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

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Introduction

- Usher Syndrome (Usher) is the most common genetic cause of deaf-blindness, characterized by the loss of vision, balance, and hearing.[1]
- 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.[2]
- Mutations in the *USH1C* gene account for 6-15% of USH1[1],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[3], 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 engineered to contain the 216A mutation also have vision, balance, and hearing deficits similar to the patients.[4,5,6]
- Treatment of USH1C mice with splice-switching antisense oligonucleotides (ASO) targeting the 216A mutation corrects *USH1C* splicing and significantly improves vision, balance, and hearing.[7,8,9]

The aim of this study was to determine the effect of 216A-targeted ASO therapy on fine motor coordination and balance in USH1C mice.

USH1C Mouse Model

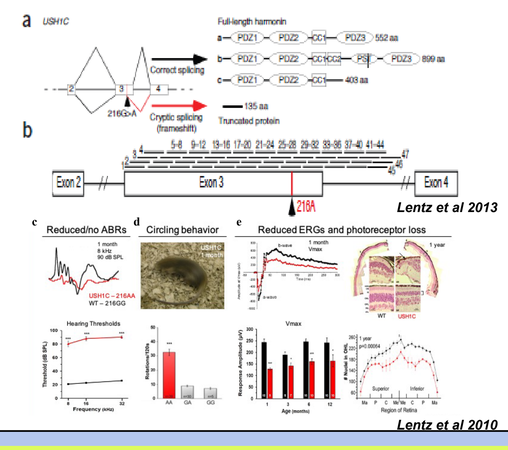

Figure 1. *USH1C* gene and mice. a,b Gene structure of *USH1C* and the location of the 216A splicing mutation. **c-d** Hearing, balance, and vision deficits observed in the USH1C mice.

Acknowledgements

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Materials and Methods

Semicircular Canal Injection for Delivery of ASO in Postnatal Day 2 (P2) Mice: **P2 mice were first anesthetized. Then a small incision is made posterior to the left ear nub. Forceps are then used to expose the semicircular canal. A 10 µL syringe is then used to inject 30µg of ASO directly into the posterior semicircular canal over the course of 2 minutes. The incision is then sutured closed and the pups are returned to the dam.**

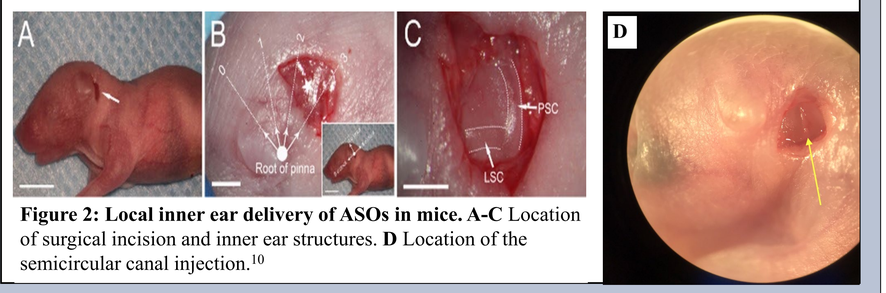

Figure 2: Local inner ear delivery of ASOs in mice. A-C Location of surgical incision and inner ear structures. **D** Location of the semicircular canal injection.[10]

Assessing Balance Behavior: **Fine motor coordination and balance were assessed using a balance beam behavioral test in USH1C-ASO, USH1C control (untreated, USH-ctl) and wild-type (WT) mice. Mice traverse 60cm across an elevated, 5° incline balance beam to enter a 3D-printed housing structure (pictured below) while being recorded with a camera (HD Webcam eMeet C960) and their movement analyzed with ANY-maze software. A map of the apparatus is drawn in the software and zones are designated to determine the time to traverse (active zone) and number of falls (fall zone). Each mouse is given 5 trials and the best 3 are scored for analysis.**

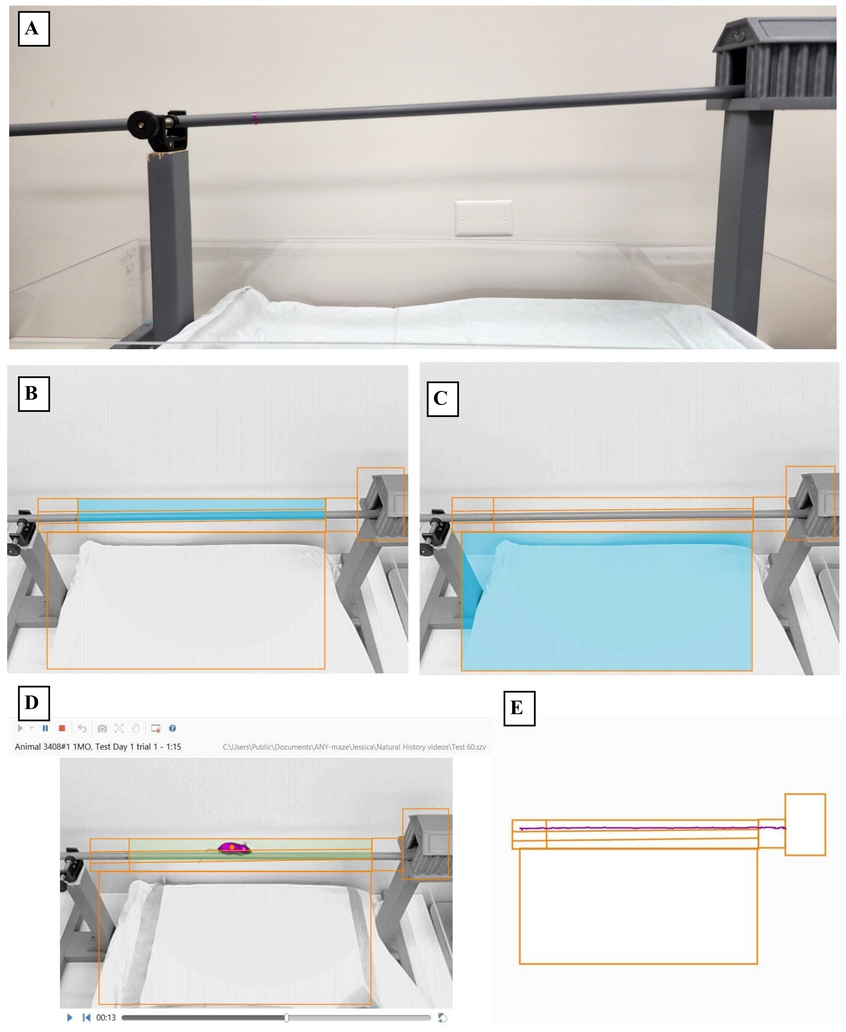

Figure 3: Balance beam behavioral test using ANY-maze. A Picture of the balance beam apparatus. **B-C** Designated "active" (**B**, blue) and "fall" (**C**, blue) zones in ANY-maze that measure the time to traverse and number of falls, respectively. **D** Representative WT mouse being tracked by ANY-maze while traversing the "Active" zone. **E** Tracking plot from D.

Results

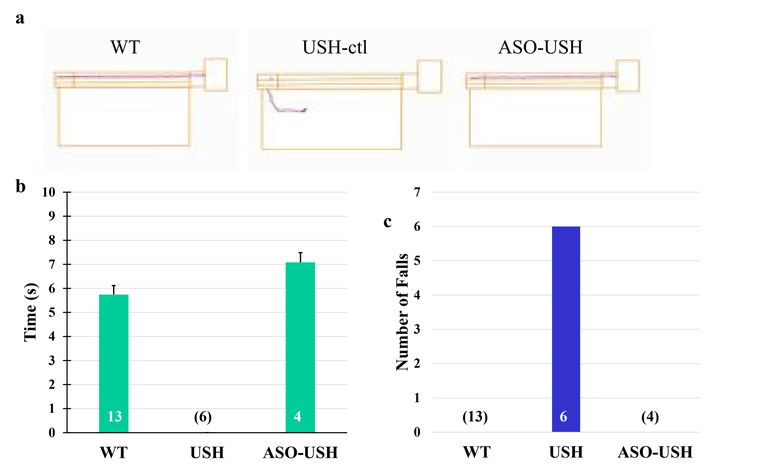

Figure 4: Balance beam test in USH1C mice. a Representative ANY-Maze tracking plots for WT, USH-ctl and ASO-USH mice. **b** Average time to traverse the balance beam in the active zone was not significantly different between wild-type (WT) and ASO-USH treated mice [5.75s and 7.08s, respectively (p=0.08002)]. **c** Number of falls during the balance beam test was significantly greater in ASO-ctl mice compared with WT and ASO-USH mice. The number of mice analyzed is indicated in the bars.

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 mice, as well as ASO treatment timing, dose, and window of therapeutic opportunity, will provide a treatment regimen and outcome measures to guide a future clinical trial and improve care for patients.**

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