Matthew D. Ward
Medical - L2
School of Medicine, LSUHSC, New Orleans, LA

Jessica Landry, Inga Kristaponyte, Ph.D., Bhagwat Alapure, Ph.D., Jason Kim
Neuroscience Center of Excellence, LSUHSC, New Orleans, LA

Jennifer J. Lentz, Ph.D.
Department of Otorhinolaryngology, LSUHSC, New Orleans, LA

"Antisense therapy significantly improves fine motor coordination and balance in Usher syndrome Type 1C mice"

BACKGROUND: Usher Syndrome (Usher) is the most common genetic cause of deaf-blindness, characterized by the loss of vision, balance, and hearing. Three clinical types (USH1-3) and 10 genes are associated with the disease. USH1 is the most severe with congenital severe-profound sensorineural hearing loss and vestibular areflexia, and childhood onset of retinitis pigmentosa. Mutations in the *USH1C* gene account for 6-15% of USH1, however, the *USH1C c.*216G>A (216A) founder mutation accounts for nearly all USH1 cases in the Acadian populations in U.S. and Canada. The 216A mutation causes aberrant splicing that results in a truncated harmonin protein, and photoreceptor and cochlear hair cell dysfunction. The vestibular dysfunction associated with USH1 leads to the loss of balance, which severely limits patients' independence and decreases their quality of life. USH1C mice that were engineered to contain the 216A mutation also have vision, balance, and hearing deficits similar to the patients. Treatment of USH1C mice with splice-switching antisense oligonucleotides (ASO) designed to target the 216A mutation corrects *USH1C* splicing and significantly improves vision, balance, and hearing. The aim of this study was to determine the effect of 216A-targeted ASO therapy on fine motor coordination and balance in USH1C mice.

METHODS**:** USH1C mice were treated locally to the inner ear via semicircular canal injection at postnatal day 2 (P2) with ASO therapy (30µg). Fine motor coordination and balance were assessed using a balance beam behavioral test in USH1C-ASO, USH1C control (untreated) and wild-type control mice. Briefly, mice traversed an elevated, 5° incline balance beam to enter a 3D-printed housing structure. The mouse's movement was recorded with a camera (HD Webcam eMeet C960) and analyzed with ANY-maze software. Five trials per mouse separated by a 10-minute rest period were recorded and the average time travelled across the beam was computed based on position of the center-point of the mouse. Mice had been previously acclimated to the beam using the same testing protocol 2-3 days prior to the experimental run.

RESULTS: The time to traverse the balance beam was not significantly different between treated USH1C-ASO and wild-type mice [25.71+/-15.10s and 25.65+/-15.19s, respectively ($p=0.9938$)], whereas USH1C-control mice could not complete the balance beam course due to severe vestibular dysfunction and imbalance.

CONCLUSION**:** Our results show that local treatment with ASO improves postural stability, fine motor coordination, and balance behavior in USH1C mice. These data suggest that ASO therapy can successfully rescue balance deficits associated with Usher. A thorough understanding of the natural progression of the vestibular dysfunction and imbalance in USH1C, as well as ASO treatment timing, dose, and window of therapeutic opportunity, will provide outcome measures to guide a future clinical trial and improve care for patients.