish continuously grows (Wan et al., 2016).

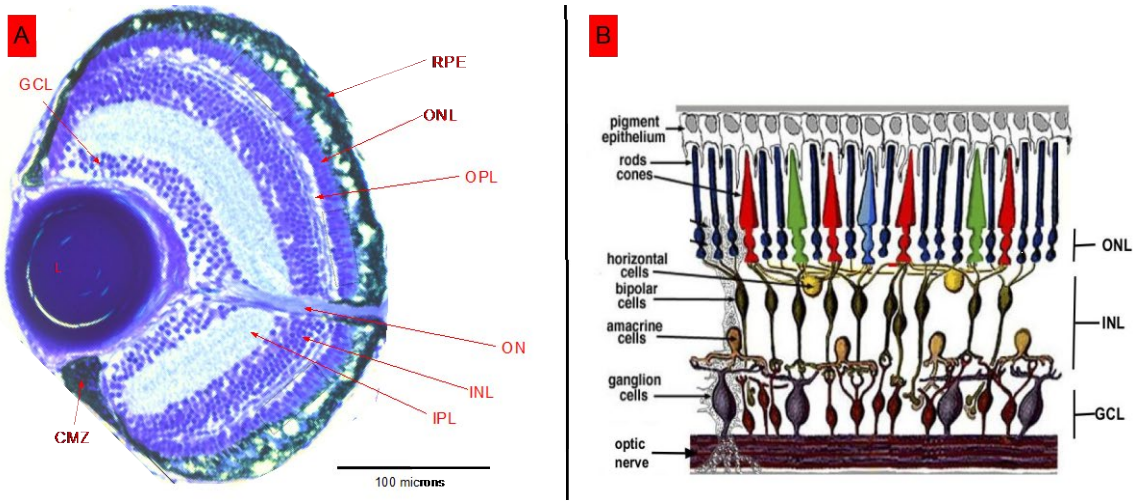

Figure 2: Retinal cell layers

(A) Magnified image of a sectioned larval zebrafish eye stained with toluidine blue. GCL; ganglion cell layer. L; lens. CMZ; ciliary marginal zone. RPE; retinal pigmented epithelium. ONL; outer nuclear layer. OPL; outer plexiform layer. ON; optic nerve. INL; inner nuclear layer. IPL; inner plexiform layer. (B) Cartoon depiction of the retinal cell layers. The outer nuclear layer (ONL), inner nuclear layer (INL), and ganglion cell layer (GCL) have been labeled on the right. The cell types within each layer have been labeled on the left. Source: Webvision.

Because vision loss in USH is due to defects in photoreceptor cells, photoreceptors will be the focus of this thesis. There are two kinds of photoreceptor cells: rods and cones (Fig. 3), which share a number of specialized structural and functional features. The outer segment (OS) of photoreceptors is closely associated with the RPE, and is made of elaborately folded membranes studded with proteins called opsins that respond to light energy. Rods feature tall, straight outer segments, whereas cone OS are shorter and tapered at the top. When light encounters the photoreceptor outer segment, chemical changes occur in these opsins that initiate the signaling cascade of the visual process, ultimately relayed through the synapse. The photoreceptor inner segment (IS) is responsible for metabolic regulation of the cell. In some animals,

including humans and zebrafish, but not rodents, fingerlike projections called calyceal processes wrap around the exterior of the cell at the IS-OS boundary.

Functionally, the different types of opsins confer distinct light-detecting abilities on the different types of photoreceptors. Rod opsins are highly sensitive to even small amounts of light, making them well suited for function in low-light conditions as well as in the detection of movement. In humans and other primates, rods are enriched in the periphery of the retina. Cones contain opsins that require brighter light levels to function, but are sensitive to specific wavelengths of the visible light spectrum, and thus are specialized for daytime color vision. Cones in the primate eye are most densely populated in the central retina, providing the highest visual acuity when the eyes are aimed at an object in front of them. Cones in the zebrafish retina are arranged in an orderly mosaic pattern across the zebrafish retina. Rods surround each UV cone, a cone that primates lack. Despite organism-specific adaptations, such as the CMZ and photoreceptor arrangement, anatomical and functional properties of retinal cells are shared between zebrafish and humans, which justifies the use of zebrafish as a USH disease model.

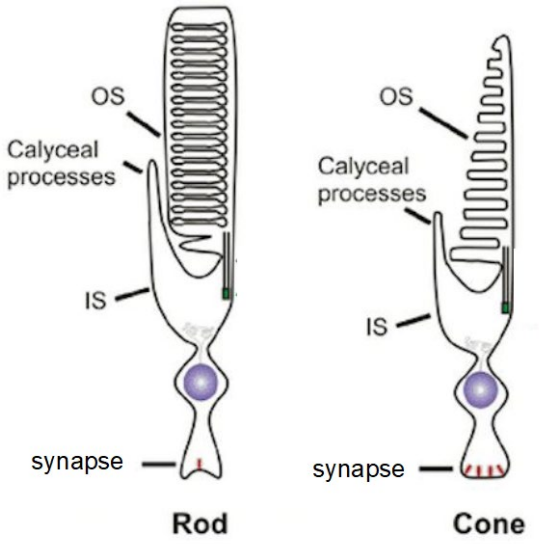

Figure 3: Structural similarities between rods and cones

OS; outer segment. IS; inner segment. The OS projects towards the RPE. The connection between the OS and IS is stabilized by calyceal processes. Source: Slijkerman et al., 2015.

1.5 Usher Syndrome Type 1F

There are 6 identified USH1 genes encoding a diverse variety of proteins that localize within cells of the eye and ear. Mutations affecting the normal function of any one of these six proteins result in molecular dysfunction that leads to the symptoms characteristic of USH1, the most severe form of the disorder (Petit et al., 2001). The symptoms associated with USH1 include severe to profound hearing loss at birth, balance issues, and onset of visual dysfunction beginning before puberty (Toms et al., 2020).

This thesis will focus on Usher type 1F (USH1F), a subtype of USH1. Besides hearing loss and balance defects, which are present at birth, the first reported symptom of individuals with USH1F is usually difficulty seeing at night, a symptom of rod photoreceptor dysfunction. A key early sign of USH1F eye pathology is abnormal

blotchy pigment in the retinal layer, which is due to displaced RPE cells resulting from a breakdown in photoreceptor organization. This pigmented tissue is characteristic of the retinal degeneration pattern that progress from peripheral to central vision loss, thus these diseases were originally referred to as 'retinitis pigmentosa'. Additional clinical signs of USH1F include a cloudy appearance to the lens and reductions in the vasculature, or network of blood vessels, in the retina. These vascular changes are often seen in retinal degeneration due to decreasing demand for nutrients as the photoreceptors die. As degeneration progresses, the RPE begins to break down, vascular attenuation becomes more severe, and the optic nerve presents with a pale appearance, a further sign of retinal cell death. Rod cell death in the periphery causes changes to the retinal environment that eventually lead to progressive loss of photoreceptor function across the retina until only a pinhole of central vision remains. Vision tests of USH1F patients show a sharp decline in visual acuity between the third and fourth decade of life, as photoreceptor death progresses toward the center of the retina. Evaluations of retinal function of USH1F patients indicate dysfunctional photoreceptor activity in surviving photoreceptors (Sethna et al. 2021).

The gene associated with USH1F is *PCDH15*, which encodes the Protocadherin15 protein (PCDH15). Mutations in *PCDH15* were found to cause 11% of the US and UK cases of USH1 (Yan et al., 2010). PCDH15 in the human retina is localized at the calyceal processes, in the area between the photoreceptor inner and outer segments (Sahly et al., 2012). The frequency of a particular USH1F gene mutation, called R245X, is especially high in the Ashkenazi Jewish population, with up to 2.48% of Ashkenazi Jews carrying the R245X variant of *PCDH15* (Ben-Yosef et al.,

2003). The R245X DNA mutation causes termination of the production of the PCDH15 protein before the entire protein is made. This early termination results in a truncated, or shortened version of the PCDH15 protein (Fig. 4). Generating mutations in zebrafish and mouse that result in truncated versions of the protein similar to that seen in R245X patients results in structural abnormalities in hair cells and photoreceptors (Phillips et al. 2021, Sethna et al., 2021).

Figure 4: Shortened PCDH15 protein due to R245X mutation

Top: graphic representation of the full-length PCDH15 protein. The region labeled N signifies the start of the protein, and the region labeled C signifies the end of the protein. The blue blocks are regions of PCDH15 that participate in protein-protein interactions outside of the cell, the narrow orange rectangle denotes the portion of the protein that interacts with the cell membrane and allows the protein to embed within it. The purple blocks are domains that enable PCDH15 to interact with other proteins inside the cell. Bottom: shortened PCDH15 protein that would result from the premature halt in production due to the R245X mutation. This truncated protein lacks the blue, purple and orange regions of the full-length protein, destroying the ability of this protein to participate in protein-protein interactions. The zebrafish mutants described in this study feature a similarly truncated protein. Source: Phillips et al., 2021.

Most mouse models of USH1F have pronounced hearing and balance defects comparable to the human condition, but do not exhibit the same photoreceptor structural defects seen in humans with USH1F (Sahly et al., 2012). This may be due to both the nocturnal nature of mice and differences in their photoreceptor anatomy: mice lack calyceal processes.

A recent study generated mice with the precise R245X mutation known to be pathogenic in humans and found that, although there was no detected structural impairment of the photoreceptors, *pcdh15 R245X* mice still displayed signs of visual dysfunction (Sethna et al., 2021). The authors used the mouse model to determine that Pcdh15 plays a role in facilitating the movement of proteins from the RPE to photoreceptor cells, and between the photoreceptor inner and outer segments. The disruption of this transport in *pcdh15 R245X* mice resulted in visual deficits. In addition, the authors noted a small but significant decrease in the thickness of the photoreceptor cell layer (ONL) in *pcdh15 R245X* mice, suggesting mild retinal degeneration in this model (Sethna et al., 2021). Unlike mice, zebrafish have calyceal processes, and zebrafish models of USH1F have been shown to exhibit defects in calyceal processes as well as disruption in the organization of photoreceptor outer segments (Miles et al., 2021 & Phillips et al., 2021).

Previously, Seiler and colleagues (2005) characterized two zebrafish *pcdh15* genes: *pcdh15a* and *pcdh15b.* Using a strain of zebrafish with a truncating mutation in the *pcdh15a* gene, they found that the Pcdh15a protein was necessary for the structural and functional integrity of hair cells in the inner ear. To understand the function of the Pcdh15b protein, they used a technique to temporarily deplete Pcdh15b protein by injecting a designed molecule called a morpholino oligonucleotide into one-cell stage zebrafish embryos to disrupt the processing of the *pcdh15b* gene product. They found that the normal structure and function of photoreceptor cells were severely disrupted in the absence of functional Pcdh15b (Seiler et al., 2005). In contrast, hearing and balance were unaffected by the morpholino. This study concluded that the two *pcdh15*

orthologues present in zebrafish perform separate functions that both contribute to the USH1F phenotype when mutated. The morpholino approach, while informative, is limited because long-term effects of losing Pcdh15b function could not be assessed, and the study was not designed to analyze specific mutations found in humans with USH1F.

The fish used in our studies were modified via gene editing to contain a mutation in a specific portion of the *pcdh15b* gene that mimics the R245X mutation that is prevalent in the Ashkenazi Jewish population (Phillips et al., 2021). Our analysis of zebrafish with two copies of this mutation showed a role for *pcdh15b* in both the retina and areas of the inner ear associated with balance, although hearing appeared unaffected. Analysis of the retinal tissue in these fish revealed abnormal calyceal processes and photoreceptor outer segments, as well as elevated death of photoreceptors (Fig. 5). Structural impairment of hair cells in the inner ear region associated with balance was also noted. The defects observed in these animals are consistent with human USH1F visual and vestibular dysfunction (Phillips et al., 2021), providing the most complete disease model to date. Another recent zebrafish study showed that Pcdh15 proteins are localized in multiple areas of zebrafish photoreceptors: the calyceal processes, at the junction between the inner and outer segment, and within the photoreceptor synapse (Fig. 6) (Miles et al., 2021). Taken together, there is strong evidence to support the hypothesis that loss of PCDH15 protein function in humans leads to the progressive degeneration of outer segments due to the structural disruption of calyceal processes, and that these abnormalities precede photoreceptor death.

Data from multiple USH1F animal models indicate that exposure to elevated light levels amplifies photoreceptor damage. Raising zebrafish USH1F mutants in

bright light conditions exacerbated abnormal photoreceptor outer segment growth and cell death in these mutants, while raising them in low light or full dark conditions attenuated outer segment abnormalities (Miles et al., 2021) and cell death (Phillips et al., 2021). In addition, UV filters had a protective effect on USH1F mutants exposed to increased light intensity by partially rescuing photoreceptor degeneration (Phillips et al., 2021). Similarly, abnormal retinal symptoms were heightened in the *R245X* mouse model of USH1F when exposed to higher light levels (Sethna et al., 2021). Therefore, it is important to monitor the light intensity of each experimental procedure designed to test retinal cell function or integrity in our *pcdh15b* mutant zebrafish .

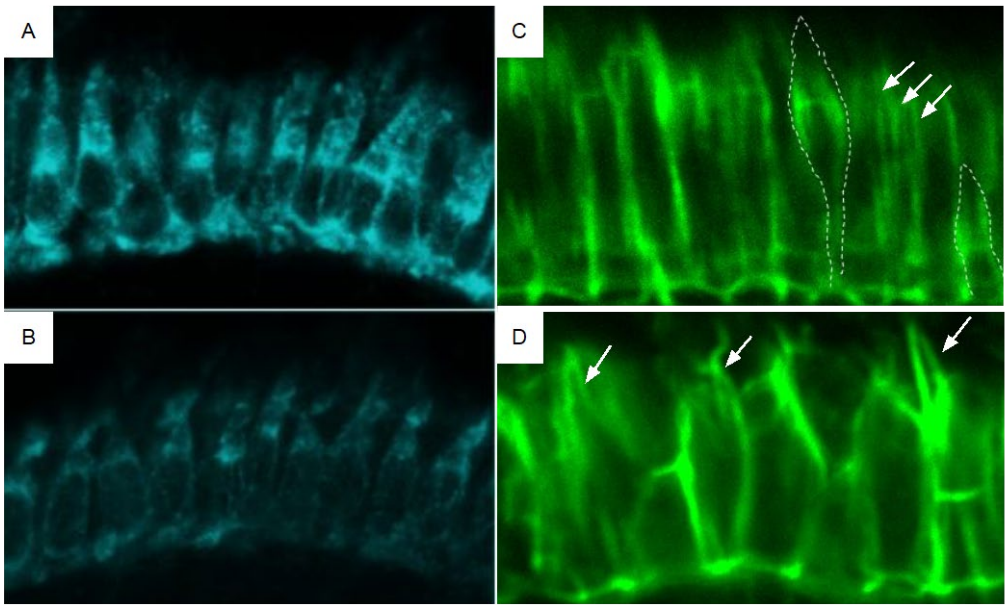

Figure 5: Zebrafish *pcdh15b* photoreceptors show disrupted outer segments and calyceal processes.

(A) Cone photoreceptor outer segments of 6-day post fertilization (dpf) wild type zebrafish are tapered and uniform, with abundant distribution of the functional protein labeled in green. (B) *pcdh15b* mutant zebrafish cones are short and disorganized, with less protein detected. (C) In wild-type photoreceptors, calyceal processes labeled by actin appear as a fine fringe around the photoreceptor (white arrows). Two cones have been outlined in white. (D) Calyceal processes in the *pcdh15b* retina are disheveled, clumped, and unevenly distributed. Source: Phillips et al., 2021.

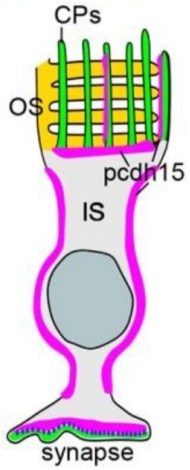

Figure 6: Pcdh15 localization in the photoreceptor

Pcdh15 (magenta) is localized at the calyceal processes and the photoreceptor synapse (both labeled green), at the base of the outer segment and associated with the inner segment cell membrane. OS; outer segment. CPs; calyceal processes. IS; inner segment. Source: Miles et al., 2021.

Based on the information collectively obtained from USH1F animal models, there is a strong likelihood that progressive structural abnormalities in photoreceptors also precede cell death in the pathology of USH1F. This hypothesis is consistent with the timeline of vision loss seen in human USH1F patients. Visual testing of individuals with USH1F within their first decades of life shows loss of vision in the periphery and under low light conditions. It is usually not until the third and fourth decades of life that visual impairment approaches its terminal phase. Similarly, although response to visual stimuli was reduced in 5-day post fertilization (dpf) zebrafish (Phillips et al., 2021), there was a detectable response. These findings indicate that photoreceptors with structural abnormalities can still muster some level of visual function, suggesting that preservation of even ailing and morphologically disrupted photoreceptors would contribute to preservation of vision.

1.6 Therapeutic Intervention

Although cochlear implants are the standard of care to treat USH1F hearing deficits, there is no treatment for the vision loss. A variety of potential avenues for treatment of the type of retinal degeneration seen in USH are currently being investigated, including gene replacement, gene editing, and small molecules that act on the affected tissue in some therapeutic way. This thesis will focus on an intervention with the potential to extend the period of visual function in the zebrafish USH1F model by slowing the process of cell death.

There are many causes of cell death, but the intervention investigated in this thesis targets death caused by oxidative stress. Oxidative stress results when metabolic products called reactive oxygen species (ROS) are not cleared from the cell efficiently, which leads to cell dysfunction and eventual cell death. Multiple disease states can contribute to a build-up of ROS, including inflammation, cancer, and metabolic imbalance. ROS have been shown to contribute to photoreceptor cell death in other types of inherited retinal degeneration (RD) (Murakami et al., 2020), and therefore could be a potential contributor to photoreceptor death in USH. Oxidative damage has been detected in mouse models of RD, and antioxidant-based therapies that decrease the levels of ROS have been shown to reduce photoreceptor cell death in one such model (Murakami et al., 2020). Furthermore, the use of an antioxidant-based drug on an USH1 mouse model slowed photoreceptor degeneration (Trouillet et al., 2018).

In an attempt to find a treatment for the retinal degeneration component of USH1F, I will use the zebrafish *pcdh15b* model generated in the Westerfield lab to test the effects of a chemical compound called hexafluoro. Hexafluoro is a small polyphenol molecule (Fig. 7) synthesized from a compound called honokiol, which is isolated from the bark of the Magnolia tree (Xian et al., 2015). Previous work tested hexafluoro in the brain of mice to see if its anti-oxidative effects could reduce inflammation and associated cognitive decline following the use of anesthesia. This study showed that hexafluoro potentially participates in cell death rescue of neurons in the hippocampus of mice via reduction of ROS (Ye et al., 2018), but it has never been tested on retinal degeneration models or in zebrafish. Hexafluoro treatment will not restore normal *pcdh15b* function in mutant zebrafish, and thus is not expected to alter the structural

abnormalities caused by the absence of normal protein activity in photoreceptors. However, hexafluoro will potentially slow the progressive events within the cell that precede photoreceptor death. This thesis seeks to understand whether hexafluoro can attenuate cell death in the retina, and whether this will preserve some visual function.

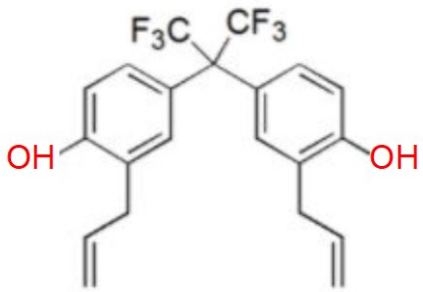

Figure 7: Chemical structure of hexafluoro

The lines in this structure depict carbon molecules bonded to other carbon molecules. Hexafluoro contains two hydroxyl groups (OH), and two trifluoromethyl groups (CF_3). The hydroxyl groups (red) make hexafluoro soluble in water, and reactive with harmful oxygen-based molecules. Source: Medra et al. 2016.

1.7 Thesis Reasoning

Analyzing whether or not hexafluoro can improve photoreceptor cell health in the zebrafish *pcdh15b* retina will provide important information about the role of oxidative stress in USH1F photoreceptor cell death, in addition to preclinical data about the effectiveness and safety of this compound. Such information is a step in bringing a treatment option to the clinic for people living with this disease. Because the blindness in USH1F and other forms of RD is progressive, and occurs over the course of decades, there is a long time frame for potential therapeutic intervention. A drug that could slow or stop the initial death of photoreceptor cells would help to maintain the health of surrounding cells, and slow the resultant degeneration of the retina. More targeted

therapies, such as gene replacement and gene editing to correct the genetic mutations underlying retinal degeneration, would be effective only if applied to the photoreceptors, thus the more advanced the degenerative process, the fewer living cells are left to treat with these methods. If hexafluoro proves to be effective in rescuing photoreceptor cells, it could preserve some measure of vision and would additionally buy time to develop other therapies that target the deficits of USH photoreceptors more directly.

The results of this study could have implications beyond the scope of USH1F, paving a therapeutic avenue based on antioxidant drug therapies for other types of USH and non-syndromic forms of retinal degeneration (Ferrari et al., 2011). The experiments described in this thesis represent preliminary findings on the impact of hexafluoro on photoreceptor cell health and vision of a zebrafish model of USH1F.

2 Materials and Methods

2.1 Animal Care

The health and safety of the zebrafish is the priority in the laboratory. All research conducted in the Westerfield Lab follows the extensive animal care and use protocols required by the University of Oregon and the National Institutes of Health, our major source of research funding. All personnel working with zebrafish must undergo institutionally mandated training on the safe and humane handling of vertebrate research animals. The top priority of researchers and fish facility staff is that the animals are well cared for and healthy for the duration of their lives.

At the University of Oregon, the zebrafish colony is kept in a temperature-controlled environment on a 14-hour light, and 10-hour dark cycle. All experiments on live animals must be preapproved by the Institutional Animal Care and Use Committee and are designed to minimize discomfort for the animals, up to and including specific protocols for euthanasia at the end of an experiment.

All of the experiments described in this thesis were conducted on young fish in the first week of life, derived from mating adult male and female fish. We identified male and female fish for mating crosses by visual observation. Females are slightly larger than age-matched males, with round white bellies, whereas males have a narrower body shape and a darker yellow streak on their bellies (Fig. 8). Male and female partners were placed in a one-gallon plastic chamber filled with water, along with green netting simulating river grass, which encourages mating behavior. Zebrafish will typically breed at the beginning of the light cycle. The chamber consisted of two separate components: an outer container and a basket-like insert. When adult fish

spawned, the fertilized eggs fell through holes in the bottom of the basket, which prevented the adult fish from eating them. When embryos were visible at the bottom of the tank, the basket insert containing the adult fish was removed and transferred to clean water, leaving the embryos in the bottom of the outer chamber for collection.

Figure 8: Male vs. Female Zebrafish

Identification of male and female zebrafish is based on visual observation. (A) Males are smaller and narrower than females, with a deep golden color and darker blue stripes. They also tend to be more active. (B) Females are slightly larger and rounder, with lighter coloring than males. Source: Chaudhary et al., 2012.

The embryos were removed from this chamber into a Petri dish filled with embryo medium (EM), a buffered salt solution optimized for the health and development of the young fish. EM consists of a combination of several salts, and pH balanced water. The dishes were maintained daily with additions of fresh EM and removal of non-viable embryos and other debris. After a few days, the embryos emerged from their egg sac, called a chorion, and became free-swimming larvae. Zebrafish reach fertile adulthood at approximately 3 months of age (Ramcharran, 2016).

2.2 Preclinical Screening of Hexafluoro

We administered hexafluoro to young fish to investigate whether reducing oxidative stress can prolong the life of photoreceptor cells in the zebrafish model of USH1F. For this experiment, adult carriers of the *pcdh15b*^{b1257} mutation were bred and the offspring collected as described. Following Mendelian genetics, 25% of the offspring from two carrier parents will be mutants, and 75% will be unaffected siblings (Fig. 1).

For each round of drug treatment, at 2 dpf we divided 200 embryos per experiment into experimental and control groups with the following conditions: the experimental group was treated with a solution containing hexafluoro, added to the EM in the Petri dishes. We added 6.6 µL of a 1 mg/mL solution of hexafluoro diluted in dimethyl sulfoxide (DMSO) to 60 mL of EM for a final concentration of 0.11 µM. This is the maximum dose shown to be tolerable to wild-type embryos and larvae in earlier dosage testing experiments. The control fish received 6.6 µL of DMSO with no active ingredient added.

For the duration of the experiment, the Petri dishes containing the fish were kept on a tray in a separate temperature-controlled room that maintains the same light cycle as the general fish facilities. Because hexafluoro is a toxic chemical, the fish were stored on a shelf that is approved for experiments involving drug treatment, and the fish receiving hexafluoro treatment were kept within a secondary container on the tray, isolated from the control fish to avoid contamination. These restrictions resulted in an environmental light intensity of only 40 lux, in contrast to the main facility conditions of about 400 lux.

Each day, through 6 dpf, the EM in each dish was removed and replaced with fresh media, with or without added hexafluoro. The animals were monitored for health and viability for the duration of the treatment. The wild-type larvae cannot be distinguished from the mutant larvae based on their observable anatomy, however, by 5 dpf mutant larvae display an abnormal swimming behavior. When startled by a loud tap on the Petri dish, wild-type larvae will swim away in a straight line, whereas the balance defects present in *pcdh15b* mutants cause them to swim in an abnormal looping and twirling pattern. At 5 dpf, we performed this test and placed mutant fish exhibiting abnormal swimming behavior into separate Petri dishes.

2.3 Optokinetic Response Assay

To test the visual function of the hexafluoro-treated and control fish, we performed an optokinetic response (OKR) assay at 5 dpf. The larvae were placed in a small Petri dish containing methyl cellulose, a viscous solution that prevents the fish from swimming away during the testing period. The Petri dish was then placed on a stationary platform surrounded by a rotating drum that contains 10 alternating black and white stripes (Fig. 9) illuminated from above with a measured intensity of 15,000 lux. The drum rotated at 9 revolutions per minute for 1 minute clockwise, and 1 minute counterclockwise while we observed and recorded the number of saccades, or controlled eye movements, completed during the testing period. This experiment was repeated for 3-6 fish from each test group: drug-treated mutants, control mutants, drug-treated siblings, and control siblings.

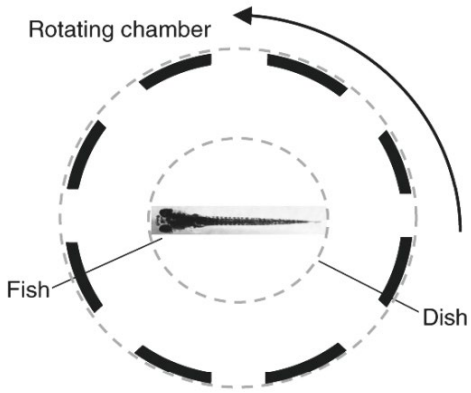

Figure 9: Optokinetic response assay set-up

Individual larva were immobilized in a viscous liquid in the center of a rotating drum with alternating black and white stripes. Eye movements were viewed through a microscope set up over the apparatus. Image source: Lagnado, 2005.

2.4 Preserving the Fish Tissue and Preparing Slides

To analyze photoreceptor cells microscopically, the fish tissue needed to be preserved and processed. All of our experiments involved visualizing cells from thin sections of zebrafish tissue mounted on glass slides, prepared as follows: First, the euthanized larvae were collected in a 1.7 mL Eppendorf tube filled with 4% paraformaldehyde (a chemical used for tissue preservation) diluted in PBS-T, phosphate buffered saline containing 0.1% Tween-20, a mild detergent to preserve the integrity of the tissue. The larvae remained in this solution for 2 hours at room temperature, or overnight at 4°C. After this fixation process was complete, the fish were rinsed in PBS-T three times for five minutes each.

For the fluorescent antibody labeling experiments, the fish were embedded in a gel matrix prior to sectioning. This method allowed us to view multiple cellular layers of tissue under high magnification. Solutions of increasing sucrose concentrations were

added to the tubes containing the fish. Sucrose stiffens the cell membranes, further ensuring preservation of tissue shape and orientation, and prevents tissue damage from the freezing temperatures required for processing in a later step. The fish were then embedded in a molten agarose-sucrose solution which cools slowly, allowing time for the tissue to be placed in the medium in the desired orientation before the matrix solidifies. Once set, the gelatin blocks containing the fish tissue were placed in 30% sucrose overnight at 4°C, until the blocks were saturated with the sucrose solution, again as a protective step for the freezing process to come. The blocks were then frozen in liquid nitrogen and maintained at a temperature below freezing while being sliced into 16-micron sections with a sharp metal blade. In addition to suspending the tissue in the desired orientation, the frozen gelatin matrix allows for evenly cut sections that are transferred to microscope slides. The slides were air dried and either used right away or stored in the freezer for future experiments.

For the toluidine blue staining experiments described in this thesis, a different sectioning method was required. Individual photoreceptors are about 2 microns thick, so the 16-micron sections of the agarose slides contain multiple layers of cells stacked on top of one another, making it difficult to visualize single cell layers and, in particular, whether any cells are missing from these layers. The procedure to obtain a section thin enough to incorporate only a single layer of photoreceptors is similar to that previously described, except the embedding medium is a water-soluble acrylic resin rather than agarose. After embedding the fish tissue, the blocks of liquid resin were hardened at 55°C for 24 hours. Then, using a sharp metal blade, the blocks were cut into 2-micron sections, and transferred to microscope slides.

2.5 Fluorescent Antibody Labeling

2.5.1 Primary Antibody Labeling

To evaluate the effect of hexafluoro on retinal cells, we used a variety of fluorescent markers to label proteins and structures of interest in the retinal tissue. First, the slides had to be properly prepared so that the reactive solutions used would bind selectively to the target tissue. The area of the slide containing the sectioned tissue was circled using a hydrophobic marker, which creates a barrier so that when solution is applied, it covers the tissue without running off the edge of the glass slide. Next, to rehydrate the tissue on the slides, they were washed for 5 minutes in PBS-T. Following the wash, blocking solution was added to each slide using a micropipette. A blocking solution is used to stabilize the tissue and increase the binding specificity of the reactive solutions. We prepared a blocking solution of 10% goat serum, 1% bovine albumin, and PBS-T. The slides were placed in a humid chamber, a plastic box with a lid containing racks to hold the slides, and lined with absorbent paper saturated with PBS-T. This set-up ensures that the solution covering the tissue will not evaporate during the incubation steps.

Following an incubation period of approximately 1 hour at room temperature, the blocking solution was removed from the slides, and the primary antibody solution was added. The antibody solution is the blocking solution into which primary antibodies targeting a protein of interest are added. We used an antibody against the protein *GNBP1,* which is specific to rod photoreceptors, diluted 1:100 in block. The slides treated with primary antibody solution were returned to the humid chamber and incubated at 4°C overnight.

2.5.2 Secondary Antibody Labeling

After the slides incubated in the primary antibody solution overnight at 4°C, they were washed four times for ten minutes each at room temperature in PBS-T to prepare for application of the secondary antibody. The secondary antibody includes a fluorescent tag and is selected to target the primary antibody used in the first incubation step (Fig. 10). The secondary antibody reveals the location of the primary antibody molecules by emitting a fluorescent signal when exposed to the correct excitation wavelength of light.

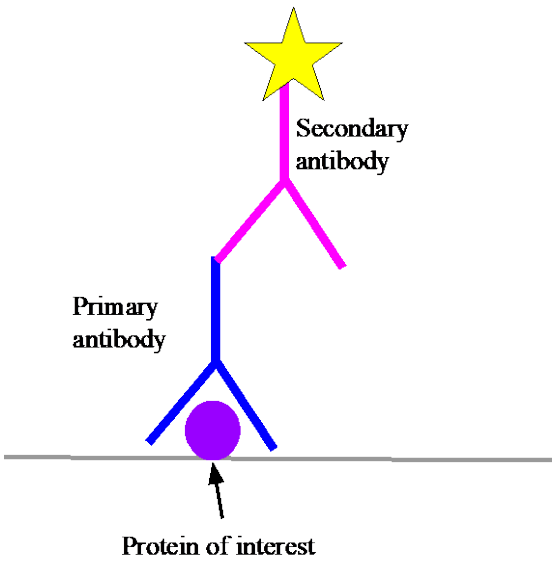

Figure 10: Cartoon depiction of a secondary antibody labeling

The primary antibody (blue) targets the protein of interest. The secondary antibody (pink) contains a fluorescent tag (yellow star), and targets the primary antibody. The protein(s) of interest can now be detected by the fluorescent signal viewed under a microscope. Figure created using Google Drawings.

Use of fluorescent tags that emit specific wavelengths allows each reagent to be visualized as a distinct color within the tissue of interest. The secondary antibody used in this experiment contained a fluorescent marker that would indicate the location of the

primary antibody bound to GNBP1 protein in rod cells. The secondary antibody was diluted 1:800. In addition to the secondary antibody, we also added fluorescently labeled phalloidin and peanut agglutinin (PNA) to the solution at dilutions of 1:100 and 1:200, respectively. Phalloidin is a chemical commonly found in poisonous mushrooms that binds to actin, a protein found in numerous cell types throughout the body, including the calyceal processes of photoreceptors. PNA binds to the membranes in the outer segments of cone cells. Both the phalloidin and PNA molecules that we used were coupled with fluorescent markers of complementary wavelengths so that, along with the secondary antibody bound to GNBP1, all three signals could be detected in separate wavelength channels when viewing and imaging.

From this point forward in the fluorescent labeling procedure, the slides were kept in the dark to avoid degradation of the fluorescent molecules by light. The slides treated with this solution were returned to the humid chamber and placed in the dark overnight at 4°C. The following day, the slides were washed 4 times in PBS-T, treated with a solution to prevent fading of the fluorescent signals, and covered with a coverslip. The slides were kept in the dark at 4°C and then imaged using a Leica SP8 Inverted scanning confocal microscope, which captures fluorescent signals using a laser calibrated to specific excitation wavelengths.

2.6 Toluidine Blue Staining

The slides stained with toluidine blue were used to count the number of photoreceptors in drug-treated mutant fish and all experimental controls. Toluidine blue is a dye with a high affinity for acidic tissue components (Sridharan et al., 2012). Photoreceptor cell bodies contain DNA and RNA, which are acidic molecules, causing

them to stain more darkly than the surrounding tissue, allowing for easy location and quantification of photoreceptor cell bodies. Tissues with lower acidity, such as cartilage and fat, are stained lighter shades of blue and purple based on their acidic profile.

The toluidine blue solution was prepared by dissolving 1 g of borax and 1 g of toluidine blue in 100 mL of water. A plastic pipette was used to transfer a few drops of the solution onto the microscope slides to cover the sectioned tissue. The slide was placed on a heat block for about one minute, or until the edges of the blue solution started to dry, after which slides were washed in several changes of deionized water to remove excess stain. Next, the slides were dehydrated in increasing concentrations of ethanol followed by clearing in xylene, a chemical solvent that makes the tissue more transparent so that everything other than the specifically stained regions is clear and colorless. Following the dehydration and clearing process, slides were treated with a mounting medium (Permount) and cover-slipped.

Sections containing a clear view of the optic nerve were selected for imaging on a Leica DMLB compound light microscope, and images were captured using the DFC310 digital camera and associated software. The optic nerve acted as a reference point to ensure that photoreceptor counts from different eyes were from the same region of each eye, and thus could be reliably compared to one another. Another set of slides was imaged on an Aperio VERSA200 imaging system, after which the imaging software QuPath was used to view scanned images of the toluidine blue slides and select sections with optic nerves for further analysis. ImageJ, an image processing software, was then used to count photoreceptor cells from the selected images.

2.7 Statistical Analysis

The results of all quantitative experiments presented in this thesis were analyzed by calculating the mean average, standard deviation, and number of data points in each group and comparing these values to one another to determine statistical significance. Statistical significance is a comparative value that determines whether differences between groups (such as photoreceptor cell numbers in control vs. mutant fish) are caused by chance or by a specific cause, in this case the drug being applied. Significance is typically expressed as a p-value, or probability value. A comparison between two groups with a p-value less than 0.05 is considered statistically significant. The smaller the p-value, the more statistically significant the results. For all quantitative experiments in this thesis, data points were plotted and analyzed using GraphPad Prism statistical software. P-values were calculated by applying a Mann-Whitney nonparametric test.

3 Results

3.1 Hexafluoro does not exacerbate balance or hearing deficits in *pcdh15b* mutants

Prior research in the Westerfield lab showed a steep dose-response for hexafluoro toxicity in wild-type larvae, and a dose of 0.11 micromolar was found to be the maximum concentration of hexafluoro that could be delivered without adverse effects. In wild-type fish, concentrations even slightly in excess of this resulted in swimming and balance defects. Given that these symptoms are already present in *pcdh15b* mutant larvae, it was important to test whether they might be more sensitive to the maximum tolerated dose of hexafluoro than wild-type fish.

Each day starting at 5 dpf when we changed the media in the dishes, we also observed the swimming behavior of the larvae and tested the startle response of each group. If we observed mutants that did not respond to the startle test, or which had more difficulty swimming beyond the looping pattern that is typical for mutant fish, we might suspect that the drug was impairing hearing or exacerbating balance problems. According to recessive mendelian inheritance, we expected 25% of the larvae to exhibit the 'looping' swimming pattern when startled. Therefore, if we observed more than 25% with this behavior, this could indicate that hexafluoro was worsening the balance of the sibling fish. Based on this trial, we did not see any elevation in the number of larvae exhibiting circling behavior, nor did the drug worsen balance or startle response in the mutant larvae.

3.2 Hexafluoro does not exacerbate retinal defects in *pcdh15b* mutants

To visualize the photoreceptors of the drug-treated and control larvae, we performed fluorescent labeling on sectioned tissues (Fig. 11).

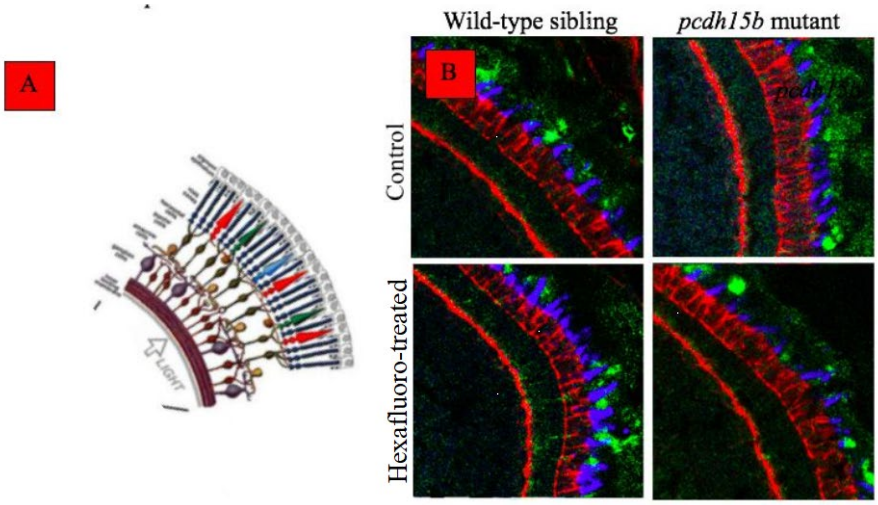

Figure 11: Fluorescent labeling of control and hexafluoro-treated wild-type and *pcdh15b* mutant zebrafish retinal tissue

(A) Cartoon depiction of retinal cell layers. Source: Webvision. (B) Confocal images of *pcdh15b* fish retinal tissue. The protein actin is labeled in red. Cone outer segments are labeled in blue. Rod outer segments are labeled in green. The cone cells (blue) in the wild-type fish are uniformly structured and oriented, whereas the cone cells of the *pcdh15b* mutants are sparser and disorganized.

We observed no differences between the rod cells (green) or cone cells (blue) between the control and hexafluoro-treated wild-type fish. Images of the *pcdh15b* mutant retina show the abnormal arrangement of cone outer segments (blue) previously characterized in *pcdh15b* mutants. Actin (red) was labeled to analyze the calyceal processes. The calyceal processes in both untreated and hexafluoro-treated *pcdh15b* are both notably disorganized when compared to control fish, similar to what is seen in Figure 5. The rods, cones, and calyceal processes of the hexafluoro-treated *pcdh15b* mutant fish show similar abnormalities to the control *pcdh15b* mutant fish, confirming

that hexafluoro neither exacerbates nor improves the photoreceptor structural defects. We next analyzed the effects of hexafluoro on visual function and photoreceptor survival in *pcdh15b* mutant fish.

3.3 Hexafluoro-treated *pcdh15b* mutants show improved visual response compared to untreated *pcdh15b* mutants

We used the optokinetic response assay to test the ability of the experimental and control group fish to track moving targets, recording the number of tracking eye movements, or saccades, in response to the rotating visual stimuli (Figure 12).

The sibling fish, whether treated or untreated, followed an average of 10-10.5 black stripes within the drum during each test period. Their eyes locked onto a stripe and swiveled to follow it until it reached the limit of their peripheral range, then snapped back to track the next stripe coming into view. These slow, coordinated eye movements are known as saccades. In contrast, the majority of the eye movements recorded from *pcdh15b* mutants treated with DMSO were abnormal, short arc movements rather than full saccades. These eye movements were much quicker and twitchier than their siblings, making it apparent that they were not properly tracking the black stripes within the rotating drum. Most mutants also exhibited a low number of more normal saccades, an average of 2.5. For this group, we counted both normal saccades and abnormal eye movements.

In contrast, the visual response was improved in hexafluoro-treated mutants. Although the number of normal saccades was still reduced compared to the sibling groups, the number was significantly greater than what we observed in untreated

mutants with an average of 8 saccades, and the abnormal twitch-like eye movements were completely absent.

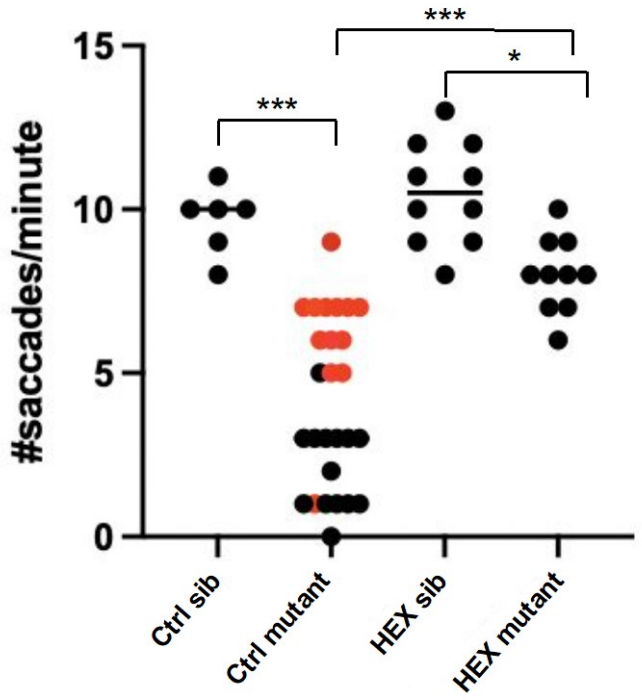

Figure 12: Optokinetic response assay results

Each dot represents the number of saccades recorded from one fish during the 1-minute testing period. There are two saccade values collected per fish (CW and CCW). Additionally, there are two different values graphed for mutant fish treated with DMSO: normal saccades are represented by black dots and abnormal short arc twitches are represented by red dots. *** = p-value of <0.0001; * = p-value of < 0.05.

3.4 Hexafluoro-treated *pcdh15b* mutants show a slight increase in the number of photoreceptor cells retained in the central region, although barely below statistical significance

After assessing difference in visual function between hexafluoro-treated and untreated *pcdh15b* mutant fish, we sought to understand whether there was a difference in photoreceptor cell death between these groups that would explain the results from the OKR assay. Photoreceptor cells in the dorsal, central, and ventral portions (Fig. 13) of

the zebrafish retina were stained with toluidine blue and counted to see whether there was a significant difference between the number of photoreceptors between hexafluoro-treated and untreated *pcdh15b* mutant fish.

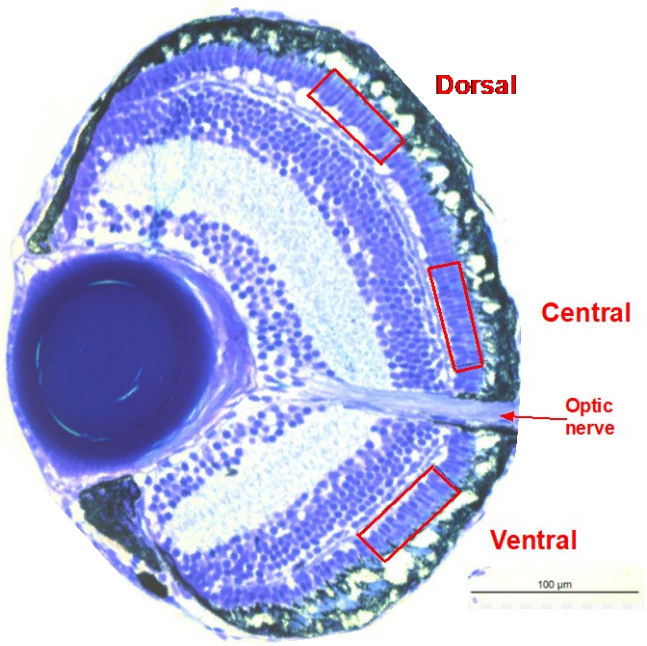

Figure 13: Dorsal, central, and ventral portions of the ONL used to count photoreceptors.

Magnified zebrafish retinal tissue stained with toluidine blue. Red boxes denote regions in which photoreceptors were counted (dorsal, central, and ventral). Each region of interest in 50 μm in length and 17-20 μm in width, depending on the width of the ONL.

There was a significant difference in photoreceptor cell counts between control siblings and control mutants in all regions (dorsal, central, and ventral), as well as between hexafluoro-treated siblings and hexafluoro-treated mutants (Fig. 14). In two replicate experiments, we saw an average increase of 4 photoreceptor cells in the central region of treated mutants compared to untreated mutants, 11-15% more photoreceptor cells compared to untreated mutants. However, given the variability in cell counts, this small increase did not rise to the level of statistical significance.

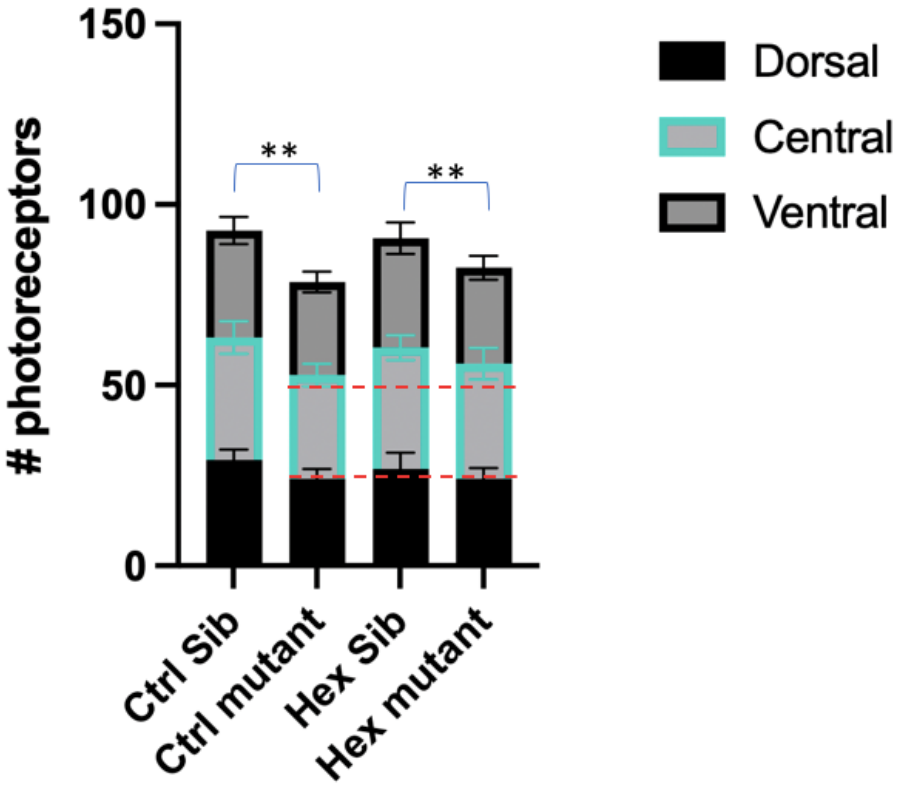

Figure 14: Photoreceptor survival in hexafluoro-treated zebrafish

The shaded regions within each bar represent average photoreceptor counts in each designated region of the eye. The red lines highlight the difference between photoreceptor counts in the central region of control mutants and hexafluoro-treated mutants. N≥ 12 for each condition. ** = p-value of < 0.005. P-value between the central region of control mutants and hexafluoro-treated mutants was 0.06.

4 Discussion and Conclusion

Based on our assessment of swimming behavior and retinal health, we conclude that the hexafluoro dosage is safe and well-tolerated by *pcdh15b* mutants. These results provide important safety data that will inform future clinical trials.

These studies also demonstrated that, even at this small dose, hexafluoro is biologically active in zebrafish larvae. Hexafluoro treatment partially restored normal optokinetic responses in *pcdh15b* mutants while completely resolving the abnormal eye movements seen in untreated mutants. We interpret this improvement in visual function as evidence that hexafluoro has a stabilizing effect on photoreceptors that lack the full length Pcdh15b protein.

Our photoreceptor cell counts showed that hexafluoro had only a small effect on photoreceptor survival. The increase in visual function seen in the OKR assay in addition to the minimal cell rescue seen in mutants suggests that improvement in visual function is not dependent on a large increase in photoreceptor density. Furthermore, this experiment confirms that visual function in our zebrafish model of early USH1F disease pathology is more reliant on photoreceptor functional competence rather than ONL cell density. This conclusion is consistent with data seen in the USH1F mouse model: USH1F mice treated with 9-*cis* retinal showed improved visual function when compared to controls (Sethna et al., 2021). These two studies taken together suggest that although the application of exogenous drugs does not repair the functional abnormalities seen in USH1F photoreceptors, they seem to stabilize function downstream of Pcdh15. This thesis provides evidence that drugs, such as hexafluoro,

have the potential to extend the period of functionality of photoreceptors, and therefore visual function, in USH1F patients.

A key element of these results is that they were obtained from fish raised in a low light setting, which has shown to be protective against retinal degeneration in *pcdh15b* mutants (Phillips et al 2021, Miles et al 2021). Remarkably, even in this protective environment, hexafluoro treatment resulted in significant visual function improvement, and slight improvement in retinal degeneration. These preliminary hexafluoro findings will help to guide future research toward finding a treatment for the retinal degeneration seen in USH1F.

5 Future Research

The results of the experiments described in this thesis provide significant data to design further studies examining the effect of hexafluoro on retinal cell function and survival.

One important avenue of inquiry is to investigate how hexafluoro functions at the molecular level. Our toluidine blue results provide information about the level of cell death in the retina, but not the overall health of the surviving photoreceptor cells. It would be beneficial to determine the level of ROS in the ONL of treated and untreated *pcdh15b* mutant fish. This can be done by using a probe that reacts with ROS to produce a fluorescent signal, such as dihydroethidium (DHE) or CellROX. Furthermore, several models of RD have shown that multiple cell components are sensitive to oxidative damage in the degenerative retina, especially in the photoreceptor layer. Antibody labeling can be used to assess the levels of oxidized lipids, proteins, and nucleic acids in both hexafluoro-treated and untreated mutants (Murakami et al., 2020). Overall, elucidating the mechanism that underlies the functional improvements noted in treated mutants will be essential to justify moving hexafluoro toward a human clinical trial.

Given the low light conditions of the experiments described in this thesis, the limits of hexafluoro's rescue ability are unknown at this time. Thus, an important next step would be to repeat the same drug trials at higher light levels. We may find that, with more light-induced photoreceptor loss in the untreated mutants, the preservative effect of hexafluoro will become statistically significant. Alternatively, hexafluoro may

not be able to out-compete the more rapid retinal degeneration characteristic of this mutant raised in elevated light conditions.

The experimental design to test the effect of various light intensities on hexafluoro-treated fish would be similar to the design presented above. However, in addition to the group raised at 40 lux, there will also be a group raised in regular facility lighting (400 lux), and a group raised in light levels equivalent to being outdoors on a cloudy day (5000 lux). This experiment will enable us to detect differences in the photoreceptor rescue of hexafluoro between low and high light conditions, and also determine whether there is a point at which hexafluoro cannot keep up with the cell loss in *pcdh15b* mutants associated with more intense light conditions. Experiments that take light intensity into consideration are important because people with USH1F experience a variety of light conditions throughout their lives. The goal of therapeutic interventions, such as hexafluoro, is to preserve or restore the quality of life for people with USH1F, and this includes outdoor, daytime activities. Testing the effect of light intensity will lead to results that are more applicable to human beings with USH1F. In addition, the USH1F zebrafish model is the only USH model in the Westerfield lab collection with photoreceptor death evident at low light levels. Therefore, we need to establish hexafluoro treatment protocols at higher light intensities so that it can be applied to other models of USH. Further experimentation with hexafluoro will reveal more about the potential for antioxidant therapies in rescuing retinal degeneration, and how this intervention could be beneficial for people with USH1F.

Glossary

CMZ	Ciliary marginal zone
DMSO	Dimethyl sulfoxide
dpf	Days post fertilization
EM	Embryo medium
GCL	Ganglion cell layer
INL	Inner nuclear layer
IPL	Inner plexiform layer
IS	Inner segment
OKR	Optokinetic response
ON	Optic nerve
ONL	Outer nuclear layer
OPL	Outer plexiform layer
OS	Outer segment
PCDH15	Human USH1F gene
PCDH15	Human Protocadherin15 protein
pcdh15	Zebrafish/mouse USH1F gene
Pcdh15	Zebrafish/mouse Protocadherin15 protein
PNA	Peanut agglutinin
R245X	USH1F gene mutation prevalent in Ashkenazi Jewish population
RD	Retinal degeneration
ROS	Reactive oxygen species
RPE	Retinal pigmented epithelium
USH	Usher syndrome
USH1	Usher syndrome type 1
USH1F	Usher syndrome type 1F

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