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The *USH2A* Gene: An Analysis of Ultrasonic Vocalizations in a Mouse Model of Usher Syndrome Type 2

An Honors Thesis

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Introduction

Usher syndrome (USH) is a complex, rare autosomal recessive genetic disorder that is presented in humans and manifests in its most common form as inherited deaf-blindness [1]. The genetic disorder is characterized by variable degrees of sensorineural hearing loss (SNHL), retinitis pigmentosa (RP), and in some cases, vestibular dysfunction [2]. Usher syndrome has a prevalence of about 1 in 10,000, indicating that it is the most common form of combined deaf-blindness that plagues the human population [1]. The syndrome presents as three clinical types (Usher syndromes type 1, 2 or 3). Patients are classified into the three types based on the diverse clinical symptoms that are observed by medical professionals. This classification is mainly based on the severity and progression of the hearing loss, and the age of onset for the retinitis pigmentosa [2].

Usher syndrome was very first described in the year 1858 by Albrecht von Gräfe, who is considered to be a pioneer of modern-day ophthalmology. He reported a case of deaf patients with retinitis pigmentosa, whose two brothers had the same exact symptoms [11]. A few years after that, one of von Gräfe's students, Richard Liebreich, examined the entire population of Berlin for an identifiable disease pattern in people with retinitis pigmentosa and deafness. Through extensive study, he found the syndrome to be recessive [12]. The syndrome was named after Charles Usher, a British ophthalmologist who established the heritability, pathology and transmission of this illness in the year 1914 [11].

In Usher syndrome type 1, patients diagnosed are defined as having congenital severe to profound sensorineural hearing loss and vestibular areflexia. Retinitis pigmentosa has an onset typically within the first decade of life for this type [2]. In Usher

syndrome type 3 patients, the known symptoms such as hearing loss, vestibular dysfunction and onset of retinitis pigmentosa are progressive, sporadic and variable, respectively [2]. In Usher syndrome type 2, the focus of this study, patients display congenital moderate to severe sensorineural hearing loss. Overall, patients have normal vestibular function and an onset of retinitis pigmentosa within the second decade of life (unlike type 1, which is within the first decade of life) [2, 3]. Usher syndrome type 2 is the most common form of Usher syndrome. It accounts for about 50% to 65% of all cases of Usher syndrome, whereas Usher syndrome type 1 accounts for only 10% to 35% of all cases [2]. Usher syndrome type 3 is quite rare, and accounts for only 2% to 5% of cases [2].

Retinitis pigmentosa is a condition where the photoreceptor cells begin to degenerate, primarily from the outer periphery to the center of the retina. First, patients afflicted tend to experience night blindness . Then, they experience a loss of peripheral vision. As retinitis pigmentosa progresses, the patient's field of vision narrows until only central vision remains [4]. This degeneration experienced is generally attributable to rod dysfunction, though cones may degenerate later in the course of development. Other clinical symptoms of retinitis pigmentosa may include, in addition to those listed above, abnormal electroretinogram responses, abnormal retinal pigmentation including peripheral bone spicules, arterial narrowing, optic-nerve pallor, and predisposition to myopia and posterior subcapsular cataracts [11].

Sensorineural hearing loss, on the other hand, is often caused by abnormal development of hair cells in the inner ear. The inner ear consists of the cochlea and the vestibular labyrinth. The former mediates sound transduction, and the latter detects gravitational force and angular and linear accelerations. Both contain hair cells which aid in

converting mechanical stimuli into afferent nerve signals toward the brain. These hair cells contain, on their apical surface, a hair bundle which contains stereocilia, which consists of organized actin-filled projections. The sensorineural hearing loss and potential balance defects in Usher syndrome result from the alteration of stereocilia [5, 11].

Usher syndrome is inherited as an autosomal recessive disorder, meaning both parents must contribute a mutation for this syndrome to appear, and it must be on a chromosome pair that is not one of the sex chromosomes. Currently, there are 12 genes known to researchers that are implicated in causing Usher syndrome. There are six genes that, when mutated, are linked to Usher syndrome type 1. They are as follows: *MYO7A, USH1C, CDH23, PCDH15, USH1G, CIB2* [2]. Mutations in the *MYO7A* gene, which encodes myosin VIIA [12], are responsible for up to 70% of cases of Usher syndrome type I [2]. However, despite its high correlation with type 1, *MYO7A* mutations are not correlated with Usher syndrome type 2 at all, which is less severe than type 1 [6]. Mutations in three other genes, *USH2A, GPR98* and *WHRN* are known to cause Usher syndrome type 2. Specifically, mutations in *USH2A* account for 85% of Usher syndrome type 2 cases, making it the most common mutation for this type. Mutations in the *GPR98* gene, which encodes for the G-protein-coupled 7-transmembrance receptor CLGR1 [11], account for only 6% of Usher syndrome type 2 [2]. In the case of Usher syndrome type 3, *CLRN1* is the most frequent mutated gene. In addition to the genes listed above that are linked to a specific clinical classification of Usher syndrome, there are two genes that when mutated, correlate with atypical cases of the syndrome that do not fit in the classifications. The genes are: *CEP250* and *ARSG* [2].

As mentioned above, the gene *USH2A* is most implicated for Usher syndrome type 2. The *USH2A* gene is responsible for encoding two isoforms of the protein usherin [7]. Usherin contains laminin EGF motifs, a pentaxin domain, and many fibronectin type III motifs [8]. This protein is a very important component of basement membranes, which are very thin, sheet-like structures that support and separate cells in many tissues. Specifically, usherin is found in basement membranes in the inner ear and in the retina (which is a thin layer of light-sensitive tissues at the back of the eye). The functions of usherin are still being studied extensively, but it is understood that the protein has an essential role in the development and overall function of inner ear hair cells and photoreceptors in the retina [9]. The gene that encodes usherin, *USH2A*, has a total of 72 exons, an overall length of 800 kb and is located at 1q41, which is the long (q) arm of chromosome 1 at position 41 [10]. There have been a very large number of mutations detected in this gene, all of which may lead to Usher syndrome type 2. One of the most common, however, is c.2299delG. It is located on exon 13, which accounts for just about 15% to 45% of all mutated alleles of the *USH2A* gene [11].

Usher syndrome is of particular importance to understand because of its public health impact. Patients with Usher syndrome may face social isolation due to their varying inabilities to both hear or see. Combined deaf-blindness can have a substantial impact on the health and educational functioning of affected children. They may have more doctor visits, more hospital-days, more time lost as school and an increased risk of repeating a grade in school [15]. Furthermore, children who suffer from Usher syndrome type 2 may have poorer self-esteem, more depression and anxiety, and more problems with learning

[16]. Therefore, it is crucial that more is understood about this syndrome to make steps towards a future where there are preventative measures that exist.

One of the first steps towards this future would be to make a correct, and early, diagnosis. The value of an early diagnosis for Usher syndrome is undeniable. While there may be adverse effects of knowing the diagnosis early in a child's life, such as hopelessness or depression, the benefits may outweigh the costs. The burden of Usher syndrome is greater than that of a hearing or visual handicap by itself – combined sensory deficit is very difficult to handle. For the parents of a child who has Usher syndrome, this may be an unfathomable burden. Since the retinitis pigmentosa does not present until the second decade of life, parents may choose to have several children without knowing the future disability that will plague them. Earlier diagnostic measures allow for informed family planning and genetic counseling. Not only that, but the nature of the retinitis pigmentosa is also dangerous. As discussed above, the very first tangible affect a child may see is the appearance of night blindness. This is very dangerous (for example, when considering outdoor activities such as bike-riding or crossing the street) and is a strong argument for earlier diagnostic measures, so children and their guardians can be prepared. Additionally, early diagnosis can assist patients in learning how to not rely on visual cues for communication. Since their vision is lost slowly and in the second decade of life, they learn to augment communication visually when only their hearing is affected. Tactile and other forms of assisted communication would be helpful to learn as a child, rather than waiting until it is necessary as a young adult. The use of assistive technologies such as braille, walking canes and service animal interactions are more easily learned by younger individuals, especially with gradual rather than sudden dependence. Lastly, an early

diagnosis can help inform children and young adults in their future careers and plans. Beginning to train for a career where vision is necessary would not be a well-informed decision. Early diagnosis would allow for greater information and support for a child who may not be aware their vision will be lost in the future [17].

In order to make steps towards an early diagnosis, a proper differential diagnosis of the syndrome is necessary. Unfortunately, there are a multitude of syndromes that may exhibit clinical signs which are similar to Usher syndrome. The best method of identifying Usher syndrome would be through a genetic test, but unfortunately research is not yet developed enough to make this occur. Not all of the genes discovered and mentioned above are directly responsible for every case of Usher syndrome. Unfortunately, there are still cases where the genetic cause is undeterminable. Furthermore, there is no current treatment available for Usher syndrome. The sensorineural hearing loss problem is supplemented with the use of hearing aids and cochlear implantation. Unfortunately, the retinitis pigmentosa has been unsolved as of yet. There are currently no therapeutic strategies to treat retinal degeneration [11]. In order to continue to develop therapeutic strategies for those afflicted, animal models must be used to uncover as much information as possible. Once the syndrome is even more understood, the scientific community can begin to develop treatments for those afflicted.

It is understood currently that Usher syndrome carriers do not exhibit any of the characteristics of the diagnosable syndrome, such as variable hearing loss, retinitis pigmentosa and potential balance defects. Heterozygotes are classified as asymptomatic, and thought to not be at risk of developing the disorder [13]. However, this idea is being questioned and studied further by a select few researchers. Carriers may in fact exhibit

subtle differences that have been mostly undetected thus far. A groundbreaking study reported some differences between 10 obligate carriers of Usher syndrome type 2 within 5 different families [14]. It was found that the carriers of the gene did exhibit sensorineural hearing loss at all frequencies, which was in excess of what was expected for their age. However, only an excess of 10 dB (on average) in hearing loss at the frequencies 0.25 – 0.5 kHz) proved to be significant. When the carriers were tested for speech discrimination scores, they were all within the hearing thresholds. There were a few other tests run that also found a general normality between the carriers and non-carriers. The researchers did discover a few vestibular abnormalities in a minority of the carrier sample, but it was not significant. A closer look at ophthalmologic findings were normal, though there were subnormal electrooculographies in ½ of the sample group [14]. In another study, researchers studied specific language impairment (SLI), which is a developmental disorder that affects an individual's spoken and/or receptive language acquisition in the absence of neurological deficits [22]. They looked specifically at copy number variants (CNVs), which are rare variants and epigenetic modifications, that may indicate the level of burden. It was found that children with SLI *and* their first-degree relatives have an increased burden of moderate-sized CNVs than population controls, and that the burden and size of CNVs did correlate with the severity of the disorder. Therefore, carriers of SLI did exhibit language problems as compared to the population controls [22]. Since these two studies, no researchers have looked at significantly whether or not carriers suffer from the characteristics of Usher syndrome in any way, though this evidence does suggest that they may exhibit deficits. This is the first focus of this study.

Deafness in infancy and childhood is well known to interfere with the normal development of speech and language [19]. Those diagnosed with Usher syndrome type 2 have moderate to severe hearing loss, but not congenital profound hearing loss. Therefore, their ability to develop speech, while more impacted than a person with no hearing loss, is less impacted than a patient with fully penetrant Usher syndrome type 2. This is the second focus of this study. Understanding if the *USH2A* gene affects vocal production in any tangible way is an important factor to consider, and whether or not carriers have any identifiable and significant difference in vocal production. Moreover, the carriers may have a different, rather than comparable but quantitatively reduced, sensory profile.

For this study, the use of a mouse model was necessary. Mouse models carrying a mutation in the *USH2A* gene have been identified and generated in order to unravel the mechanisms underlying the Usher syndrome type 2 phenotype. When the *USH2A* gene is targeted for disruption in mice, they exhibit similar symptoms to humans. They show a moderate but non-progressive hearing impairment, as well as progressive photoreceptor degeneration, mimicking the visual and hearing deficits in Usher syndrome type 2 patients [18]. In parallel, the use of mouse ultrasonic vocalizations has become more prevalent in research. They are often used as a testing measure to observe and extrapolate vocal communication defects. Wild-type male mice have been recorded and known to produce very abundant vocal signaling in the ultrasonic range. In the presence of an oestrus female, or female pheromones, adult males will emit a large number of ultrasonic vocalizations. In this paper, an examination of the total time spent vocalizing, and the shape of the individual vocalization calls, were both used to help determine if there are any notable differences in

vocal production for mice either carrying a homozygous mutation of the mouse homolog (usher2a), heterozygous mice ("carriers"), or controls.

Methods

Mouse Breeding

For this study, *USH2A* knock-out male and female mice were obtained by MTA from Johns Hopkins University. The original *USH2A* knock-out mice were generated using methods described by Liu et al., 2007 [18]. At the University of Connecticut, the *USH2A* knock-out mice were bred with wild type mice (strain: 129s4/SvJae), generating a group of male and female *USH2A* heterozygous (Het) mice as the resulting litters. Heterozygous breeding pairs were re-derived on a 129s4/SvJae background at the Gene Targeting and Transgenic Facility (GTTF) at UConn Health Services. The testing mice were then generated by performing Het x Het breeding. This allowed for the subjects to have a genotype ratio and breakdown of 1:2:1, meaning 25% wild-type (WT), 50% Het and 25% knock-out (KO). Offspring were genotyped by PCR, and chosen by their genotype. This was done by punching their ears, and then running a PCR on the DNA of the ear punch using Common (GTGAATACAGGCACCTCTGAATGTGAC), Wild-type Reverse (GTCACGGCTGAATCCCGAAGC) and Mutant Reverse (GAGATCAGCAGCCTCTGTTCCAC).

There was a total of 36 male mice subjects, although one of them (number 32) died during the experimental process. Of the 35 male mice whose data was collected and analyzed, 12 were WT's, 12 were Het's and the last 11 were KO's. After the mice were weaned, they were housed separately because of inter-male aggression in single standard cages with ample food and water. All procedures were conducted in compliance with the

National Institutes of Health and approved by the University of Connecticut's Institutional Animal Care and Use Committee (IACUC). The data collectors were blind to the genotype of the tested animals during the experimental process. The data analyzer was also blind to the genotype of the subjects during the analysis – they only had the subject number of the animals when analyzing audio files.

Behavioral Procedure

The ultrasonic mouse vocalizations were recorded by an ultrasonic microphone (B & K). The vocalizations of the male mice were recorded when they were in the presence of a female mouse in oestrus. To begin, bedding from one of two female mice was spread along the bottom of a clean cage. This was to ensure the male mouse was inundated with the scent of the female. Then, the subject was placed in the cage. After a few moments of habituation, one of the two unfamiliar female mice in oestrous was introduced to the subject. The next 5 minutes of interactions were recorded by the microphone. In the case of this experimental paradigm, the female mice are expected to vocalize very minimally. Therefore, it is assumed that the vocalizations recorded are only from the males [20]. These vocalizations were later examined for the total time spent vocalizing, as well as analysis of the shapes of the vocalizations themselves. The shapes were identified according to a previous study, using the guide in Figure 1 [21].

Data Analysis Procedure

Once the audio recordings were saved and verified to have collected the vocalization data correctly, they were then analyzed. The first measure that was analyzed was the total

time spent vocalizing. To do this, the data analyst combed through the vocalization recordings (5 minutes in length) on the computer application, *Adobe Audition*. The analyst sought to delete the empty space in the vocalization recording, where the animal did not make any calls, to determine total amount of time spent vocalizing. If the space between the calls was greater than 2 milliseconds, it was deleted from the audio. If the empty space between two calls was less than 2 milliseconds, it was kept and that time was counted towards the total time spent vocalizing. From there, once all of the empty/quiet space was removed, the length of the audio file indicated how much time the animal spent vocalizing in seconds.

The next analyzation step was to identify and categorize the shapes of the calls. A single experimenter blind to the condition of the animals went through each call manually, identified the shape that they saw best fit, and manually recorded it. A few categories of calls were identified from a previous study. They are as follows, from Ey et. al, 2013 [20]:

- *Short:* duration shorter than 5 milliseconds and frequency range $\leq$ 6.25 kHz
- *Simple*: duration longer than 5 milliseconds and frequency range $\leq$ 6.25 kHz
    a. Contains flat, up, down, chevron and reverse shapes
- *Complex*: frequency modulations in more than one direction and frequency range $>$ 6.25 kHz, or inclusion of one or more additional frequency components but no constraint on frequency range
- *One Frequency Jump*: inclusion of one jump in frequency without time gap between the consecutive frequency components
- *Two Frequency Jump*: inclusion of two jumps in frequency without time gap between the consecutive frequency components

- *Noisy*: no pure tone component identifiable

The vocalization calls were analyzed one by one, with the shapes in Figure 1 as a guideline. The final call of category type was left to the discretion of the blinded data analyst.

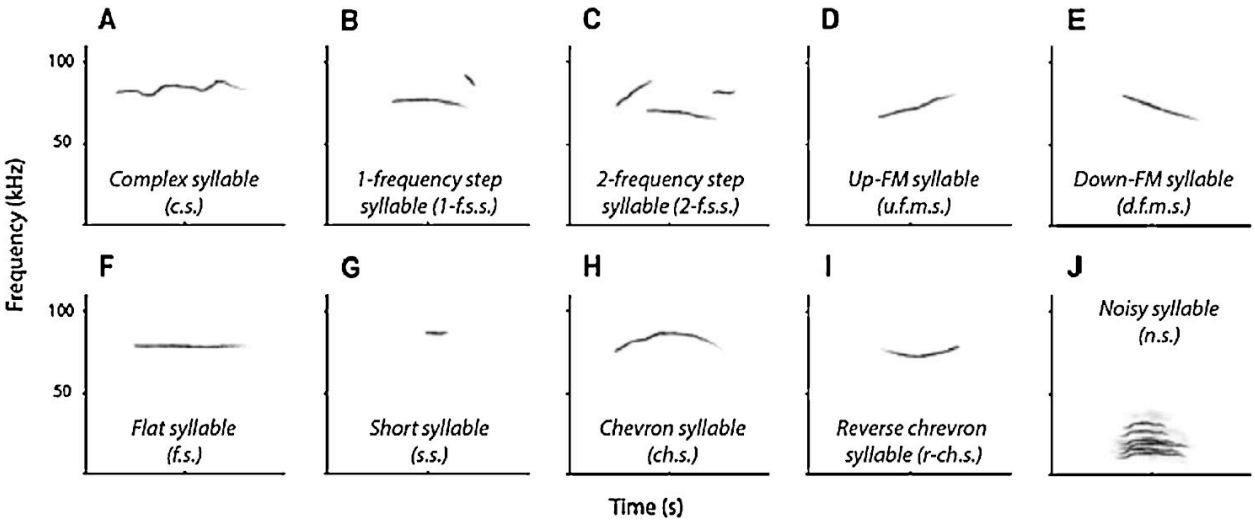

Figure 1. Presentation of the various call types, with spectrograms of examples of vocalizations within each call type. Image taken from Heckman et. al, 2016 [21].

Results

The data analysis was run with IBM SPSS Statistics, a computer program, using an ANOVA with repeated measures to determine if genotype had a significant effect on time spent vocalizing. The results showed that Genotype had no significant effect on total time spent vocalizing overall (p= 0.583). These results can be seen in Figure 2.

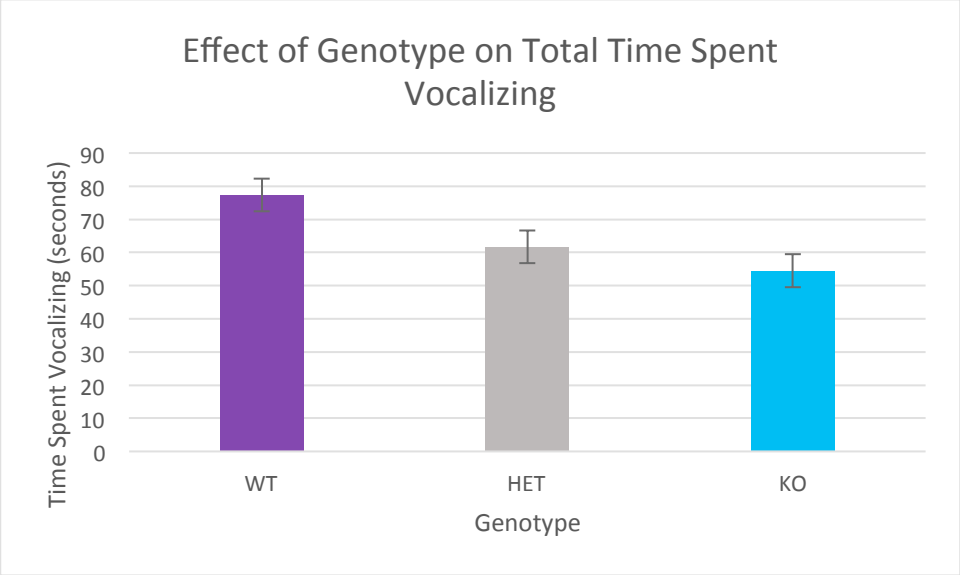

Figure 2. Effect of genotype on total time spent vocalizing. No significant results were found.

Next we ran an ANOVA test on the different call types of the mice, but no significant effect of Genotype on call type was found. A second ANOVA repeated measures test was run with time spent vocalizing as a covariant, and the results remained the same – no significant effect of Genotype on call type category. There were no significant between-subject or within-subject effects of Genotype on call rates. The p-values ranged from p=0.180 for the call type chevron to p=0.953 for the call type short – again, indicating no significant effect. The average amount of calls in each category are organized by Genotype

in Figure 3. Typically, an asterisk sign above the bars on the graph would indicate significance. In the case of this data set, there were no significant results.

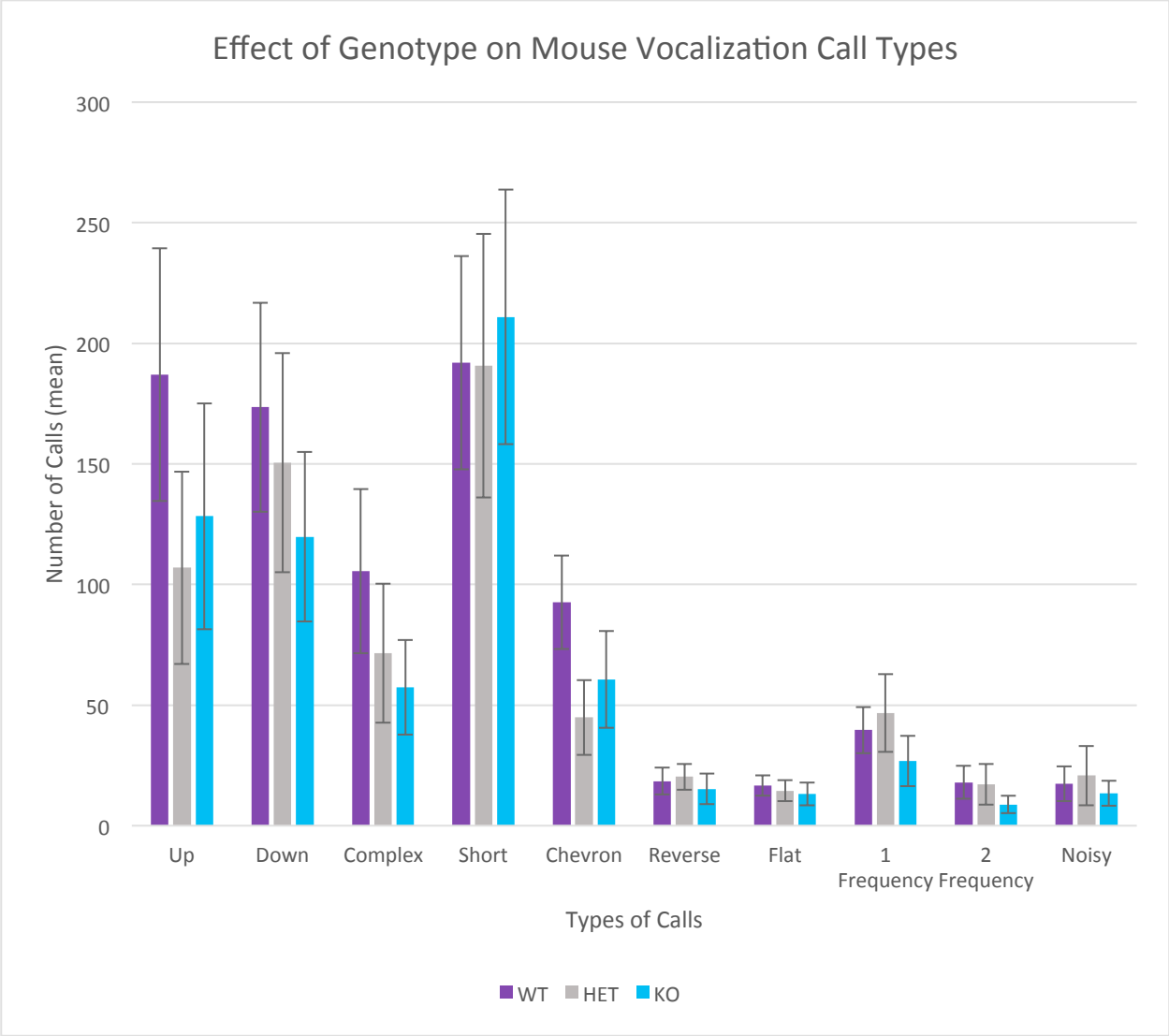

Figure 3. Effect of genotype on Mouse ultrasonic vocalizations using the ANOVA test. No significant effect of genotype on call type was observed.

Discussion

The lack of significant effects of genotype on time spent vocalizing and the shape of call types is interesting, in and of itself. A different part of this same study, analyzed and performed by other members of the lab, found receptive Genotype differences that depend on auditory task and frequency. Specifically, *USH2A* Het mice showed auditory deficits on tasks using a lower frequency range (10-15kHz) but were comparable to WT on tasks using a higher frequency range. Furthermore, the *USH2A* KO mice showed auditory deficits on tasks using higher frequency range (10-40kHz), but were comparable to or better than WT on tasks using a lower frequency range. These results are consistent with previous in-depth studies on carriers of Usher syndrome type 2 and *USH2A* carriers [14].

The insignificant vocalization data presented in this project, in conjunction with the findings of carrier auditory deficits in other studies, may indicate something important. Although receptive differences were seen between WT, Het and KO mice, there were no significant vocal production differences between the genotypes. This is a surprising discovery, and is worth taking another look at. Findings suggest the subjects were able to produce vocalizations normally, despite difficulties hearing these sounds.

There were several limitations of the data collection that may have influenced the lack of significant results. Each subject was recorded for only one 5-minute trial. Multiple trials allow for consistency and reliability in results. A lack of several trials limits confidence in the results. The lack of significant findings can be indicative of no vocal production differences between genotypes, which is an interesting finding that warrants further research, or it could be a potential flaw in the data collection process. The lack of multiple trials does not allow for either conclusion to be made confidently. Furthermore,

there were only 35 subjects total. In the case of research such as this, more subjects are always more beneficial. It allows for the examination of more data, and greater representation of each genotype studied. Another limitation of the data is the lack of female subjects. For this study, only male subjects were studied. In the world of research, when an entire portion of the population is left out of the study, this is limiting in nature. Including female mice in the study could have allowed for us to determine the effect of Genotype on call types by sex, which could have provided more insight. Lastly, there was only one experimenter who analyzed the data. They went through each call manually, labeling the call type structure individually and tallying up the total time spent vocalizing. While there are benefits to having one experimenter analyze the data, if they are not consistent or categorize the calls incorrectly, this can affect the data. The experimenter also took several months to analyze the data. The method of analyzing could have differed from the first subject that was reviewed (Subject 1) to the last one (Subject 36). All of the above are limitations that must be considered when interpreting the results, since they may have impacted the lack of significance.

Future Directions

There are many further inquiries that can be made into the *USH2A* gene and Usher syndrome type 2. First and foremost, it would be important to replicate this study. As mentioned previously, the lack of significant differences between genotypes in vocal production may be an important discovery. Unfortunately, because each subject only had one trial, the results are not entirely dependable. An exact replication of this study with a new set of subjects, and a different experimenter analyzing the data will provide insight on

whether or not these results are reliable and accurate. Furthermore, histological analysis should be performed to further analyze genotype effects. An anatomical review may provide surprising or interesting findings, which will not be known until it is done and analyzed. On the question of carriers, further behavioral testing should be performed to see if there are any other deficits that they may exhibit in hearing or vision loss. This is a new area of research that studies are beginning to look into – it is definitely a topic worth pursuing further.

One important way to take this study one step further is to study vocal production in the human patient, carrier and non-carrier population. A study that can both show that carriers have varied hearing loss that is not as severe as someone who is diagnosed with Usher syndrome, and that there are no vocal production differences across patients, carriers and non-carriers would be very intriguing. This idea is worth taking a further look at. Lastly, it is very important to remember why all of this research is being done. The more that is understood about Usher syndrome, the closer and closer the scientific community comes to finding a more definitive method of diagnosis, better treatment interventions, and hopefully one day soon, a cure. Ultimately, all research is done to better the outcomes of human beings who are afflicted. This research, while innovating and exciting in and of itself, is simply one cog in a machine focused on curing the enigma that is Usher syndrome.

References

[1] Wolfrum, U. & Nagel-Wolfrum, K. (2018). The Usher Syndrome, a Human Ciliopathy. *Klin Monbl Augenheilkd, 235*(3), 273-280.

[2] Khalaileh, A., Abu-Diab, A., Ben-Yosef, T., Raas-Rothschild, A., Lerer, I., & Alswaiti, Y, Chowers, I., Banin, E., Sharon, D., Khateb, S. (2018). The Genetics of Usher Syndrome in the Israeli and Palestinian Populations. *Investigative Opthalmology & Visual Science, 59*(2), 1095-1104.

[3] Kimberling, W., Hildebrand, M., Shearer, A., Jensen, M., Halder, J., Trzupek, K., Cohn, E., Weleber, R. G., Stone, E. M., Smith, R. J. H. (2010). Frequency of Usher syndrome in two pediatric populations: Implications for genetic screening of deaf and hard of hearing children. *Genetics In Medicine, 12*(8), 512-516.

[4] *Usher Syndrome | NIDCD*. (2018). *Nidcd.nih.gov*. Retrieved 17 February 2018, from https://www.nidcd.nih.gov/health/usher-syndrome

[5] Isgrig, K., Shteamer, J., Belyantseva, I., Drummond, M., Fitzgerald, T., & Vijayakumar, S. et al. (2017). Gene Therapy Restores Balance and Auditory Functions in a Mouse Model of Usher Syndrome. *Molecular Therapy, 25*(3), 780-791.

[6] Zong, L., Chen, K., Wu, X., Liu, M., & Jiang, H. (2016). Compound heterozygous *MYO7A* mutations segregating Usher syndrome type 2 in a Han family. *International Journal Of Pediatric Otorhinolaryngology*, *90*, 150-155.

[7] Aller, E., Larrieu, L., Jaijo, T., Baux, D., Espinós, C., & González-Candelas, F. et al. (2010). The USH2A c.2299delG mutation: dating its common origin in a Southern European population. *European Journal Of Human Genetics*, *18*(7), 788-793.

[8] *USH2A usherin [Homo sapiens (human)] - Gene - NCBI*. (2018). *Ncbi.nlm.nih.gov*. Retrieved 3 March 2018, from https://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=ShowDetailView&TermToSearch=7399

[9] National Institutes of Health. (2018). USH2A Gene. *U.S. National Library of Medicine.*

[10] Weston, M., Eudy, J., Fujita, S., Yao, S., Usami, S., & Cremers, C. et al. (2000). Genomic Structure and Identification of Novel Mutations in Usherin, the Gene Responsible for Usher Syndrome Type IIa. *The American Journal Of Human Genetics*, *66*(4), 1199-1210.

[11] Millán, J., Aller, E., Jaijo, T., Blanco-Kelly, F., Gimenez-Pardo, A., & Ayuso, C. (2011). An Update on the Genetics of Usher Syndrome. *Journal Of Ophthalmology*, *2011*, 1-8.

[12] Liebreich, R. (1861). Abkunft aus Ehen unter Blutsverwandten als Grund von Retinitis pigmentosa. *Dtsch. Klin*. **13**: 53.

[13] Lentz, J., & Keats, B. (2016). Usher Syndrome Type I. *University Of Washington, Seattle*. Retrieved from https://www.ncbi.nlm.nih.gov/books/NBK1265/

[14] Aarem, A., Cremers, C., Pinckers, A., Huygen, P., Hombergen, G., Kimberling, B. (1995). The Usher syndrome type 2A: clinical findings in obligate carriers. *International Journal of Pediatric Otorhinolaryngology, 31*. 159-174.

[15] Boyle, C., Decouflé, P., & Yeargin-Allsopp, M. (1994). Prevalence and Health Impact of Developmental Disabilities in US Children. *Pediatrics*, *93*(3), 399-403.

[16] Boulet, S., Boyle, C., & Schieve, L. (2009). Health Care Use and Health and Functional Impact of Developmental Disabilities Among US Children, 1997-2005. *Archives Of Pediatrics & Adolescent Medicine*, *163*(1), 19-26.

[17] Kimberling, W., Möller, C. (1995). Clinical and Molecular Genetics of Usher Syndrome. *Journal of the American Academy of Audiology*, *6*(1), 63-72.

[18] Liu, X., Bulgakov, O., Darrow, K., Pawlyk, B., Adamian, M., Liberman, M., & Li, T. (2007). Usherin is required for maintenance of retinal photoreceptors and normal development of cochlear hair cells. *Proceedings Of The National Academy Of Sciences*, *104*(11), 4413-4418.

[19] Kumar, A., & Dhanda, R. (1997). The identification and management of deaf children. *The Indian Journal Of Pediatrics*, *64*(6), 785-792.

[20] Ey, E., Torquet, N., Le Sourd, A., Leblond, C., Boeckers, T., Faure, P., Bourgeron, T. (2013). The Autism *ProSAP1/Shank2* mouse model displays quantitative and structural abnormalities in ultrasonic vocalisations. *Behavioural Brain Research, 256*, 677-689.

[21] Heckman, J., McGuinness, B., Celikel, T., & Englitz, B. (2016). Determinants of the mouse ultrasonic vocal structure and repertoire. *Neuroscience & Biobehavioral Reviews*, *65*, 313-325.

[22] Simpson, N., Ceroni, F., Reader, R., Covill, L., Knight, J., Hennessy, E. (2015). Genome-wide analysis identifies a role for common copy number variants in specific language impairment. *European Journal of Human Genetics, 23*, 1370-1377.