

CLINICAL IMPLICATIONS OF BASIC RESEARCH

Elizabeth G. Phimister, Ph.D., *Editor***Rescue from Hearing Loss in Usher's Syndrome**

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Attempts to restore or prevent genetically based hearing loss by means of biologic intervention have been largely unsuccessful because of the limitations inherent in both regenerating lost mechanosensory cells and salvaging the remaining function of partially damaged cells. Although the current treatment for hearing loss relies primarily on the use of cochlear implants (the Lasker–DeBakey Clinical Medical Research Award of 2013 was bestowed on the pioneers of this technology), the implants are still far from ideal. Elucidation of the genetic basis of many forms of hearing loss and advances in gene delivery may change this picture. Earlier this year, Lentz et al.¹ reported the rescue of low-frequency and midfrequency hearing and vestibular function in a mouse model of Usher's syndrome with the use of antisense oligonucleotides.

Usher's syndrome includes a group of recessive disorders characterized by hearing impairment, blindness resulting from retinitis pigmentosa, and the presence or absence of vestibular dysfunction. Three types (1, 2, and 3) have been described. The various types of Usher's syndrome have been traced to 10 genes; a mutation in any of these genes is sufficient to cause the disease.

The genes encode protein components of the hair cells of the inner ear, which transduce the mechanical oscillations of cochlear structures into neuronal impulses in the auditory nerve. Two of these proteins, cadherin 23 and protocadherin 15, form the tip links of the stereocilia, projections that extend from the surface of the hair cell and are deflected by cochlear vibrations.² The tip links tether the tips of the stereocilia to one another in rows and are stretched, or "tensioned," in such a way that the stereocilia deflections open and close cation channels adjacent to the tip links. This action results in the release of neurotransmitters that interact with the terminals of the auditory nerve, causing it to

fire. The protein harmonin, a component of the protein complex that anchors the tip links, is also known to regulate Cav1.3 calcium channels and exocytosis.³

Patients with mutations in *USH1C*, which encodes harmonin, have type 1 Usher's syndrome, the most severe form of the disease. It is characterized by profound hearing loss and the absence of vestibular function from birth; retinitis pigmentosa appears in the early teens. Although estimates indicate that Usher's syndrome occurs worldwide, with a prevalence of 3 to 6 cases per 100,000 persons, carriers of mutant *USH1C* have thus far been reported only in the Acadian population of Louisiana and in Pakistani and Lebanese families. A loss-of-function allele of *USH1C*, which creates a cryptic 5' splice site in exon 3, is unique to Acadians. Mice genetically engineered to carry this form of Usher's syndrome survived Hurricane Katrina in New Orleans⁴ and now serve as a model for the development of therapy for this condition.

Antisense oligonucleotides are short, chemically modified synthetic nucleic acids that bind RNA by means of base-pair hybridization. These oligonucleotides modulate posttranscriptional regulation, either by silencing genes or by altering RNA metabolism. They are being developed to treat a variety of conditions, including neurodegenerative diseases, and some are now in clinical trials.⁵ Because the Acadian *USH1C* target mutation leads to a splicing defect, Lentz et al. designed an antisense oligonucleotide intended to restore correct splicing.

The approach taken by the investigators was deceptively simple (Fig. 1). Using in vitro screens, they tested dozens of antisense oligonucleotides for their ability to block the cryptic splice site and thereby the aberrant splicing of *USH1C* messenger RNA. Once they established which oligonucleotides obstructed cryptic splicing, promoted correct splicing, and increased harmonin levels

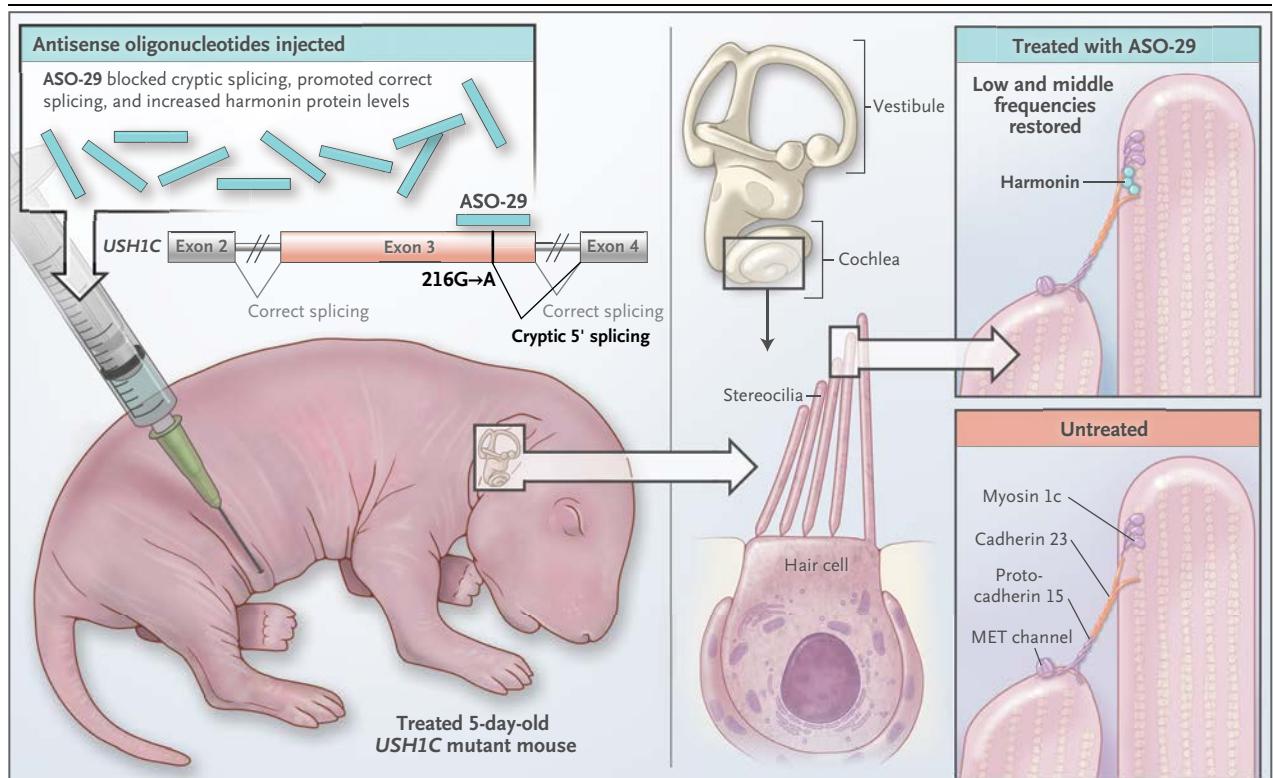


Figure 1. Rescue from Hearing Loss and Circling Behavior in a Mouse Model of Type 1 Usher's Syndrome.

The mutation *USH1C* 216G→A introduced in mice creates a cryptic 5' splice site that is a replica of the Acadian *USH1C* mutation found in humans that causes hearing loss, balance dysfunction, and blindness. Lentz et al.¹ reported that the intraperitoneal injection of the antisense oligonucleotide ASO-29 into these mice within a few days after birth led to a correction of the splicing defect and the restoration of functional harmonin in the tip links of the stereocilia, which contain myosin 1c, cadherin 23, protocadherin, and mechano-electrical transduction (MET) channels. The hearing thresholds of the treated mice at 8 and 16 kHz, which are associated with hair cells in the apex of the cochlea, were similar to those of nonmutant mice and were maintained for 6 months. The basal cells of the cochlea remained damaged, and high-frequency hearing did not develop. Balance defects related to damage of the vestibular hair cells, represented by head tossing and circling, were absent in the treated mutant mice.

in mutant cell lines, they were ready to test the approach in the mouse model. The “winning” antisense oligonucleotide was ASO-29, which had the greatest ability to correct splicing and increase expression of harmonin. Mutant mice were injected intraperitoneally with approximately 0.75 mg of ASO-29.

The results were dramatic: normal hearing and balance developed in the treated mutant mice, which expressed normal levels of harmonin. These mice also had more hair cells and more ordered hair-cell bundles than the untreated mutant mice. Hearing at low frequencies and midfrequencies, measured on the basis of the auditory brain-stem response, was the same as that in nonmutant mice. However, hearing at high frequencies was not restored. There was a

clear correlation between a residual hearing deficit and the number of abnormal stereociliar bundles remaining in the base of the cochlea — a finding with inauspicious ramifications for the correction of the loss in high-frequency hearing associated with aging and exposure to noise. The timing of the delivery of the oligonucleotides was crucial: delivery 3 to 5 days after birth was the most effective, although vestibular function was rescued when mice were treated as late as 13 days after birth. The effects were durable: at 6 months of age, hearing and vestibular function remained intact.

Is this approach applicable to other forms of hearing loss? Hereditary hearing impairment is highly heterogeneous. The oligonucleotide therapy used by Lentz et al. was directed at splicing

defects, which are responsible for only some forms of genetic hearing loss. How might their strategy translate to treatment in humans? Given that normal human newborns, unlike mice, are able to hear at birth, the therapeutic window for oligonucleotide therapy in humans may be during gestation, a more complex period in which to undertake the process. However, there is a great deal of anticipation regarding the use of oligonucleotides for the treatment of the later-onset blindness in the Usher syndrome model. The relatively slow progression of vision loss in humans may render its prevention more amenable to intervention, should the proof-of-principle work of Lentz et al. translate to a clinical trial.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

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