Optimizing antisense oligonucleotide chemistry for the treatment of hearing loss and imbalance in Usher syndrome

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Introduction

Usher syndrome (Usher) is a rare autosomal recessive disorder characterized by the loss of hearing, vestibular, and visual function. Usher patients exhibit varying degrees of impairment and the 3 main clinical types (Usher 1, 2, 3) are classified by severity and onset of hearing and vision loss. Usher type 1 is the most severe with profound congenital sensorineural hearing loss and vestibular dysfunction, and progressive vision loss due to retinitis pigmentosa beginning in early childhood. USH1C is the most common Usher 1 subtype in the Acadian populations of Louisiana and Canada due to a founder mutation in the *USH1C* gene (c.216G>A (216A)). The 216A mutation creates a cryptic 5' splice site in exon 3 of the *USH1C* gene that is favored over the authentic splice site. Aberrant splicing results in a 35 base pair deletion and frameshift that results in truncated harmonin proteins. Harmonin is a scaffolding protein crucial to the function of stereocilia bundles in cochlear and vestibular hair cells and maintenance of photoreceptor cells. Our long-term goals are to restore a functional, full length harmonin protein at an early disease stage to minimize disease morbidity and prevent or delay progression. Antisense oligonucleotides (ASOs) bind to complementary base pairs on pre-mRNA and can be utilized to correct splicing, restoring normal protein expression and function. We have previously shown that a novel antisense oligonucleotide (ASO-29) therapy targeting the 216A mutation in the *USH1C* gene has high therapeutic efficacy in transiently restoring hearing, balance, and vision when administered early in the critical developmental period in a murine model of USH1C. To further develop this drug, various ASO chemistries targeting the 216A mutation were tested for efficacy in improving hearing and balance in USH1C mice.

Methods

Mice: *USH1C* c.216G>A knock in mice and wildtype littermate controls were bred at LSUHSC.

Antisense oligonucleotides: 2'-*O*-methoxyethyl-modified (2'MOE) targeting the *Ush1c* c.216G>A mutation (ASO-29, 5'-AGCTGATCATATTCTACC-3') were generated as previously described by Lentz *et al*. (2013). Morpholino ASOs (MO-29) were also designed to target the human *USH1C* c.216G>A mutation and synthesized and purified by Gene Tools, LLC.

Intraperitoneal Injection: USH1C mice were treated systemically via intraperitoneal injection at postnatal day 2 with 300 µg of ASO-29, MO-29, or a combination of the two chemistries (COMBO) each at 150 µg.

ABR: Mice were anesthetized with ketamine/xylazine and normasol, placed on a heating pad to maintain core temperature, and put into a sound attenuating chamber. Thresholds were detected visually by the lowest sound pressure level (SPL) in which a recognizable and reproducible waveform was present. Responses were recorded at ascending frequencies (5.6, 8, 11.3, 16, 22.6, 32 kHz) and descending intensities in 6 dB increments (90-18 dB SPL).

Rotarod: Mice were acclimated to the Rotarod with a short training session 2-3 days prior to experimental trials. Mice were placed in physically separated individual lanes at a baseline rotation of 4 RPM, accelerating to a maximum of 40 RPMs over 240 seconds. Time stopped when a mouse fell, spun continuously in quick succession, or reached 300 seconds. Five trials separated by 10-minute rest periods were conducted, and the highest 3 times were averaged.

Balance Beam: Mice were acclimated to the Balance Beam with a short training session 2-3 days prior to experimental trials. Mice were tracked across a two-dimensional digital active zone created with Any-maze software. They traversed 60 cm across a 16 mm wide elevated beam to enter a 3D-printed housing structure. 5 trials were sequentially conducted, and the fastest 3 times were averaged.

Statistical Analyses: All data are shown as mean ± SEM. Univariate analysis of variance (ANOVA) was used to determine intergroup differences.

ABR Analyses

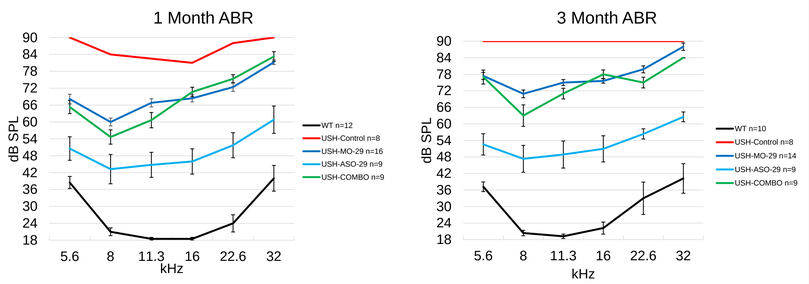

Figure 1. Average ABR Threshold (dB SPL) to pure tones ranging in frequency from 5.6 to 32 kHz in 1- and 3- month mice. In USH Control mice, thresholds were elevated or undetectable for all frequencies at both time points. WT mice had very low thresholds, especially in the middle frequencies, and were often able to hear the lowest intensities played. USH1C mice treated systemically with ASO-29, MO-29, or COMBO therapy showed moderate rescue of ABR thresholds compared to untreated USH1C mice ($p<0.05$). WT mice were statistically different from all other groups ($p<0.05$). ASO-29 mice were statistically different from MO-29 and COMBO mice. ($p<0.05$). Error bars indicate SEM.

Rotarod Analyses

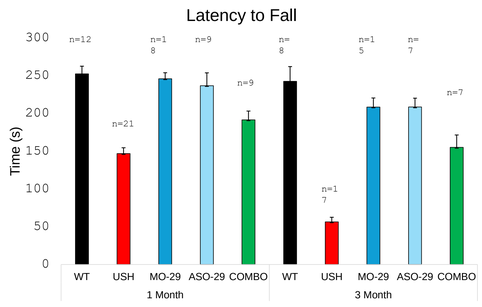

Figure 2. Rotarod latency to fall (s) in 1- and 3- month mice. USH1C mice treated with ASO-29, MO-29, or COMBO therapy had longer latency to fall compared to untreated USH Control mice ($p<0.05$). Latency to fall was not statistically different from WT mice at either time point following treatment with ASO-29 or MO-29, whereas mice treated with COMBO were statistically different from WT mice ($p<0.05$). Error bars indicate SEM.

Balance Beam Analyses

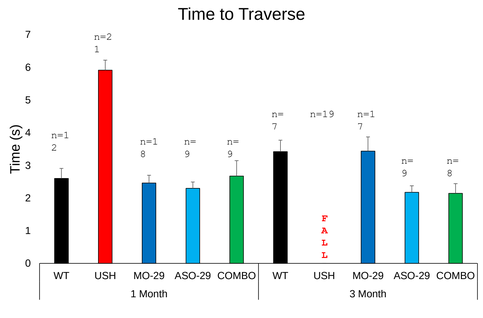

Figure 3. Balance beam time to traverse active zone (s) in 1- and 3- month mice.. USH1C mice treated with ASO-29, MO-29, or COMBO therapy crossed the active zone faster than untreated USH Control mice ($p<0.05$) and were not statistically different from WT mice at both time points ($p<0.05$). At 3 months, USH Control mice were only able to cross in 3/95 total trials (3.16%), while 100% of WT, ASO-29, MO-29, and COMBO crossed successfully. Error bars indicate SEM.

Conclusion

- **Systemic treatment with AS0-29, MO-29, or COMBO therapy significantly improved hearing thresholds to low, mid, and high frequency stimulation in USH1C mice when delivered early in the neonatal critical period.**
- **Systemic treatment with a 2'MOE ASO (ASO-29) showed a 10-15 dB SPL increase in threshold shift compared with a morpholino (MO-29) or the COMBO therapy, suggesting better hearing rescue in USH1C mice.**
- **Systemic treatment of USH1C mice with a 2'MOE ASO (ASO-29) significantly improved hearing thresholds to low, mid, and high frequency stimulation that was stable for at least 3 months-post treatment, whereas treatment with a morpholino (MO-29) or the COMBO therapy showed a loss in sensitivity (10-15 dB SPL) between 1-3 months post-treatment, suggesting ASO chemistry may affect the duration of hearing rescue.**
- **Systemic treatment with ASO-29, MO-29, or the COMBO therapy significantly improved balance behavior in USH1C mice, suggesting that 2'MOE or MO ASOs can improve fine- and gross-motor coordination and balance.**

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