Usher Syndrome Type 1C Research Update

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Outline

- **Usher syndrome review**
  - Characteristics and Prevalence
  - Types and subtypes
  - Clinical management and therapies under investigation
- **Lentz Lab Mission**
  - *USH1C* gene
  - Knock-in mouse model
- **Antisense Therapy for Acadian USH1C**
  - Targeting 216A mutation
  - Treatment of USH1C mice with ASOs
- **Gene Therapy for all USH1C**
  - USH1C gene therapy development
  - Treatment of USH1C mice with gene replacement therapy

Usher syndrome review – Prevalence and Types

- Usher syndrome (USH or US) is the leading genetic cause of concurrent hearing and vision impairment. Some individuals also have imbalance.
- Estimated 1 in ~20,000 individuals in the world have Usher
- Currently, there are 3 clinical types and 11 subtypes (genes):

Types:	Type 1 (USH1)	Type 2 (USH2)	Type 3 (USH3)
Subtypes:	USH1B (*MYO7A*) USH1C (*USH1C*) USH1D (*CDH23*) USH1F (*PCDH15*) USH1G (*SANS*) USH1J (CIB2)	USH2A (*USH2A*) USH2C (*ADGRV1*) USH2D (*WHRN*)	USH3A (*CLRN1*) USH3B (*HARS*)

Usher syndrome review - Diagnosis

- Diagnosis is established with clinical features based on-
  - Severity of sensorineural hearing impairment (HI)
  - Presence of vestibular areflexia (imbalance)
  - Age of onset of retinitis pigmentosa (RP) – progressive visual loss that begins with night-blindness

Type 1 (USH1)	Type 2 (USH2)	Type 3 (USH3)
Severe-profound HI	Mild - severe HI	Post-lingual HI
Vestibular areflexia	RP beginning in late adolescence	Variable Balance
RP beginning in early adolescence		RP beginning in adulthood

Usher syndrome review – Genetic Testing

- Diagnosis is established with clinical features
- Genetic testing confirms diagnosis and determines the specific mutation that is the cause of the patient's hearing and visual symptoms
- Multigene genetic testing panels are most commonly used-
  - Usher syndrome multigene panel
  - Inherited Retinal Dystrophy panel
  - Hereditary Hearing Loss panel

Type 1 (USH1)	Type 2 (USH2)	Type 3 (USH3)
USH1B (*MYO7A*)	USH2A (*USH2A*)	USH3A (*CLRN1*)
USH1C (*USH1C*)	USH2C (*ADGRV1*)	USH3B (*HARS*)
USH1D (*CDH23*)	USH2D (*WHRN*)	
USH1F (*PCDH15*)		
USH1G (*SANS*)		
USH1J (*CIB2*)		

Usher syndrome review – Clinical Management

- Treatments
  - USH1 – cochlear implants or sign language; occupational and physical therapy; low vision aids
  - USH2 – hearing aids, cochlear implants; low vision aids
  - USH3 – hearing aids, cochlear implants; low vision aids

- Therapies under investigation
  - UshStat retinal gene replacement of *MYO7A* for USH1B patients
    - Trial began in 2014 and has completed the dose escalation phase, however it is currently not recruiting
    - Clinicaltrials.gov (NIH) identifier : NCT02065011

  - QR-421a retinal antisense (ASO) treatment for USH2A patients with *USH2A* exon 13 mutations
    - ProQR Therapeutics
    - 3-month interim findings (March 2020) showed the ASO was safe and well tolerated in 8 USH2A patients, 2 patients also showed improvements in retinal sensitivity, retinal structure, and visual fields
    - More information:
      - www.proqr.com (website)
      - Clinicaltrials.gov (NIH) identifier : NCT03780257
    - Recruiting

Usher syndrome review – Clinical Management

- Therapies under investigation, cont.
  - CL-17-01 retinal antioxidant treatment for RP with Usher syndrome (Australia)
    - Nacuity Pharmaceuticals, Inc.
    - More information:
      - www.nacuity.com
      - Clinicaltrials.gov (NIH) identifier : NCT04355689
    - Not yet recruiting
  - NPI-001 oral antioxidant given to patients with RP (including all USH)
    - Nacuity Pharmaceuticals, Inc.
    - Completed a 30-patient study that showed it was well tolerated and improvements in retinal sensitivity
    - Currently conducting an extension study
    - More information: www.nacuity.com

Usher syndrome review – USH1C Pre-clinical Therapeutic Development

- NIH National Eye Institute
  - Dr. Tiansen Li and Dr. Anand Swaroop
  - Developed USH1C retinal organoid models from several USH1C patient fibroblast skin cells
  - Currently screening known small molecule and other drug candidates
  - Sponsored by Usher 2020 Foundation

- Johannesburg Gutenberg, University of Mainz & LMU Munich
  - Dr. Uwe Wolfrum and Dr. Nikolai Klymiuk
  - Developed a transgenic USH1C pig model with hearing, balance, and visual deficits
  - Currently characterizing the pig model, studying mechanisms of USH1C disease, and creating a breeding herd to test new therapies
  - Sponsored by Usher 2020 Foundation and FAUN Foundation

Usher syndrome review – USH1C Pre-clinical Therapeutic Development

- Oregon Health & Science University
  - Dr. John Brigande
  - Developing an USH1C non-human primate model and genetic therapy approaches
  - Sponsored by NIH/NIDCD

- Odylia Therapeutics
  - Founded by MEEI and Usher 2020 as a non-profit company to bring rare retinal disease therapies to the clinic
  - In collaboration with Drs. Uwe Wolfrum, Kerstin Nagel-Wolfrum, Nikolai Klymiuk, Pigmod, and other experts, developing a comprehensive drug development plan to bring USH1C gene replacement therapy to clinical trials
  - Currently testing AAV gene therapy in the USH1C pig model
  - Sponsored by Usher 2020 and FAUN Foundation

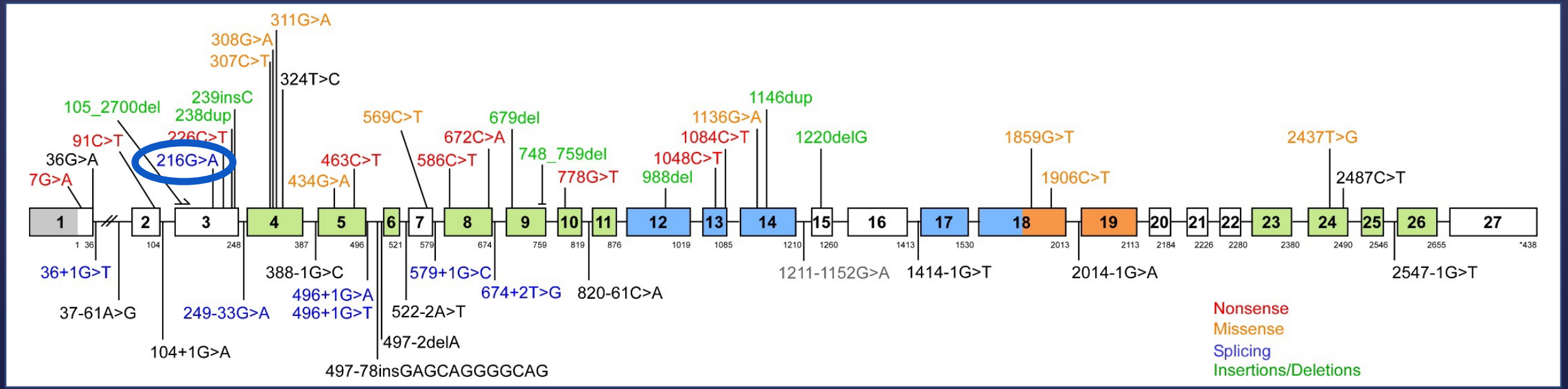
Usher syndrome Research - Lentz Lab Mission

- To understand disease mechanisms – how genetic changes cause hearing, balance, and vision impairments
- To develop new therapies for the treatment of hearing impairment, imbalance, and visual loss associated with Usher syndrome
- Focus on subtype USH1C

Types:	Type 1 (USH1)	Type 2 (USH2)	Type 3 (USH3)
Subtypes:	USH1B (*MYO7A*) **USH1C (*USH1C*)** USH1D (*CDH23*) USH1F (*PCDH15*) USH1G (*SANS*)	USH2A (*USH2A*) USH2C (*ADGRV1*) USH2D (*WHRN*)	USH3A (*CLRN1*) USH3B (*HARS*)

Subtype USH1C is caused by mutations in the *USH1C* gene

- *USH1C* gene is on chromosome 11 and contains 27 exons that are used to encode 3 families of Harmonin proteins (Harmonin-a, -b, -c)
- Harmonin proteins are found in the ear and eye
- ~47 mutations known* to cause USH1C

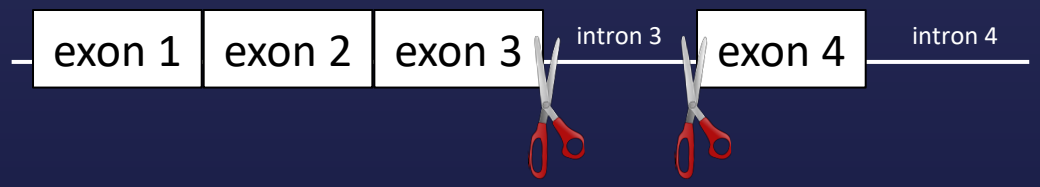


* Listed as “Pathogenic” in at least 1 US or European Database: ClinVar; HGMD; UMD-USHbases; LOVD (2020)

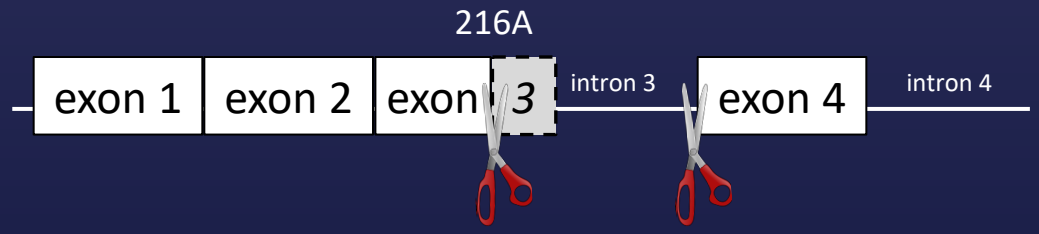
216A mutation is a splicing mutation

- Splicing is the process by which introns are removed to produce a mature messenger RNA that only contains exons used to make a protein
- 216G>A splicing mutation is a founder mutation in the Acadian populations
- The G → A change at position 216 in the *USH1C* gene causes aberrant splicing that results in a truncated mRNA and protein; and no functional protein in the eye and ear

Correct Splicing



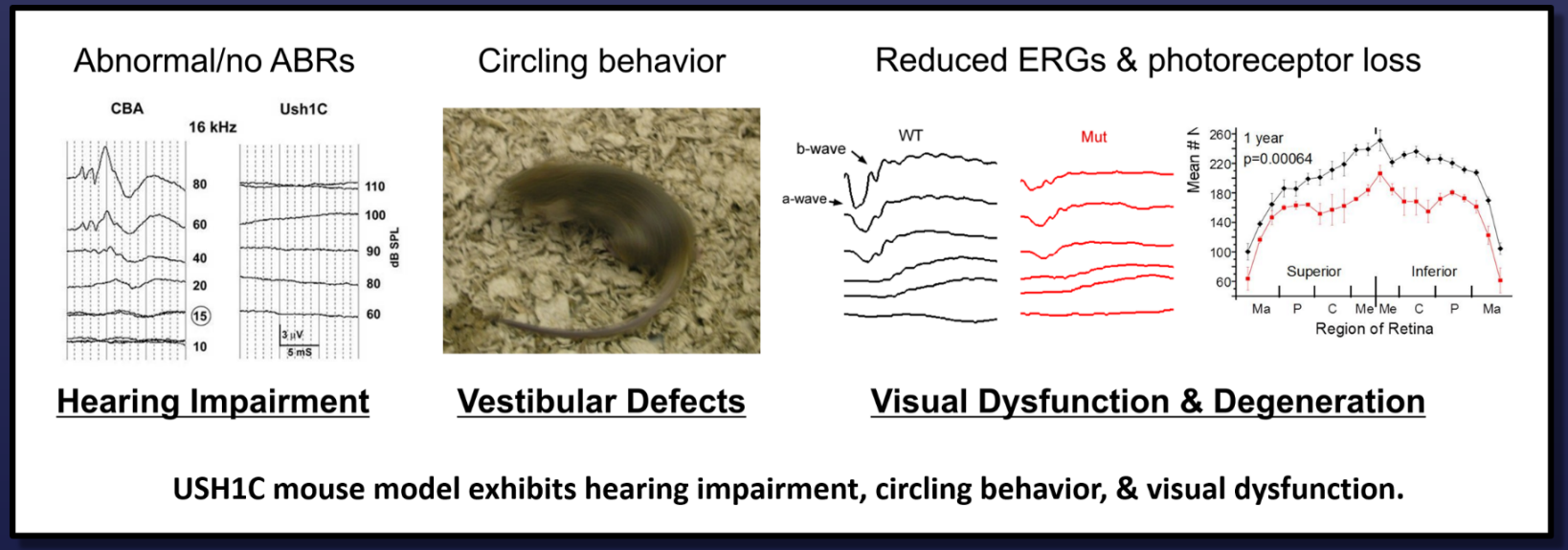
Aberrant Splicing



Lentz et al 2005

USH1C mouse model in the Lentz Lab

- Knock-in mouse model of the Acadian USH1C c.216G>A
- Similar symptoms as patients
  - Severe-profound hearing loss → abnormal/no auditory brainstem response (ABR)
  - Balance problems → circling in cage and head tossing
  - Mild vision loss → reduced electroretinogram (ERG) and slow photoreceptor loss

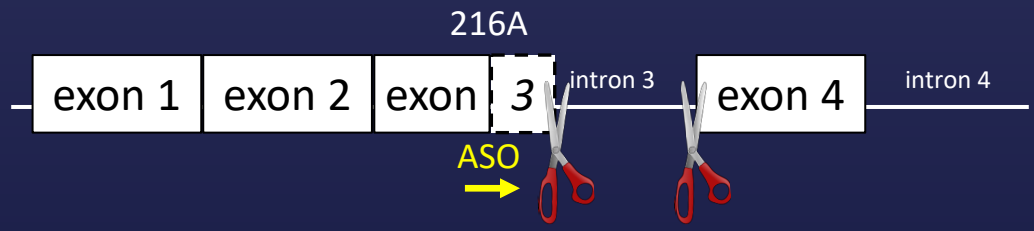


Lentz et al 2007, 2010

Antisense Therapy for Acadian USH1C

- Designed an Antisense Oligonucleotide (ASO) to target the 216A mutation in the pre-spliced RNA and correct splicing
- ASOs are short pieces of nucleic acids (DNA/RNA) that bind to their target
- 216A-targeted ASO is designed to bind to the 216A mutation which blocks splicing proteins from cutting at the wrong place, and forces correct splicing

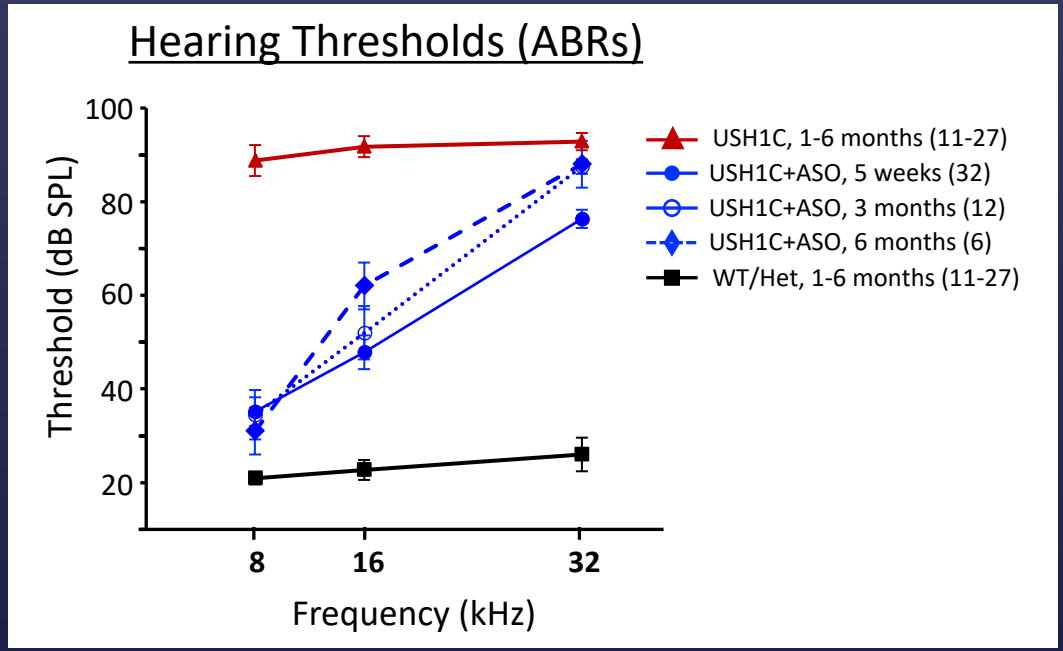
ASO-corrected 216A Splicing



ASOs restore hearing in USH1C mice

- ASOs injected systemically restore hearing thresholds (ABRs) in USH1C mice
- Treatment must be given before hearing begins-

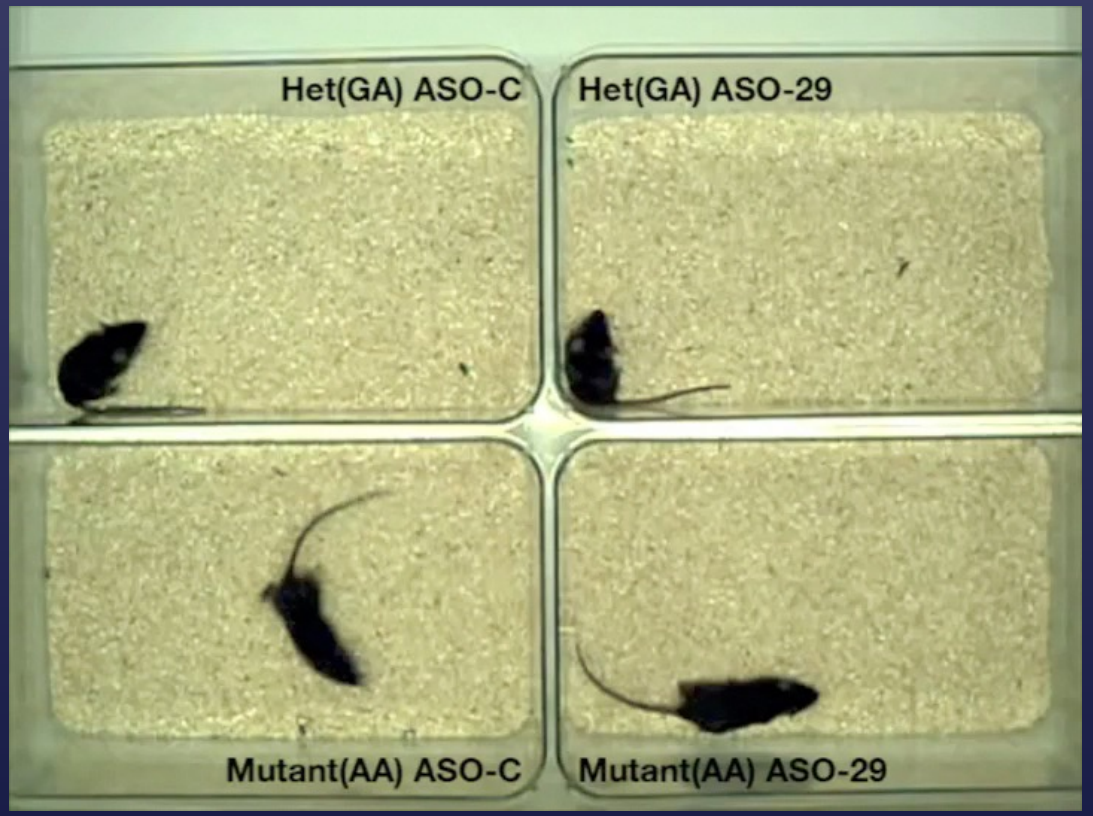
Single Systemic ASO Treatment
given on post-natal day (P)1



Lentz et al 2013, 2017

ASOs restore balance in USH1C mice

- ASOs restore balance behavior in USH1C mice
- Treatment timing?

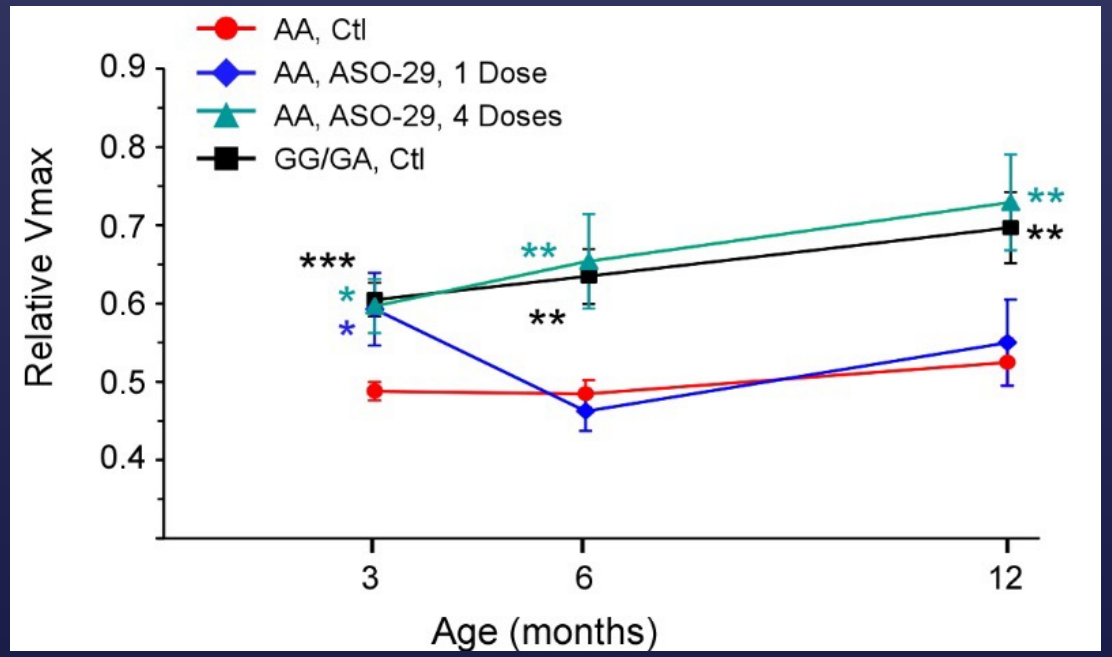


Lentz et al 2013

ASOs restore vision in USH1C mice

- ASOs injected one time directly into the eye by intravitreal injection restore visual function in USH1C mice for 3 months
- Continued treatments maintain visual benefits for up to 1 year

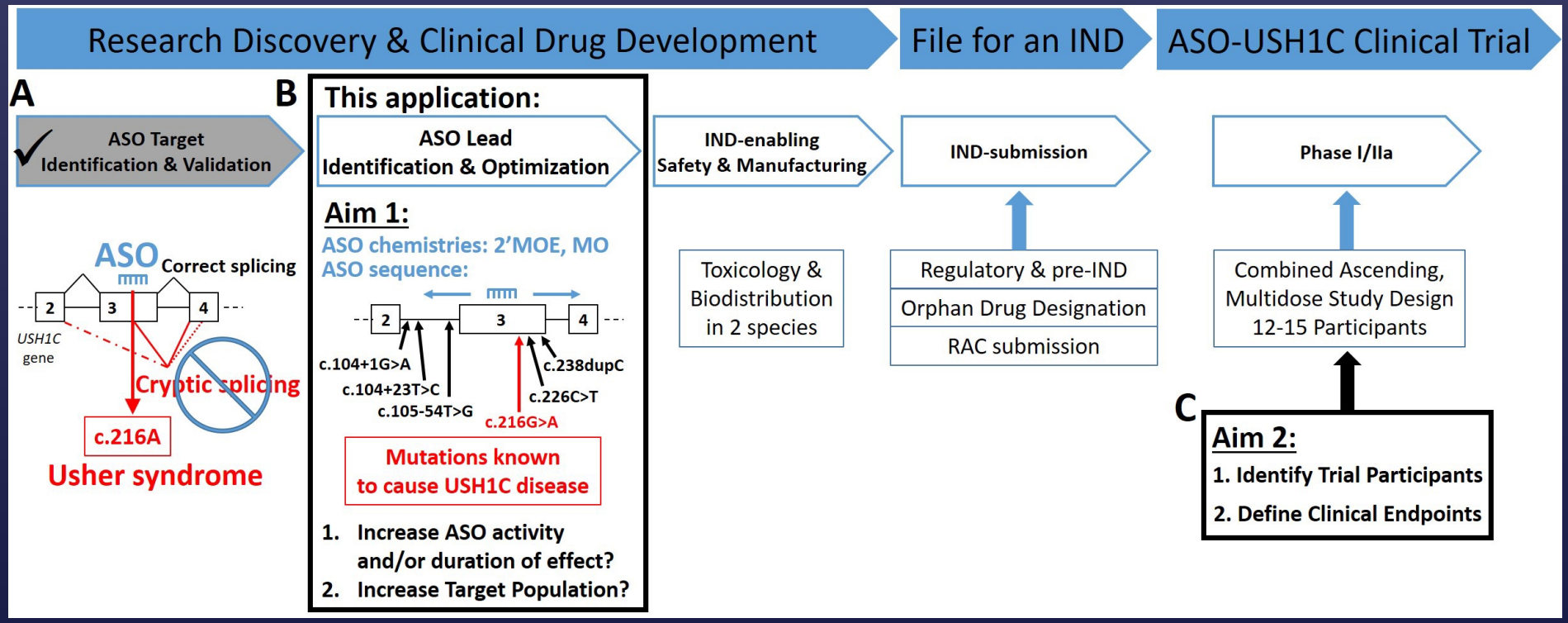
Multiple Treatments
4 Doses – P21, 3, 6, 9 months



Lentz unpublished data

Development of ASO therapy for Acadian USH1C

- Next steps to develop the ASO as a treatment for visual loss in USH1C
  - NIH funded grant to
    1) Optimize the ASO drug
    2) Identify USH1C patients and determine clinical outcomes measures



Optimization of ASOs for Acadian USH1C – Aim 1

- Current best performing ASO:
  - Single treatment improves vision by ~ 20 - 40% for 3 months
- Can we increase ASO activity or duration of effect?
  - Test 100-200 new ASOs with slight modifications in sequence and chemistry
- Currently, we have completed ~65% of the testing and are waiting for long-term studies for some of them (duration of effect)
- Once the testing is complete and have the ASO with the highest activity and/or longest effect, the next steps are to prove it's safe

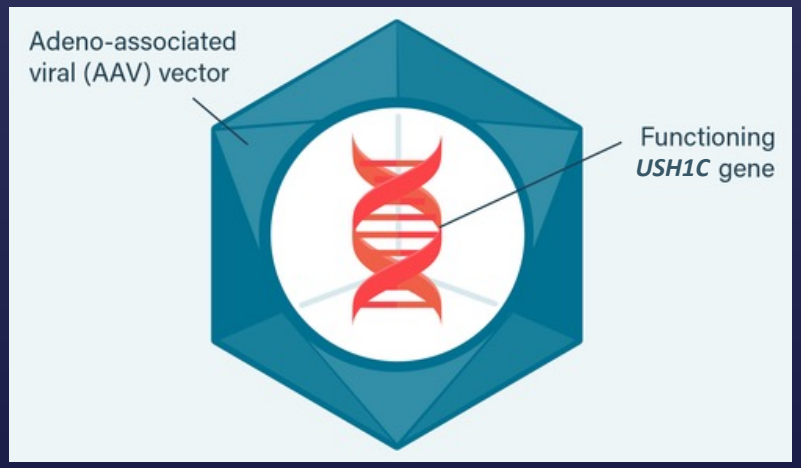
Prospective Natural History of Visual Loss in USH1C – Aim 2

- Identify Acadian USH1C patients
  - *Retrospective* NHS, enrolling:
    - Louisiana residents – All USH
    - Non-Louisiana residents – All USH1C
- Prospective NHS to determine clinical outcomes measures that could be used to guide a clinical trial
  - Enrolling USH1C patients age 12 – 65 years
  - 4 clinic visits – 1 visit every 6 months for 2 years
  - Clinics –
    - Dr. Maria Reinoso, LSUHSC, New Orleans, Louisiana
    - Dr. Robert Koenekoop, MUHC, Montreal, Canada
    - Dr. Wadih Zein, NEI, Bethesda, Maryland

Retrospective **Natural History Study of Usher Syndrome in Louisiana (2014-present) Patient Population**	
Total Enrolled	**103**
Louisiana	75
Canada	12
Other	16
% Males	50% (52/103)
Age range	18 mo – 93 years
With genetic confirmation	70% (72/103)
USH1	90/103
USH1B	1/67
USH1C	**65/67**
USH1D	1/67
USH2	8/103
USH2A	2/2
USH3	3/103
USH3A	3/3
Atypical-USH	1/103
Other	1/103

Gene therapy for USH1C

- **Gene of interest**
  - *Normal* *USH1C* gene delivery to restore functional harmonin protein
- **Viral vector**
  - Adeno-associated virus (AAV)
    - Replication deficient (cannot reproduce without a *helper virus* present)
    - Not known to cause disease in humans

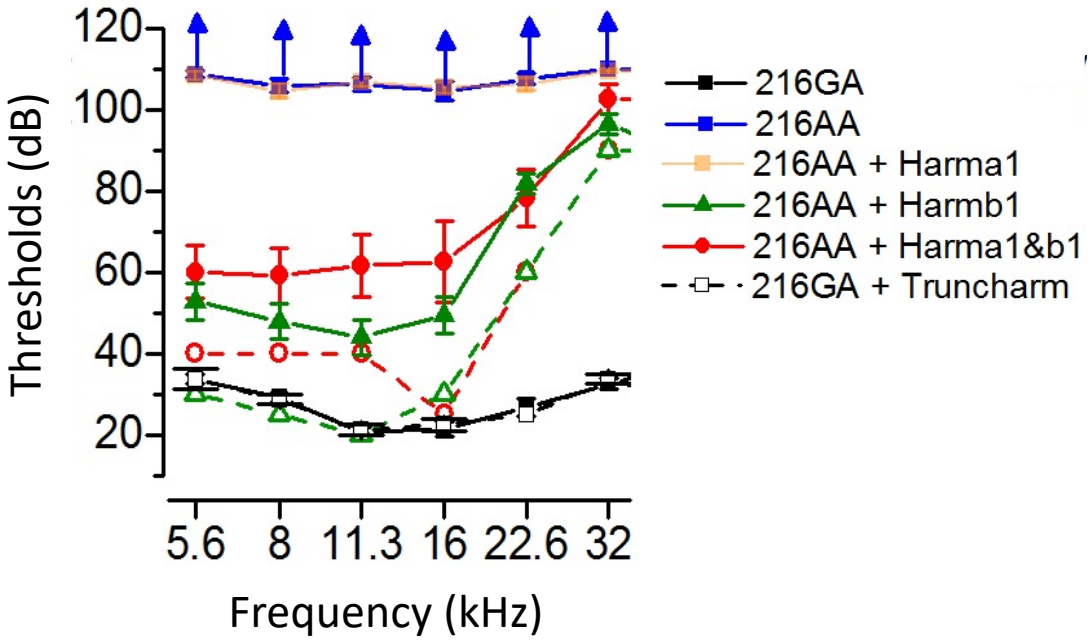


Gene therapy restores hearing in USH1C mice

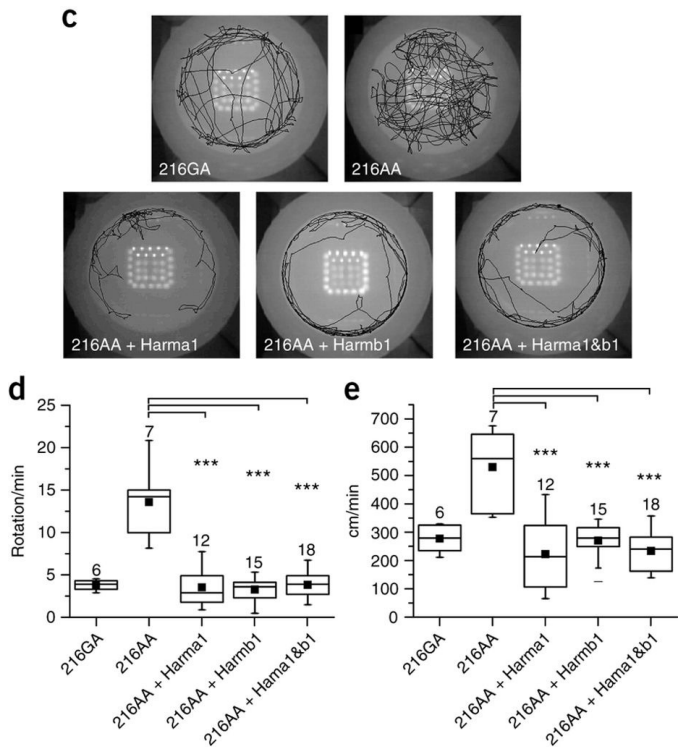
Gwen Geleoc, PhD

- Gene therapy is injected directly into the ear
- Gene therapy (AAV-Ush1c-b) restores hearing and balance in USH1C mice
- Treatment must be given before hearing begins

Hearing thresholds (ABRs)



Balance Behavior (Open-field)

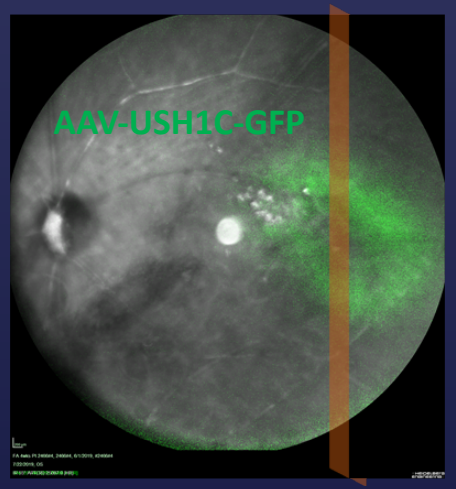


Pan et al. 2017

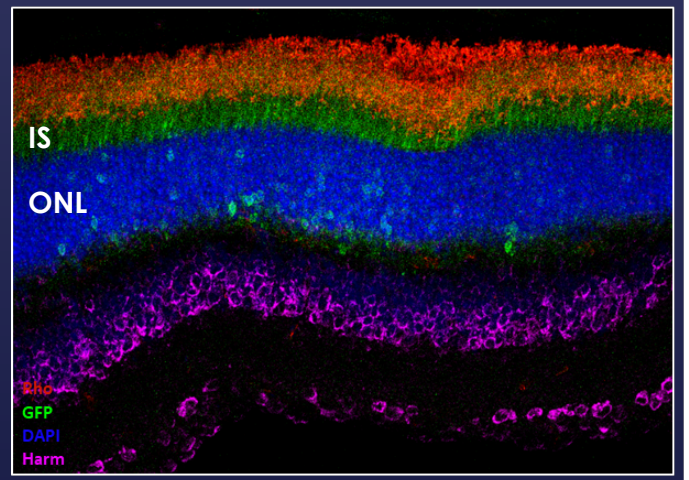
Does gene therapy restore vision in USH1C mice?

- Gene therapy is injected directly into the eye by subretinal injection
- Expression of full-length *Ush1c* in the AAV treated eye, but not the untreated eye, of USH1C mice
- Improves vision in USH1C mice?

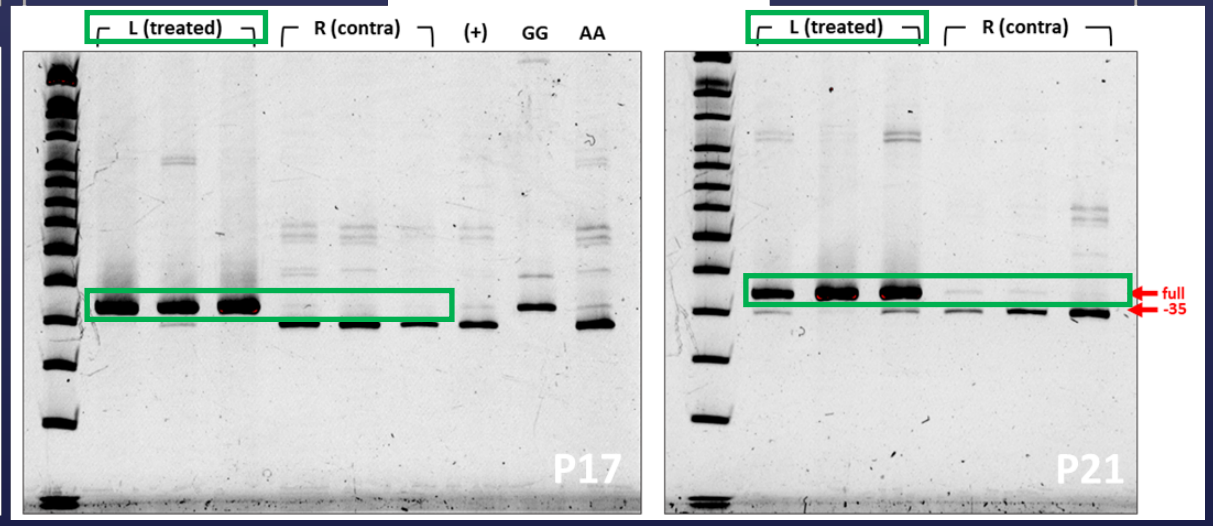
Fundus 4wks post injection



Retina IHC 4wks post injection



PAGE Ush1c transcript analysis



Lentz unpublished data

USH1C Research Summary and Next steps

- ASO therapy restores hearing, balance, and vision in USH1C mice
- Currently optimizing the ASO drug to improve its activity and/or duration of effect as a treatment for visual loss in Acadian USH1C patients
- Gene replacement therapy restores hearing and balance in USH1C mice
- Currently testing gene replacement therapy for visual loss in USH1C mice
- Natural History Studies for patients-
  1) *retrospective* NHS to improve our understanding of the natural clinical history of Usher syndrome in Louisiana
  2) *Prospective* NHS of visual loss in USH1C
  3) *Prospective* NHS of imbalance in USH1C
  - Contact Dr. Lentz for more information about participating – jlentz@lsuhsc.edu)

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