Usher Syndrome Coalition | Developing a drug therapy for both vision and hearing loss in individuals diagnosed with Usher III, Usher III Initiative

The following presentation on the history and progress of the Usher III Initiative was written by its President and Co-founder Cynthia Elden, and is being read for you by Georgia Horn, a member of the Usher III Team.

When I was five years old, I began experiencing mild hearing loss in one ear, which doctors treated with a single hearing aid, and later with auditory rehabilitation to strengthen my speaking skills. At that time in the early 1970s, genetic testing was not available to us. Instead, my doctors could only hypothesize that my hearing loss was the possible result of my mother's exposure to the German measles during her pregnancy. It would take many years to figure it out, but my hearing loss was actually the result of Usher syndrome.

Though first documented anecdotally in the mid-19th century and identified clinically in the early 20th, knowledge of this disease, its etiology and manifestations, has progressed slowly. In fact, Usher III was not even identified as a distinct genetic mutation of the syndrome until the 1980s. And Usher IIIA, the mutation I have, was only discovered in the late 1990s, almost 30 years after I first experienced hearing loss at age five.

When I was 12, my auditory function declined dramatically, this time in both ears. And in addition, although I was not physically aware of it at the time, my eyesight was declining as well throughout this period. Over the subsequent three years, I underwent a battery of neurological and hearing tests to try and determine what had caused the significant deterioration in my hearing.

In one test, a neurologist performed a simple exercise to study my peripheral vision by moving her fingers to the side of my head and asking me to count how many she was holding up. I couldn't see her fingers at all. Lack of peripheral vision and night blindness are both telltale early signs of Retinitis Pigmentosa, or RP. My parents were told that with RP, my field of vision would progressively reduce to a small pinhole, a condition known as tunnel vision, an illustration of which is shown here, and that I would begin to experience night blindness. I remained unaware of the diagnosis until I was 18.

Because there were no doctors specializing in RP in Chicago at the time my parents initially received my diagnosis, my father, Richard Elden, became determined to find the best retina specialist in the country. This led my parents to Dr. Elliot Berson, a Harvard Medical School professor, clinician at Massachusetts Eye and Ear Hospital, and one of the pioneers in 20th century research on inherited retinal diseases. Based solely on the clinical characteristics described in my case history, Dr. Berson

was the first clinician to recognize that my symptoms fit the profile of Usher syndrome. He gave my parents a probable diagnosis of Usher II. I did not learn about my diagnosis until I went to Boston to meet with Dr. Berson at age 18.

While the component of auditory loss in Usher syndrome came as no surprise, prior to this visit I had no concept of the fact that what I saw was literally different from what my parents or my peers could see. Growing up in the brightly lit city of Chicago, I had been able to go out at night without many problems and never needed to get a driver's license. Although I could sometimes be clumsy, tripping on curbs or missing steps, I had no reason to believe that this clumsiness was the result of visual problems.

During this meeting, Dr. Berson turned off the lights in his office. And when I was the only person in the room unable to identify the number of fingers my parents were holding up, it was the first time I realized that I saw dramatically less than others. It is a parent's natural inclination to want to protect their child, and I realized that by keeping my diagnosis from me my parents were trying to do just that. But I believe that despite their best intentions, my parents would have done me a greater service by being honest with me as soon as they had learned of my diagnosis.

There is no guidebook on how best to tell your child that they will go both deaf and blind one day, but based on my own experience, I am a big supporter of transparency. I had grown up knowing about and dealing with my hearing loss, and during this period I developed many of the complex skills necessary to navigate the world without full auditory function. I felt that by not giving me the opportunity to apply these tools to my full diagnosis as soon as they had received it, my parents had underestimated the strength of my coping mechanisms and my own resilience. I believe that if your child is strong enough and smart enough to ask the questions, they should be given honest answers.

I was in my 30s before I was finally diagnosed correctly with Usher III. Usher III is the rarest type of Usher syndrome, making up about only 2% to 5% of Usher syndrome cases worldwide. In general, the three types are distinguished by three factors-- age of onset, severity of sensory loss, and impact on vestibular function. The chart on screen outlines a simple summary of these distinctions. Usher III tends to manifest later in life, between adolescence and mid-age.

Within each type, there are also subcategories that reflect the specific genetic cause. Because Usher syndrome is heterogeneous, even within these subcategories the way the disease impacts each individual patient is not always consistent. These factors make treatment extremely complicated. I received my Usher III diagnosis shortly after it was first distinguished as a separate genetic mutation,

and this meant that there was very little research available. The dearth of information was compounded by the fact that it is an extremely rare disease.

From the time I first experienced hearing loss, my father had worked tirelessly to understand my condition and seek out treatment options. After my Usher III diagnosis, we amplified these efforts by establishing the consortium that would later become the Usher III Initiative in 2007. My father, pictured here, is no longer with us, but I am tremendously grateful for the perseverance and determination that he instilled in me and the Initiative team, without which we never would have made it this far.

As an investor, my father pioneered low-risk diversification by establishing the first fund of funds in the United States. He applied this methodology to medical research as we embarked on the work of finding a treatment for Usher III by supporting multiple treatment techniques, including stem cell, gene therapy, and small molecule development, until one became more obviously viable than the others. In short, as we started, the ethic of the Usher III Initiative was to diversify our efforts.

Stem cells were at a very early stage then and seemed to require a longer timeline than desired, so they were ruled out early on. But gene therapy and small molecule development both showed promise and a more desirable timeline for getting to patients as quickly as possible. Following the example of the Prostate Cancer Foundation, the Initiative's model emphasizes collaboration over competition. We assembled a brilliant team of researchers from six organizations. The idea behind the consortium was to accelerate the pace of progress by incentivizing data sharing between several experts across the world.

Previous studies had established that Usher III is caused by single-point mutations in a clarin-1 gene, which encodes the clarin-1 protein. Clarin-1 protein is found in the retina and hair cells in the inner ear. The retina and the hair cells perceive external sensory signals, namely light, sound, and movement, and convert these signals into electrical impulses which are then transmitted to the brain, allowing us to see, hear, and balance. We believe that clarin-1 plays a crucial role in maintaining the structure of these sensory cells, which determines their ability to successfully perceive the external environment and communicate with the brain.

Although the mechanism of action is not yet fully understood, researchers have observed a clear correlation between defective clarin-1 and the deterioration of both vision and hearing, and occasionally balance. There are 15 known pathogenic mutations in clarin-1 that have been reported worldwide. The two most common single mutations are the founder mutations pY176X in the Finnish

population and the pN48K mutation in North America, which is particularly prevalent amongst those of Ashkenazi Jewish descent. I was diagnosed with Usher IIIA due to N48K mutated clarin-1.

The diagram on screen shows the position of this mutation, as well as other mutations in the clarin-1 protein. Clarin-1 is present at the cell surface and is comprised of a chain of 232 amino acids. All known amino acids are abbreviated by a single letter. N48K refers to the replacement of the amino acid asparagine, abbreviated to the letter N, with lysine, K, at position 48, the glycosylation site, shown here in yellow.

For most proteins during synthesis, sugar is added to specific amino acids in the protein, a process known as glycosylation, which increases the protein's stability and enhances its function. Unfortunately, in the N48K mutation, glycosylation cannot be carried out, thus altering the three-dimensional structure of the mutated clarin-1 and causing it to fold improperly, further decreasing its stability and preventing its transportation to the cell's membranes, where it must be localized in order to perform its function.

This diagram illustrates clarin-1's failure to translocate to the cell's surface. Building on the existing genetic research, the consortium's first hurdle was to better understand the pathway of the disease, how this single genetic abnormality leads to the progressive loss of hearing and vision. The key was to understand the behavior and function of the mutated gene product, i.e. clarin-1 protein. Our researchers began by studying clarin-1's synthesis, transportation, and function compared to unmutated clarin-1 through biochemical, cellular, and animal studies. From a treatment perspective, Usher III is a good candidate for gene therapy as it's driven by single genetic mutations.

Gene therapy is a way of instructing mutated cells to produce healthy proteins. In order to achieve this, scientists must design a vector, typically a virus, that when injected directly into the problem site is able to penetrate the cell and reach its nucleus without killing the cell itself. Promising early efforts to develop gene therapy were spearheaded by Dr. William Hauswirth, a brilliant biochemist with previous experience using gene therapy to treat degenerative ocular diseases, and Dr. Lawrence Lustig, an innovative otolaryngologist with expertise in the study of inherited genetic defects in the ear.

Together with their colleagues, they were able to demonstrate that gene therapy, using a single injection of the viral vector they designed, could effectively preserve the hair cells in the cochlea in an Usher III mouse model. Unfortunately, mouse models do not display the visual phenotype of the disease. Had we continued to pursue gene therapy, this would have meant that in order to deliver a

therapy that addressed both senses, we would need to start over in order to try and find, characterize, and test a viable animal candidate with the visual phenotype.

In addition, gene therapy can only be delivered by localized injections at each target site. There are only a few clinics capable of injecting the retina and inner ears, and the process is unpleasant for patients and carries a degree of risk, such as infection. When we started, gene therapy was still in its early stages of development. There were many vital questions that remain to be answered, and there was no FDA approved gene therapy for the eye. For all these reasons, the Initiative decided to focus on developing our program of a small molecule.

In comparison to gene therapy, the process for small molecule development had been refined and studied for almost 100 years, since the discovery of penicillin in 1928, but this does not mean that it is straightforward or simple. Finding a small molecule is like looking for a needle in a haystack. First, via a high throughput screening of over 5,000 molecules, we identified a small handful of candidates to test individually. Eventually, this led to a single hit that the Initiative then optimized, BioFocus 844, or BF844.

The molecular structure of BF844 is shown on the left-hand side of this slide. Once the compound had been optimized, two members of the consortium, Dr. Yoshikazu Imanishi, an accomplished retina specialist, and Dr. Kumar Alagramam, a molecular biologist with expertise in otolaryngology, began with in vitro assays to examine the cellular distribution of clarin-1. Then they used a transgenic mouse model of the N48K mutation to confirm the degradation of the hair cells and measure the impact BF844 had on hearing loss.

To assess hearing in a mouse, they first determined thresholds for the Auditory evoked Brain Responses, or ABR, which measures the time it takes for the brain to respond to ear sound stimulation. By recording the brainwaves generated from sounds at different frequencies in anesthetized mice, they established the controls for the study. They measured the mices' hearing at postnatal days 22, before drug administration, 46, and 55. BF844 was administered intraperitoneally once daily at 30 milligrams per kilogram from postnatal day 30 to day 45.

The chart on screen illustrates how BF844 significantly improved the thresholds for the auditory evoked brain responses measured in decibel Sound Pressure Level, or SPL. At postnatal day 55, healthy mice responded to a sound pressure level as low as 35 decibels, approximately equivalent to a whisper. By comparison, untreated Usher III mice required the use of high-intensity sound, about 100 decibels, which is roughly equivalent to the sound in a wood shop, in order to stimulate any type

of response.

Mice that had been treated with BF844 had markedly improved hearing capacity. A 30 to 40 decibel level triggered a visible response in brain activity. At postnatal day 55, treated mice showed a 30 to 40 decibel sound pressure level lower threshold compared to that of untreated mice, indicating a 1,000 to 10,000-fold increase in sensitivity to sound across different frequencies. These findings were published in the peer-reviewed scientific journal *Nature Chemical Biology* in 2016.

BF844 was designed to provide a systemic therapy that rescues mutated clarin-1 in both the eye and the ear. BF844 corrects the dysfunctional behavior of the mutated protein by stabilizing it and helping it to exit the endoplasmic reticulum and locate to the plasma membrane. This mechanism of action has been shown to preserve the hair cells in the ear, and we believe it will have the same impact in the retina.

Additional in vitro experiments demonstrated that BF844 has the ability to rescue mutations in rhodopsin, an important pigment protein that is extremely sensitive to light, and thus enables vision in low-light conditions. The mutated rhodopsin leads to defects in glycosylation and triggers progressive retinal degeneration. BF844 stabilized and increased the expression of glycosylation-deficient rhodopsin, further supporting the theory that it should be as effective in preserving vision in Usher III patients as it was with hearing.

Finally, we have determined that when administered orally, BF844 is capable of crossing both the blood-cochlear and the blood-retinal barriers, meaning that it reaches the nervous system. This is very significant because many drugs aren't able to do so.

We have achieved remarkable progress, and today we are closer than ever before to treating this disease. After 18-plus years funding basic science research at over six academic institutions and developing the assay for our novel compound, we are now working with a team of chemists and drug developers to take our next steps. Our dedicated internal team, board members, and scientific advisors have made it possible to get this far. Each member of the team is truly outstanding, and I am tremendously grateful to them.

So what's next? We are in the process of applying to meet with the FDA prior to IND submission in order to discuss the remaining preclinical toxicology studies that are necessary to start clinical trials, which we have begun designing. We will continue to optimize and scale up material production of BF844 to meet the FDA's Good Manufacturing, or GMP, standards.

We are aware of the challenges ahead. From start to finish, it costs about $2 billion and takes an average of 12 years to develop a small molecule into a drug therapy. We have made incredible progress in this endeavor. And as a non-profit, we are able to partner with researchers across academia and the private sector in order to accelerate this process. As our costs increase, we have expanded our network and have begun seeking out new avenues for funding and potential partnerships.

I hope that other families and patients with Usher III will continue to join me and work towards this potential reality of treating patients who are losing their hearing and vision to Usher III. If we do not work together and try to get this done, nobody else will. This is a critical time for the Usher III Initiative, and I believe we have a real shot at treating this in the near future. Together, by encouraging patients to get genotyped and register with the Usher Coalition, supporting medical research through donations, increasing awareness of the disease, and continuing to collaborate with specialists across the board, there are so many ways each of us can help work to make this happen.

Thank you for listening, and please consider connecting with us via the Usher III website shown here. We would love to hear your feedback and answer any questions. Please feel free to learn more there, where you can read articles and interviews, get involved, and donate directly to Usher III research. Thank you to our incredible staff, Dr. Mahdi Farhan, Tracey Fletcher, and Georgia Horn.