Motor function, coordination, and balance are improved in Usher syndrome Type 1C mice treated with antisense oligonucleotides

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Introduction

- Usher syndrome (Usher) is an autosomal recessive genetic disease characterized by concurrent sensorineural hearing loss and retinitis pigmentosa[1].
- Usher has 4 clinical presentations (USH1-4) that differ in the severity and onset of symptoms[1,3].
- The underlying genetics of Usher is well understood, but treatment options are limited.
- The c.216G>A mutation (216A) in the *USH1C* gene accounts for nearly all the USH1 cases in the Acadian populations of Louisiana and Canada.
- The 216A splicing mutation leads to a severely truncated harmonin protein which is required for the development and function of inner ear hair cells and maintenance of retinal photoreceptors[4,5].
- The Lentz laboratory created an USH1C mouse model carrying the 216A mutation. The USH1C mice have hearing loss, imbalance, and visual dysfunction similar to patients.
- Antisense oligonucleotides (ASOs) designed to target the 216A mutation have been shown to transiently rescue hearing, balance, and balance in the short-term in USH1C mice[6-10].

The aim of this study was to determine the effect of ASO therapy on motor function, coordination, and balance behavior in USH1C mice.

USH1C gene and ASO targeting

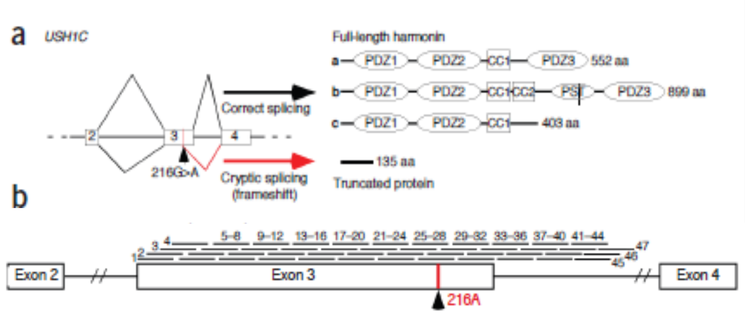

Figure 1. Targeting the 216A mutation with ASOs. a *USH1C* gene diagram and location of the 216A mutation in exon 3, correct and cryptic splicing that result in full-length and truncated harmonin proteins[6]. **b** Location of ASOs (1-47) targeting the 216A mutation.

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Materials and Methods

USH1C mice:

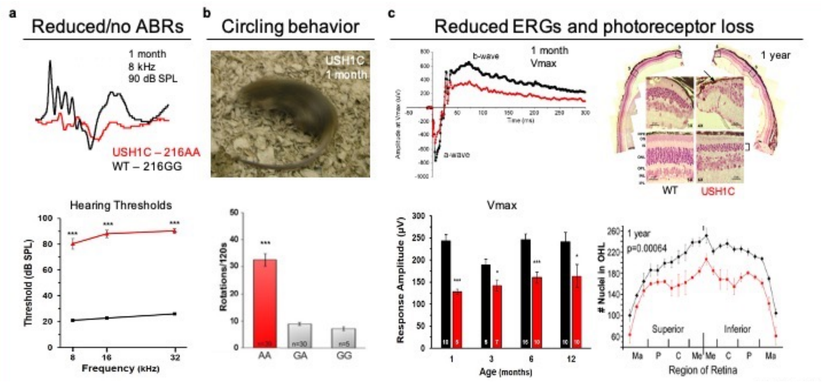

Figure 2. USH1C mice have hearing loss (a), imbalance (b), and visual dysfunction (c).

ASO treatment: USH1C mice were treated locally to the inner ear via semicircular canal injection at postnatal day 2 with ASOs targeting the 216A mutation.

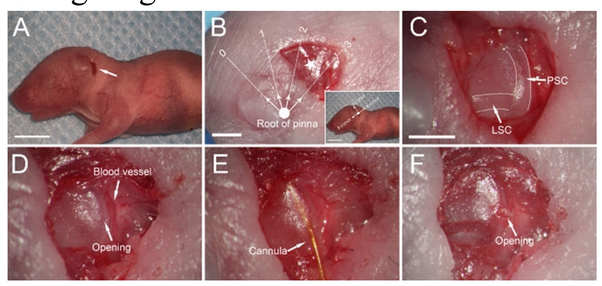

Figure 3. Semicircular canal injection in mice at P2. Post auricular incision is made (A). Orientation of the semicircular canals (B). Posterior semicircular (PSC) is identified (C). Injection location is determined (D). Small canula is used to deliver the ASO into the PSC (E).

Rotarod test: Motor function, coordination, and balance behavior were assessed by rotarod testing in USH1C-ASO, USH1C-control (untreated), and wild type littermates at 12 months of age.

Figure 4. Picture of rotarod apparatus for mice. Mice are placed in individual lanes at a baseline rotation of 4 RPM that accelerates to 40 RPM over 240 seconds. Three trials per mouse separated by a 10-minute rest period were recorded and the average latency to fall was calculated. Mice had been previously acclimated to the rotarod using the same testing paradigm 2-3 days prior to the experimental run.

Latency to Fall

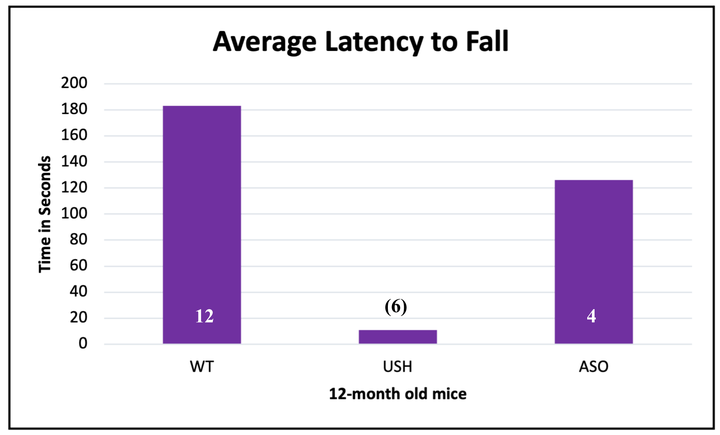

Figure 5. USH1C mice treated with ASOs have improved balance behavior as measured by rotarod testing. The latency to fall for the USH1C mice was significantly shorter comparted to wild type littermate control mice (P-value = 0.00048); whereas USH1C mice treated with ASOs were not significantly different from wild type littermates (P-value = 0.179). Number of mice analyzed is indicated in the bars.

Results and Conclusions

- Latency to fall in USH1C mice treated with ASOs locally to the inner ear at postnatal day 2 is not significantly different from wild-type littermates at 1 year-of-age, indicating a long-term benefit to motor function, coordination, and balance.

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