Effect of Antisense Oligonucleotide (ASO29) Treatment on Usher 1C Type Mice Hearing and Vestibular Function

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Introduction

Usher Syndrome (USH) is the most common form of congenital combined deafness and blindness. Usher type 1C, a particularly prevalent form of this recessive genetic disorder among the Acadian population in Southeast Louisiana, is caused by a mutation in the USH1C gene coding for Harmonin, a scaffolding protein expressed in the retina and labyrinth. Consequently, USH1C also presents with vestibular dysfunction. Previous research showed that antisense oligonucleotide (ASO) corrects defective pre-mRNA splicing USH1C gene transcripts, leading to symptom rescue. The aim of this study is to test the effect of a specific dosage (300 µg I.P. injection) of ASO29 on hearing and vestibular function in USH1C knock-in mouse, a model for human symptomology.

Experimental Design

Mice: USH1C c.216>A knock-in mice and WT littermate controls were bred at LSUHSC. All procedures met the NIH guidelines for care and use of laboratory animals and were approved by the Animal Care and Use Committee at LSUHSC.

ASO29: ASO's are short strands of synthetic DNA or RNA that bind to complementary sequences to treat mutations. ASO29 is specifically designed to target the mutated genes associated with USH1C with the goal of recovering hearing and vestibular function. ASO29 was tested on a group of mice and compared to untreated USH1C and WT control groups.

Circling Behavior: Mice were put into an open-field area where ANY-maze tracking software recorded mouse movement and circling behavior over 120 seconds.

ABR: To record auditory brainstem responses to tone stimuli, mice were anesthetized with a ketamine/xylazine, placed on a heating in a sound isolation booth, and given SQ saline after the procedure. The sound thresholds for each ABR frequency were measured by testing each frequency (5.6, 8, 11.3, 16, 22.6, and 32 kHz) at decreasing amplitudes from 90 to 18 dB (SPL).

Balance Beam: Apparatus consisted of a 60 cm elevated beam (16 mm diameter) with a small dark house structure at the end. The goal is for the mice to traverse the beam as quickly as possible to show vestibular function. Mice that fall, fail the trial and are not counted. The mice were tracked with the ANY-maze software. 5 trials were conducted sequentially, and the 3 fastest times were averaged.

Rotarod Analysis: The rotarod machine features a suspended spinning rod that goes across several sections for individual mice. The spinning starts at 4 RPMs and then gradually increases to 40 RPMs over 240 seconds. 5 trails were conducted sequentially, and the 3 fastest times were averaged. The trials stopped when the mice fell off the rod or if they reached 300 seconds.

Statistical Analyses: The data are shown as the average ± standard error (SEM). The analyses were concluded by using a single-factor ANOVA on Excel to find whether the different treatment groups were statistically similar or different.

References

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Results

Vestibular Assay: Circling Behavior

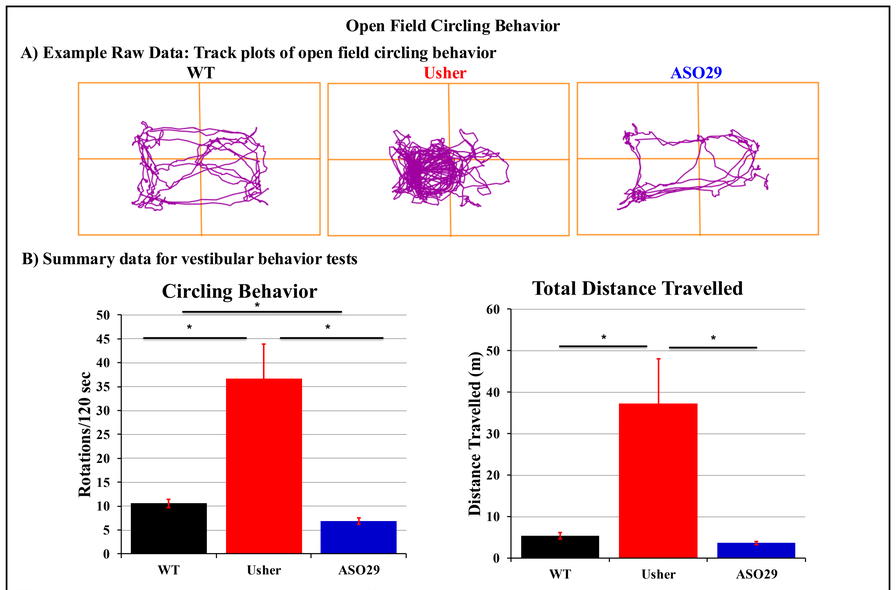


Figure 1: Antisense Oligonucleotides (ASOs) treatments improved the Circling behavior in Usher mice model (USh1c-216AA). **A)** Track plot of WT, Usher and ASO treated Usher mice, **B)** Bar graphs showing two vestibular behavioral assays by WT (**Black bar, n=9**), Usher (**Red bar, n=11**) and ASO treated (**Blue bar, n=9**) mice. ASO treated mice exhibited significantly improved circling behavior: reduced from mutants and similar to that in wild type controls. ***P-Value < 0.05; Error bars indicate SEM**

Auditory Brainstem Response

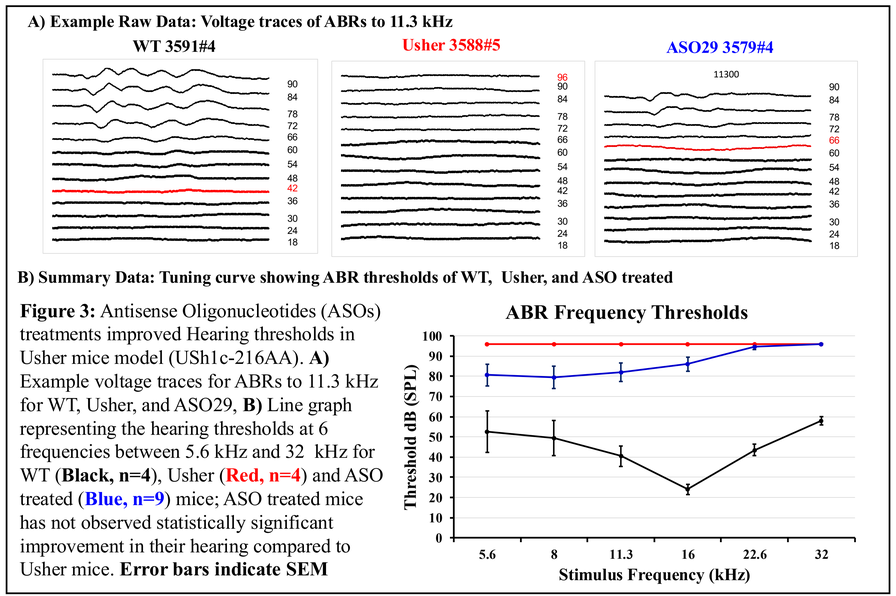


Figure 3: Antisense Oligonucleotides (ASOs) treatments improved Hearing thresholds in Usher mice model (USh1c-216AA). **A)** Example voltage traces for ABRs to 11.3 kHz for WT, Usher, and ASO29, **B)** Line graph representing the hearing thresholds at 6 frequencies between 5.6 kHz and 32 kHz for WT (**Black, n=4**), Usher (**Red, n=4**) and ASO treated (**Blue, n=9**) mice; ASO treated mice has not observed statistically significant improvement in their hearing compared to Usher mice. **Error bars indicate SEM**

Balance Beam

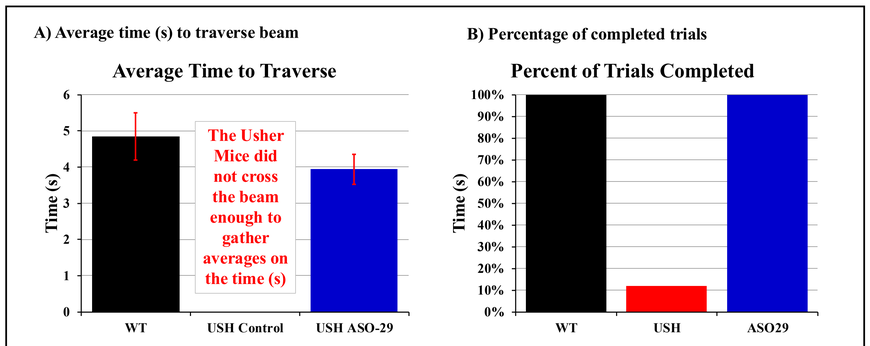
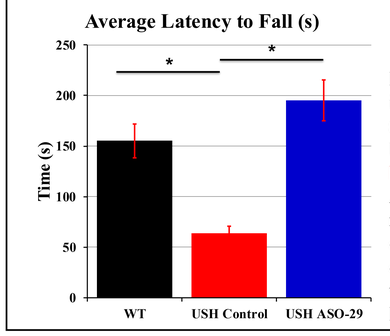


Figure 2: Antisense Oligonucleotides (ASOs) treatments improved vestibular behavior in Usher mice model (USh1c-216AA) as shown by the balance beam test results. **A)** WT (**Black bar, n=10**), Usher (**Red bar, n=5**) and ASO treated Usher (**Blue bar, n=9**) mice that shows the time it took for the mice to traverse, **B)** Bar graph representing the percentage of completed trials by WT (**Black bar**), Usher (**Red bar**) and ASO treated (**Blue bar**) mice; ASO mice are not statistically significantly different compared to the WT group, consistent with drug induced rescue of vestibular function. **Error bars indicate SEM**

Rotarod Analysis



Average Latency to Fall (s) of Mice on Rotarod Tests

Figure 4: Antisense Oligonucleotides (ASOs) treatments improved the Rotarod behavior results in Usher mice model (USh1c-216AA). The bar graph represents the average latency to fall (s) by the WT (**Black bar, n=9**), Usher (**Red bar, n=9**) and ASO treated (**Blue bar, n=9**) mice; The ASO29 treated mice has observed significantly improved rotarod behavior and normal the average latency as compared to Usher mice. The WT mice are not significantly different compared to the ASO29 treated mice, consistent with recovery of vestibular function. ***P-Value < 0.05; Error bars indicate SEM**

Results

- **Distance Travelled**: statistically significant difference between the Usher and ASO29 mice groups (Figure 1B). There is **no** significant difference between the WT group and the ASO29 group (Figure 1B).
- **Circling behavior**: on average, ASO29 group made less rotations/120 sec than even the WT group did and there is a significant difference between the ASO29 group and the Usher group (Figure 1B). The reduced number of rotations for the ASO29 group shows an improvement in vestibular function.
- **Balance Beam:** the untreated USH group did not complete enough trials without falling to measure an average (Figure 2A). The ASO29 and WT groups are not significantly different (P> 0.05; Figure 2A). When looking at the percentage of trials completed, only the USH group completed less than 100%, suggesting ASO29 drug can help to recover vestibular function of the mutant mice (Figure 2B).
- **Rotarod Data:** ASO29 group is significantly different compared to the USH group but not different compared to the WT group (Figure 4). The ASO29 group had a greater latency to fall (s) which shows a recovery of vestibular function (Figure 4).
- **Auditory Brainstem Response**: In two-way ANOVA, there were significant main effects of mouse type (P<0.001) and stimulus frequency (P=0.0006) on thresholds. In post-hoc analysis of mouse type, ASO29 treated mutant mice thresholds were significantly lower than untreated mutants (P<.001) suggestion some, although incomplete, rescue (Figure 3B).

Conclusion

- **The circling behavior, balance beam, and rotarod analysis tests indicate that ASO29 treatment dosage (300 µg I.P. injection) rescued vestibular function in USH1C c.216>A knock-in mice.**
- **The ABR tests indicate a significant, but incomplete rescue of auditory thresholds, as treated mice were more sensitive to tone stimuli than untreated mice, but not as sensitive as those for wild type mice.**
- **The results here contribute to the dataset for optimizing ASO treatment protocols, enabling development translation therapy for clinical populations affected by Usher type 1C.**

Acknowledgements

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