Advances in Drug Therapy for Usher Syndrome

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Usher Syndrome Family Conference

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LSUHealthNewOrleans
HEALTH SCIENCES CENTER

- Overall goal
- Usher syndrome update and current hypotheses
- New therapeutic approaches
- Read-through small molecules
- Antisense oligonucleotides (ASOs)
- Future Directions

Overall Goal for the scientific and medical Usher community

- Develop tools that provide a medical benefit to every individual with Usher syndrome

Usher Syndrome Update

- Prevalence
  - Autosomal, recessive, genetic disease = males and females are equally affected; and you have to inherit 2 copies of a mutation (1 copy from mom and 1 copy from dad)
  - Leading genetic cause of deaf-blindness
  - ~ 1 in 20,000 individuals worldwide
  - 1 in 6,000 in 2 pediatric populations (hearing impaired children)
  - Both rare and common

Usher Syndrome Update

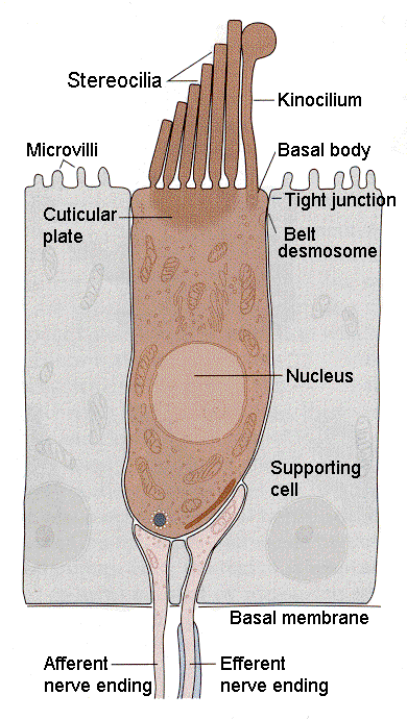
- Significant progress has been made to identify genes and mutations that cause Usher
- 3 clinical types – USH1, USH2, USH3
- 1 new clinical type – atypical USH (CEP250); early onset hearing impairment, mild RP
- Usher genes
  - 16 loci (different places in our genome)
  - 13 causative genes and 1 modifier gene (PDZD7) have been identified

Usher syndrome – Types and Genes

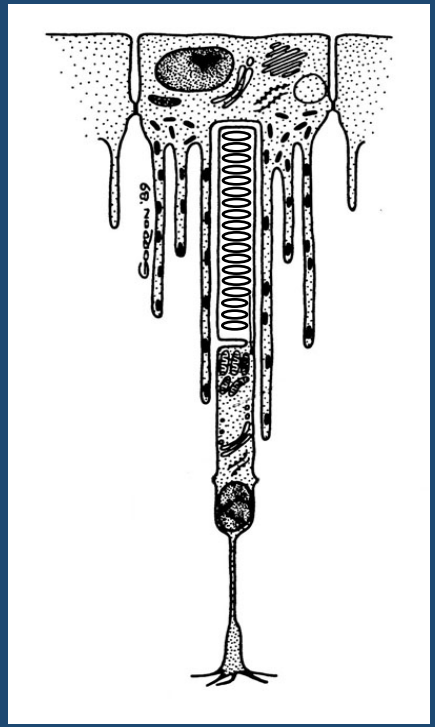
Type	Presentation	Locus	Gene	Protein
USH1 **35-45%**	Congenital, severe-profound HI Vestibular Areflexia Adolescent onset RP	USH1B	MYO7A	Myosin VIIa
		USH1C	USH1C	Harmonin
		USH1D	CDH23	Cadherin 23
		USH1F	PCDH15	Protocadherin 15
		USH1G	USH1G	Sans
		USH1J	CIB2	Calcium- and integrin-binding protein 2
USH2 **55-65%**	Congenital, mild-severe HI Late adolescent-early adult onset RP	USH2A	*USH2A*	Usherin
		USH2C	*ADGRV1 (GPR98)*	G protein-coupled receptor 98
		USH2D	*DFNB31*	Whirlin
USH3 **5%**	Post-lingual, progressive HI Adult onset RP Variable Vestibular Responses	USH3A	*CLRN1*	Clarin-1
		USH3B	*HARS*	Histidyl-tRNA synthetase

Usher proteins

Cochlear Hair Cells

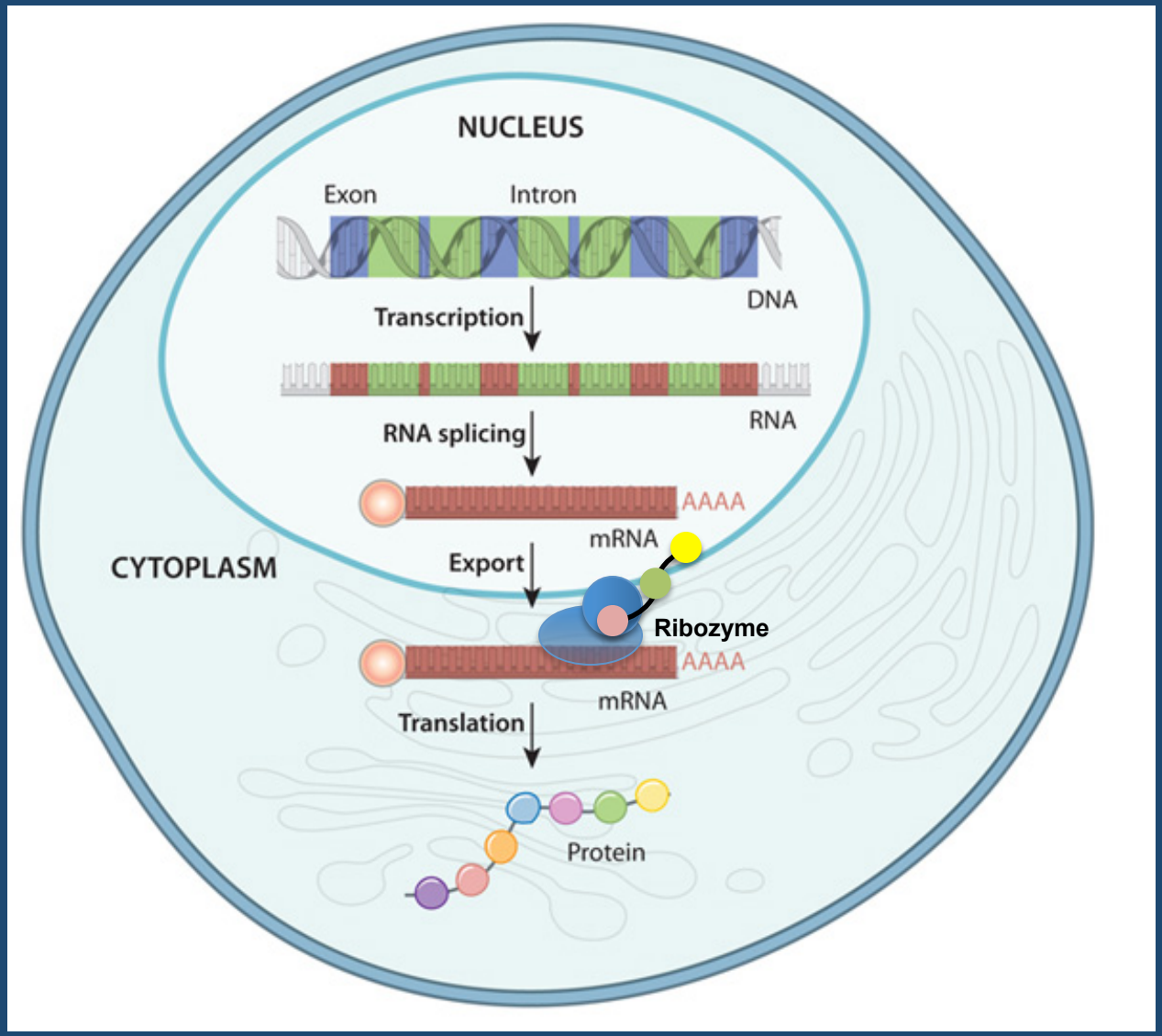


Retinal Photoreceptors & Retinal Pigment Epithelium



HYPOTHESIS: mutations in Usher genes result in defects in hair cell development and photoreceptor maintenance

Gene (code) → RNA (message) → Proteins (perform functions)



Mutation types

Nonsense – a mutation that prematurely stops the making of a protein

Missense – a mutation that changes the code of the protein by 1 amino acid

Splicing – a mutation that alters the processing of the RNA used to code for a protein

Insertions/Deletions (INDELS) – insertions or deletions of part of a gene, which then alters the code of a protein

HYPOTHESIS: New data suggest a strong genotype – phenotype correlation that is based on mutation type.

Genotype – Phenotype Correlation

- Relationship between gene/mutation (genotype) and symptoms (phenotype)

- For at least 4 USH1 genes (MYO7A, CDH23, PCD15 and USH1C), there appears to be a genotype-phenotype correlation in patients. Nonsense/indel/ splicing mutations that results in essentially no protein being made cause USH1; whereas missense or splicing mutations that result in a small amount of protein cause hearing loss alone and not RP.

- All USH genes make multiple forms of their encoded protein. Single gene makes several mRNAs (splicing), which encode several forms of a protein. These different forms of a protein have different functions in hair cells and photoreceptors. Different types of mutations in different areas of the gene may affect all of the forms of a protein or only some, and this may contribute to the differences in phenotypes.

- Natural History Studies aim to determine a genotype-phenotype correlation. Understanding natural history will allow us to be able to predict timing and severity of symptoms; which is needed to conduct clinical trials because it tells us when to give therapy and how to determine if the therapy is working.

New Therapeutic *Strategies*

- Target deafness and/or blindness in general
  - Treatment for any type of Usher
  - ➢ Stem cell therapy to replace cochlear and/or retinal cells
    - Skin or blood sample, turn them into stem cells, then coax them into becoming retinal cells
  - ➢ Optogenetics (gene therapy)
    - Deliver genes that give light sensitivity to different cells of the retina

- Target a particular gene
  - Treatment would target one type of Usher regardless of mutation
  - ➢ Gene replacement therapy – deliver a normal copy of a gene to replace the one with a mutation
    - Viral mediated – USH1B (clinical trial); USH1C; USH2A (dual vector); USH3A
    - Nanotechnoloty – USH2A

New Therapeutic *Strategies*

- Target a particular mutation type
  - Treatment of any Usher type caused by a nonsense mutation
    - Translational read-through inducing drugs – USH1C
- Target a particular mutation in a particular gene
  - Treatment for one type of Usher caused by one specific mutation
    - Antisense oligonucleotides – USH1C (USH1C c.216G>A)
    - Small Molecule Chaperone Therapy – USH3A (CLRN1 p.N48K)

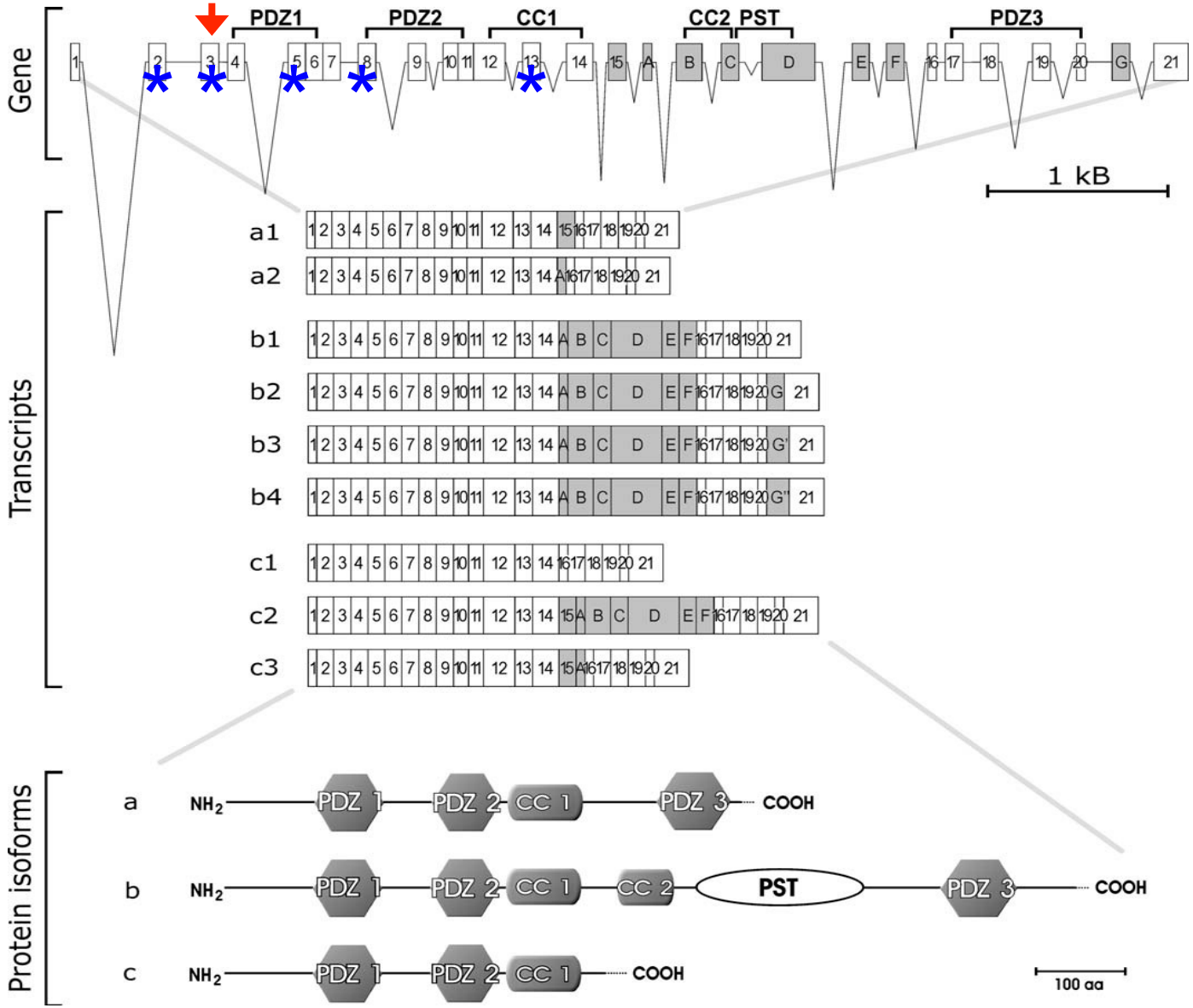
Therapeutic Strategies under development for USH1C

There are 2 strategies in the preclinical testing phase in the research laboratory-

1. Translational read-through inducing drugs (TRIDs)
2. Antisense Oligonucleotides (ASOs)

USH1C gene and Harmonin Protein Isoforms

c.216G>A Splicing mutation (ASO) ∗ Nonsense mutation (TRID)



USH1C gene

mRNA splice variants

Harmonin isoforms

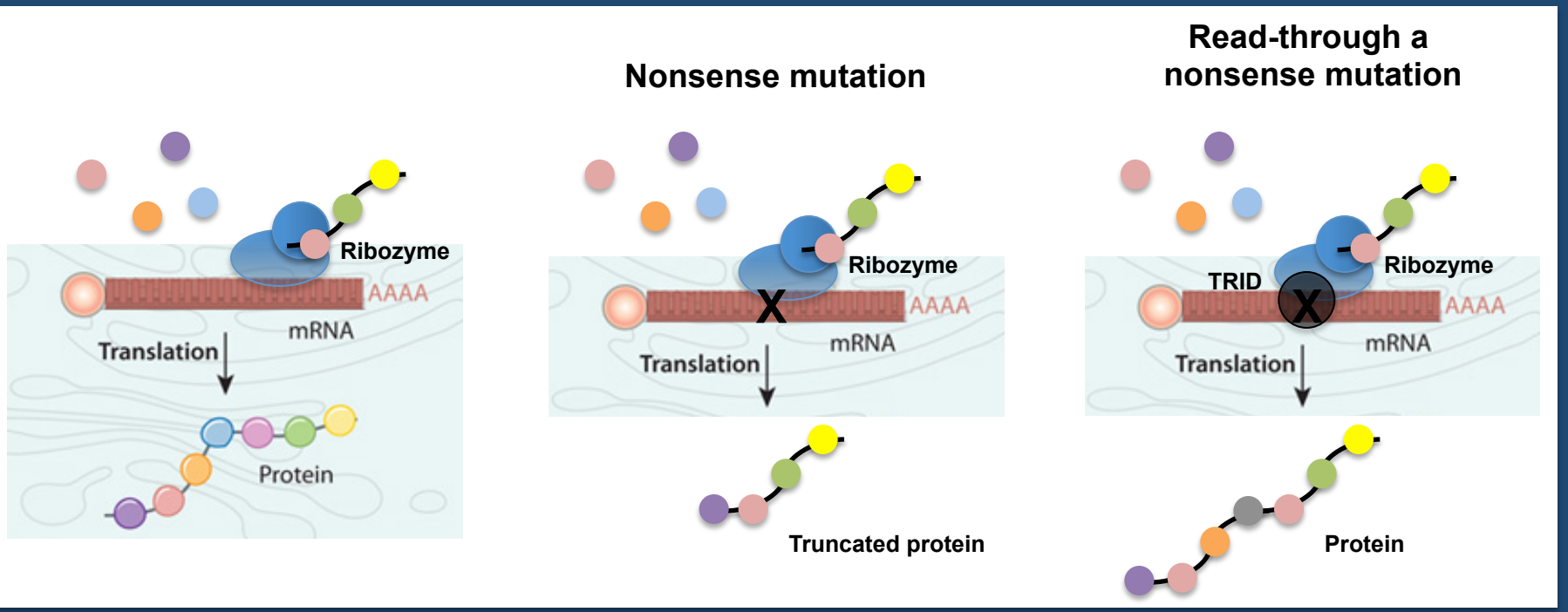
Reiners et al 2006

Translational read-through inducing drugs (TRIDs)

TRIDs – type of aminoglycosides (antibiotics)

Target ribozymes, which are the proteins responsible for translating mRNA into proteins; site of protein synthesis.

Insert a random amino acid at the nonsense mutation site to prevent stopping translation and a truncated protein.



Translational read-through inducing drugs (TRIDs)

Currently being tested in the research laboratory on nonsense mutations in the *USH1C* gene, but would work on any nonsense mutation in any USH gene

~ 12% of USH mutations are nonsense mutations

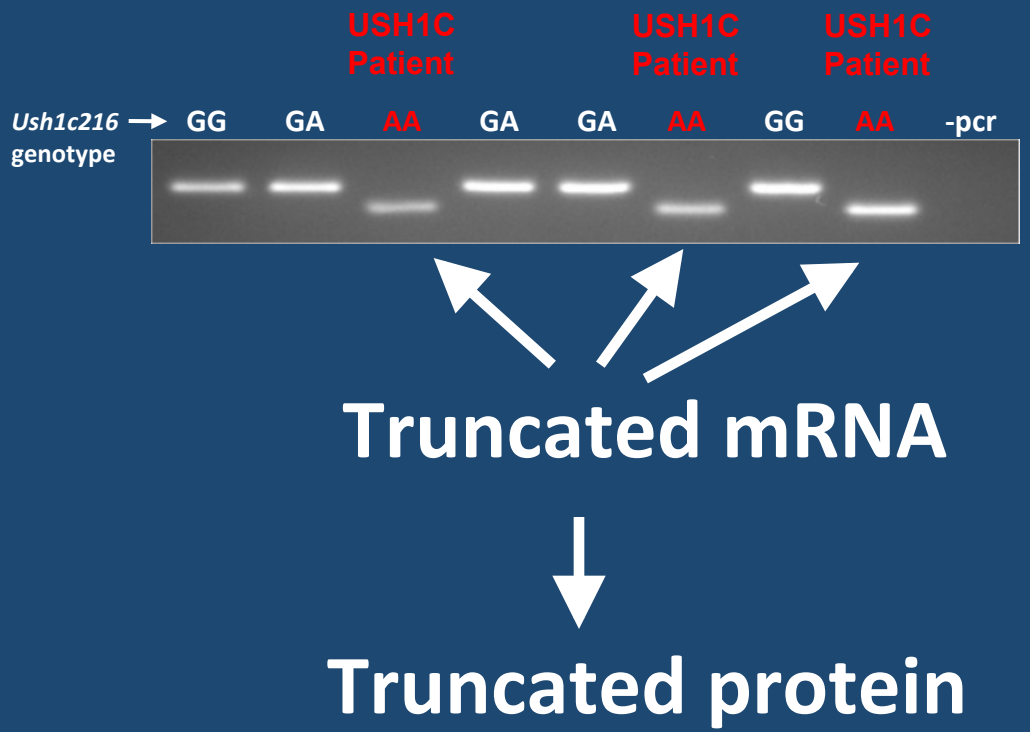
2 different TRIDS have been tested to correct the p.R31X mutation in *USH1C* in the eyes of laboratory animals; and full length Harmonin protein was detected (Goldmann et al, 2012)

Status: developing a better model to test for efficacy
- animal model with retinal degeneration
- patient cell lines

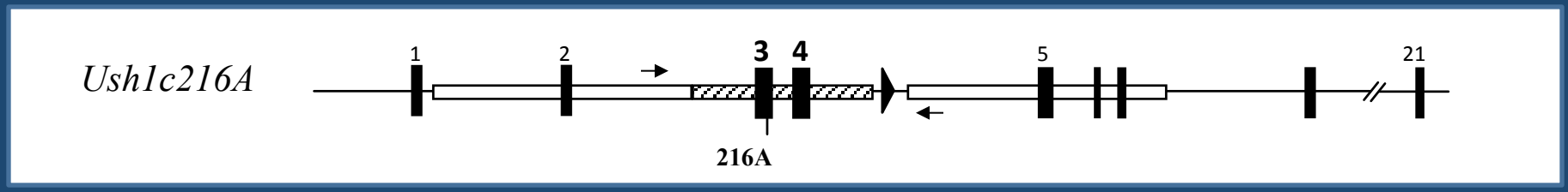
Development of Antisense oligonucleotide therapy for USH1C in Louisiana

- Nearly all type 1 Usher in Louisiana is caused by the c. 216G>A mutation in *USH1C*

<u>*USH1C* gene expression in USH1C Patients</u>



Put in the human 216A mutation into the Mouse *Ush1c* gene (knock-in)



Cochlea

Ush1c genotype →

GG GG AA AA GA GA GA GA -RT -PCR

← Truncated mRNA

Retina

Ush1c genotype →

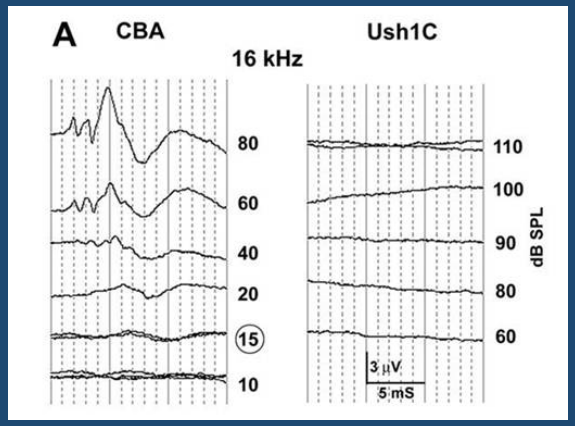
GG GG AA AA GA GA GA GA -RT -PCR

← Truncated mRNA

Lentz et al 2007

USH1C 216AA Mice

Abnormal/no ABRs

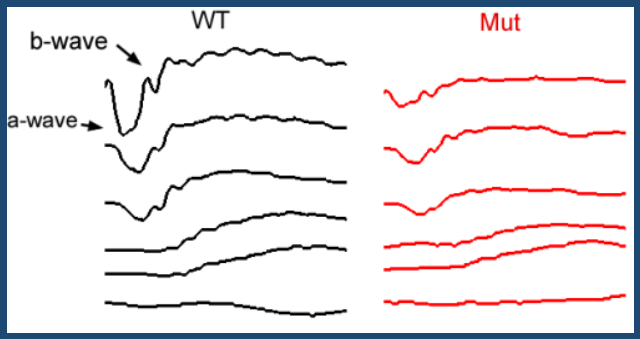


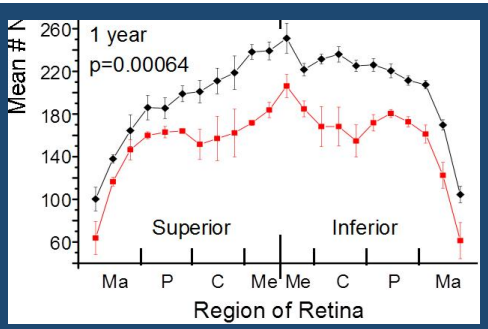
Deaf

Circling and head tossing behavior

Vestibular Defects

Reduced electroretinograms (ERGs) & photoreceptor loss



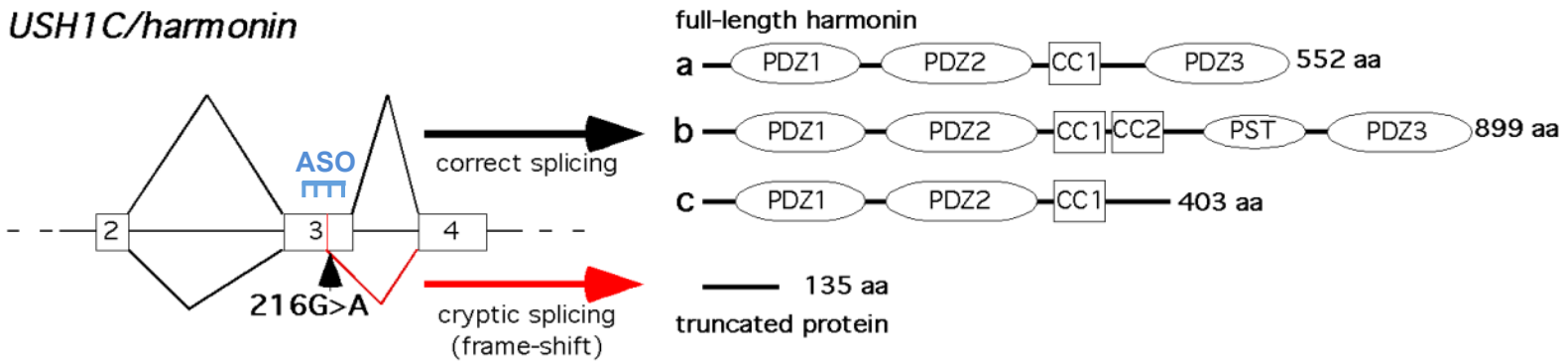


Visual Dysfunction & Degeneration

Lentz et al 2010

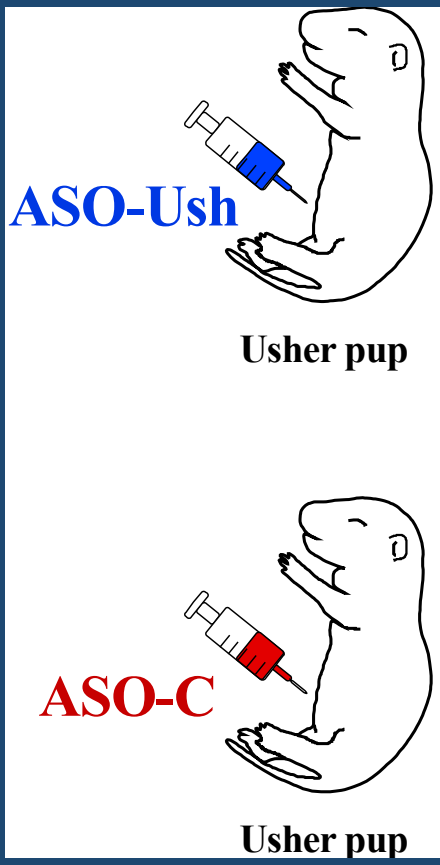
Antisense Oligonucleotides (ASOs)

- ASOs - Short, modified RNA molecules
- Targets complementary RNA in cell
- USH-ASO targets USH1C 216A-RNA to correct splicing
- Treatment for USH1C caused by the *USH1C* c.216G>A mutation



Lentz et al 2013

Laboratory Treatment Model with ASOs



Behavior

Vestibular function (Open-field, swimming)

Physiology

Hearing function (ABR, DPOAE)

Visual function (ERG)

Structure

Hair cell morphology (IHC)

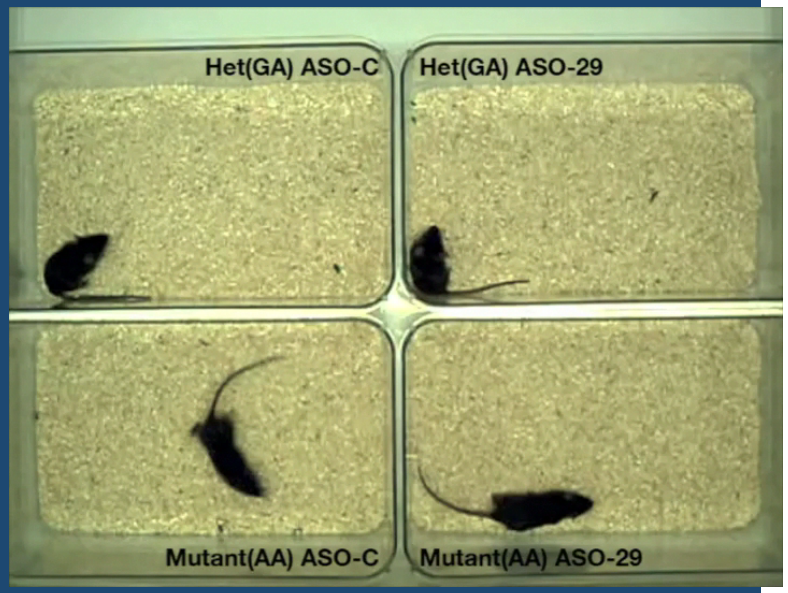
Photoreceptor cell morphology (IHC)

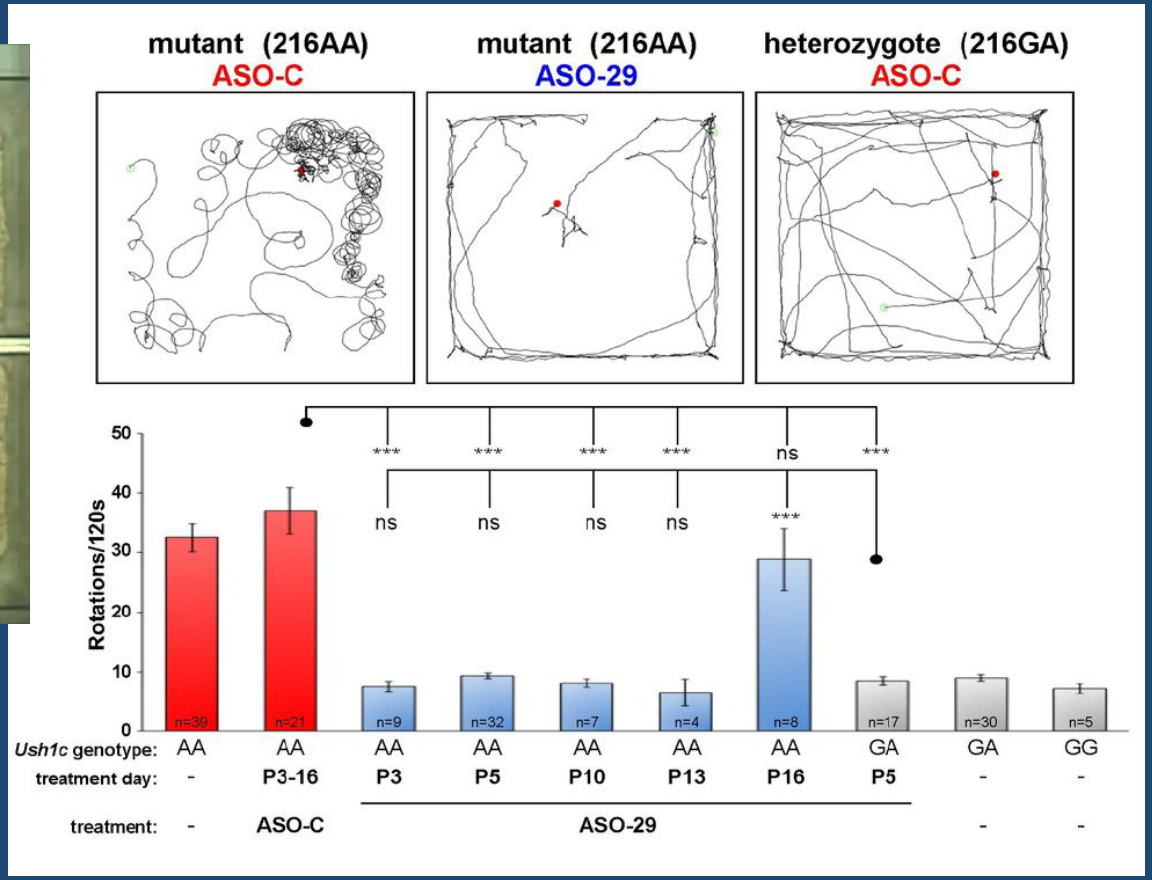
Molecular

Ush1c and Harmonin expression

ASO treatment improves balance in Usher mice

Open-field Chamber to measure vestibular function

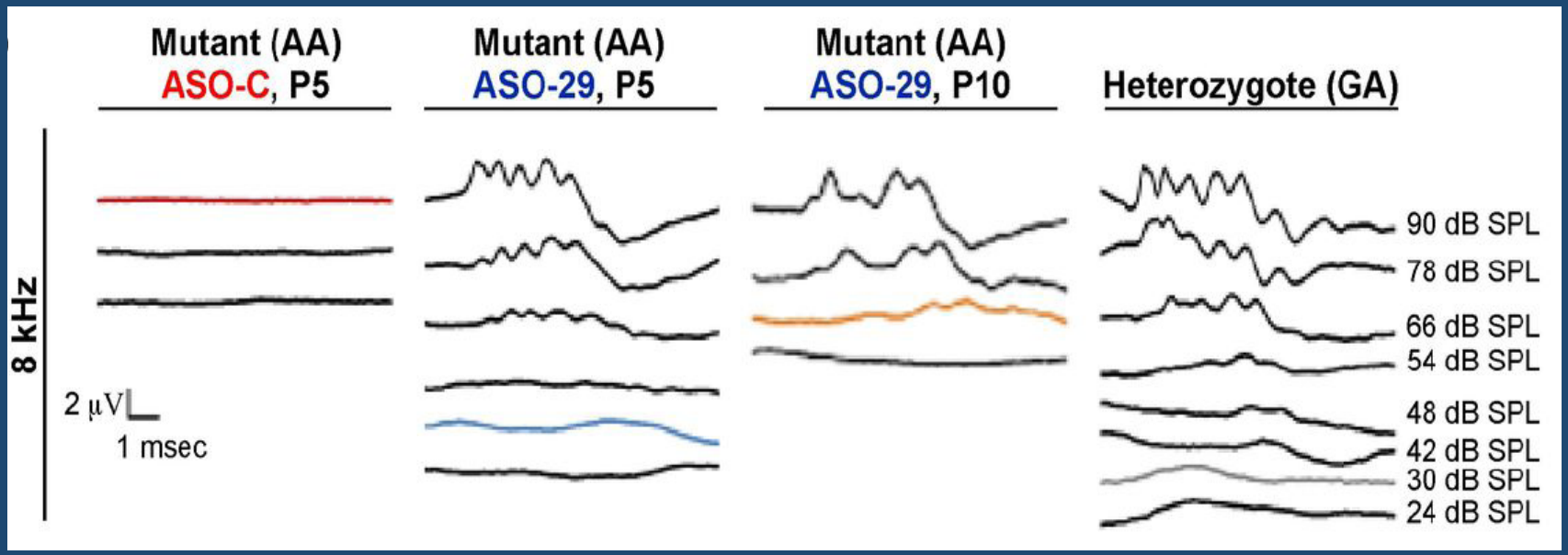




Lentz et al 2013

ASO treatment improves hearing in Usher mice

Example Hearing Audiograms (ABRs)



Lentz et al 2013

Development of ASO Therapy for USH1C

- Treatment for the Acadian *USH1C* c.216G>A mutation
- Treatment of Usher mice improves hearing and balance (Lentz et al 2013); currently testing for vision improvements

Status:

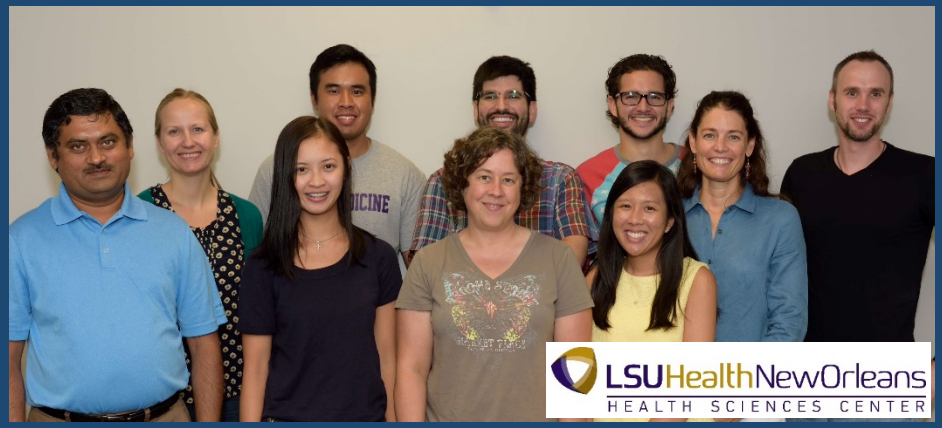
- preclinical animal testing in progress
  - testing effects of different doses
  - testing effects of timing of doses
  - testing for how long improvements last

- Natural clinical history in patients

Progress with success or promise in the development of treatments for Usher syndrome

USH1B - Ush-Stat clinical trial: gene replacement therapy
USH1C – ASOs, TRIDs, gene replacement therapy
USH2A – Gene therapy (viral, nanotechnology)
USH3A – Gene therapy, small molecule chaperone therapy

ACKNOWLEDGEMENTS



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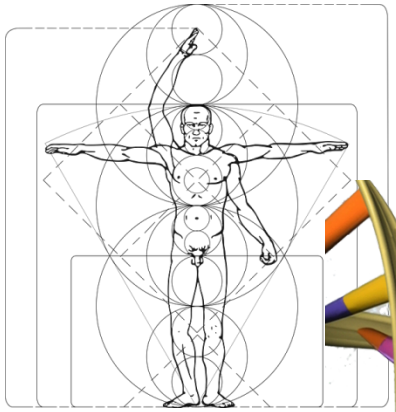
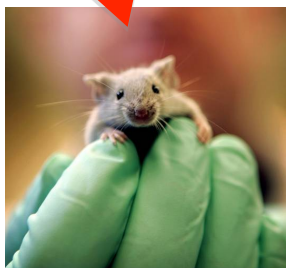
Further develop Ush-ASO : treat deafness/blindness in Usher mice



2000
USH1C.216G>A causes Usher syndrome type 1C

Bitner-Glindzics et al
Verpy et al
nature genetics

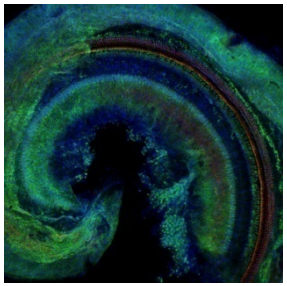
USH1C.216G>A



2005
Knocked-in 216A

2010
Deaf, vestibular defects
Retinal degeneration

Lentz et al
Developmental Neurobiology



2013
Rescue of deafness, vestibular defects

Lentz et al
nature medicine