Exon-skipping as a therapeutic approach for *USH2A* patients

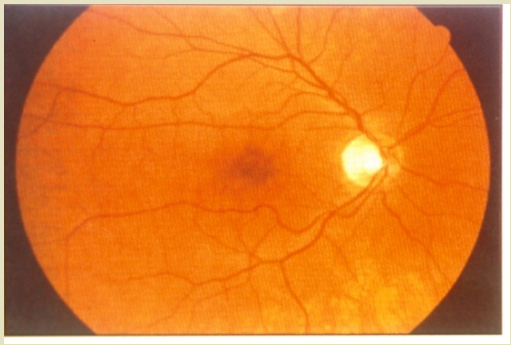
Prof. Dr. Hannie Kremer / Dr. Erwin van Wijk

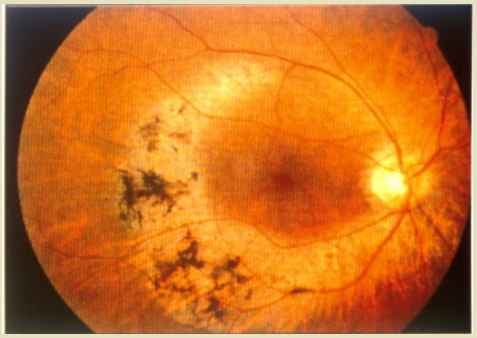
Human Genetics Nijmegen

Radboudumc
university medical center

Usher syndrome

- hearing loss
- impaired vision
- vestibular impairment
- 3-6 per 100,000
- recessive inheritance
- clinical heterogeneity

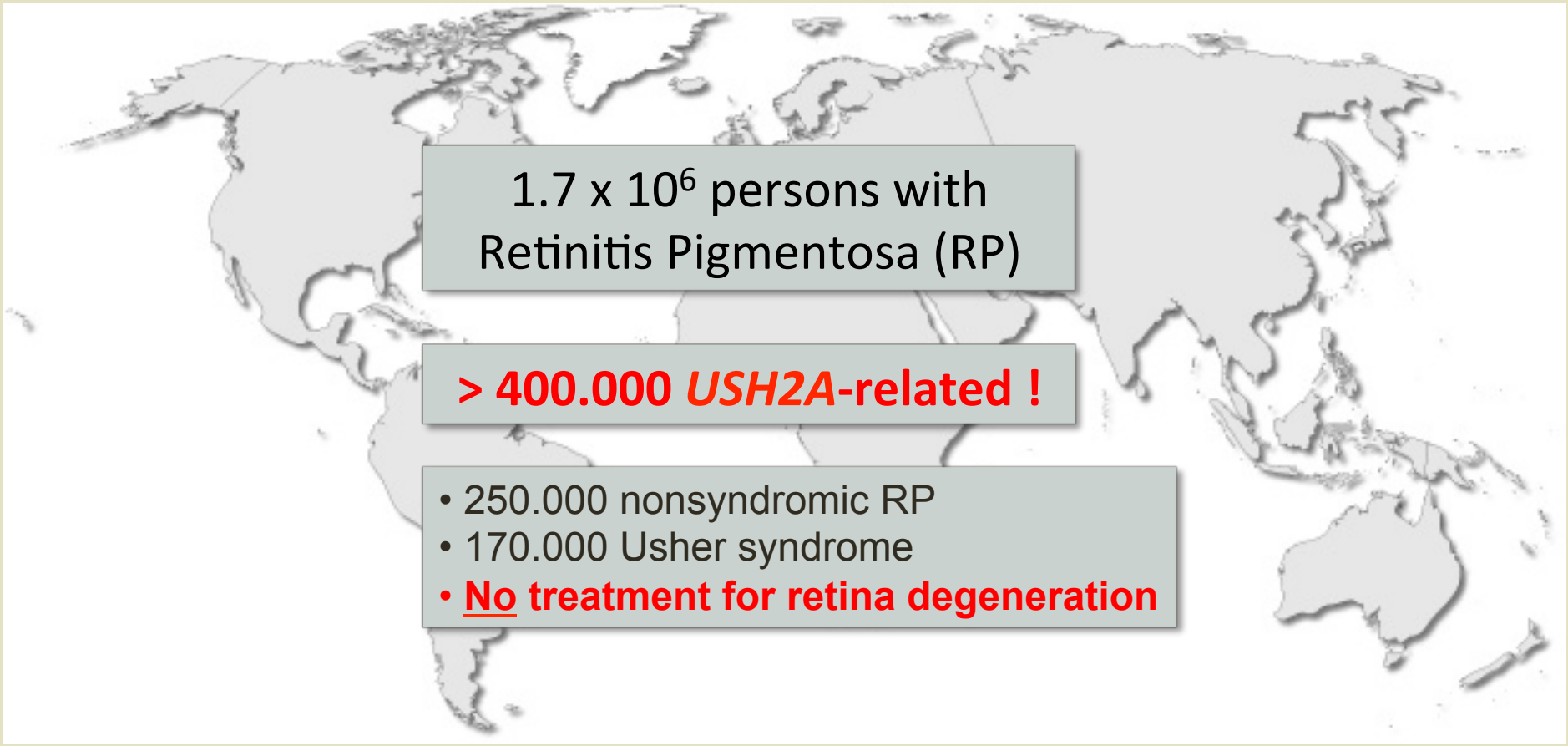




Genetic heterogeneity

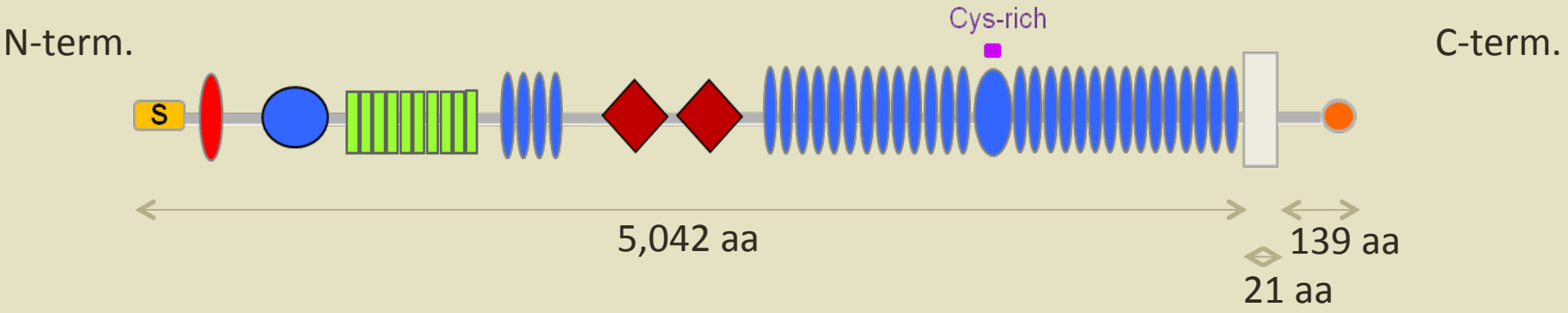
Type	Subtype	Chromosome	Gene
Usher I	USH1b	11	*MYO7A*
	USH1c	11	*USH1C*
	USH1d	10	*CDH23*
	USH1e	21	-
	USH1f	10	*PCDH15*
	USH1g	17	*USH1G*
	USH1h	15	-
	USH1j	15	*CIB2*
	USH1k	10	-
Usher II	USH2a	1	*USH2A*
	USH2c	5	*GPR98*
	USH2d	9	*DFNB31*
Usher III	USH3	3	*USH3A*

USH2A gene Involvement



Usher Research in Nijmegen - USH2A

- **5,202 amino acids**, from 72 coding exons

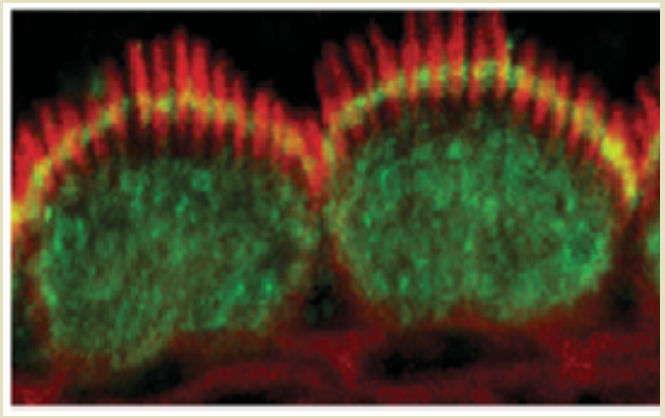


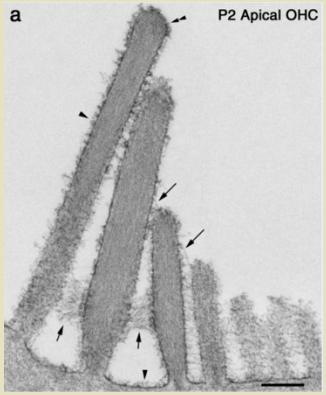
- Expressed in photoreceptor cells

S : signalsequence (S)
: Laminin G-like/thrombospondin domain (LamGL/TspN)
: Laminin N-terminal domain (LamNT)
: Laminin-type EGF-like domain (EGF Lam)
: Fibronectin type 3 domain (FN3)
: Laminin G domain (LamG)
: transmembrane region (TM)
: PDZ binding motif (PBM)

USH2A protein in the cochlea

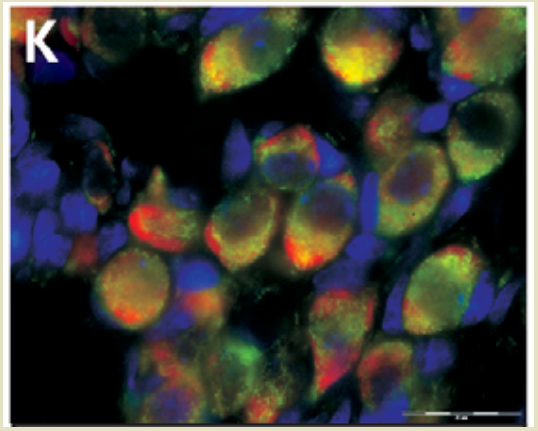
Hair cells - Hair bundle





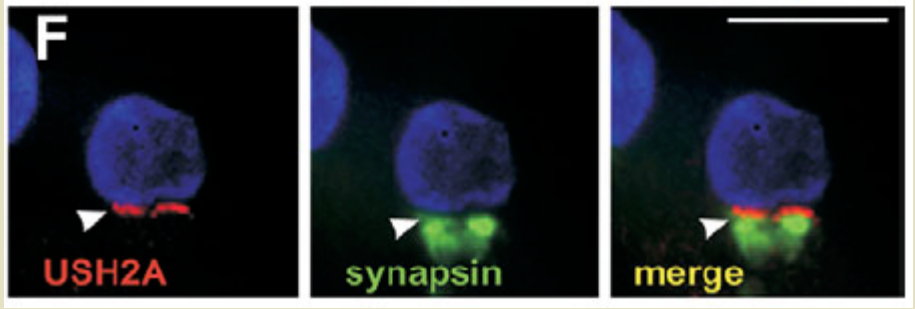
Ush2a - actin

Cochlear nerve cells



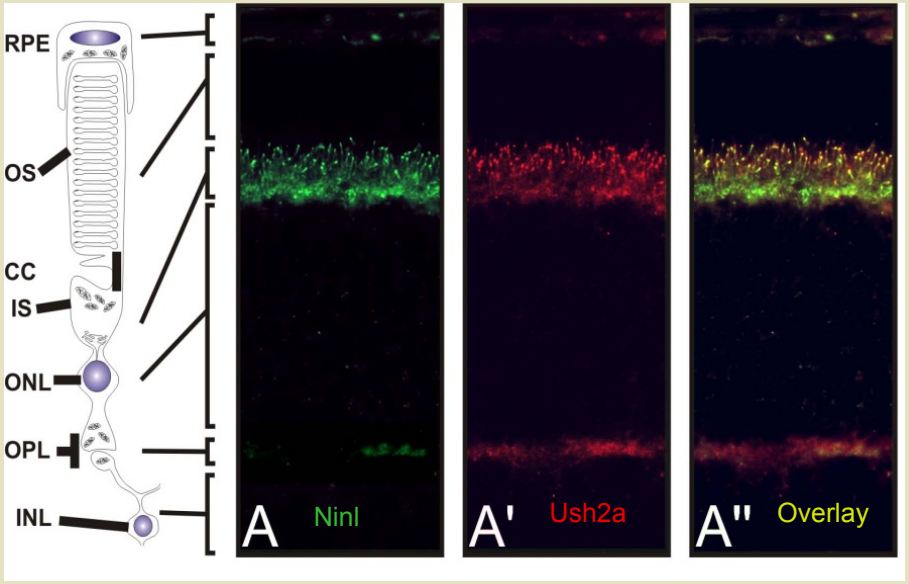
Ush2a - whirlin

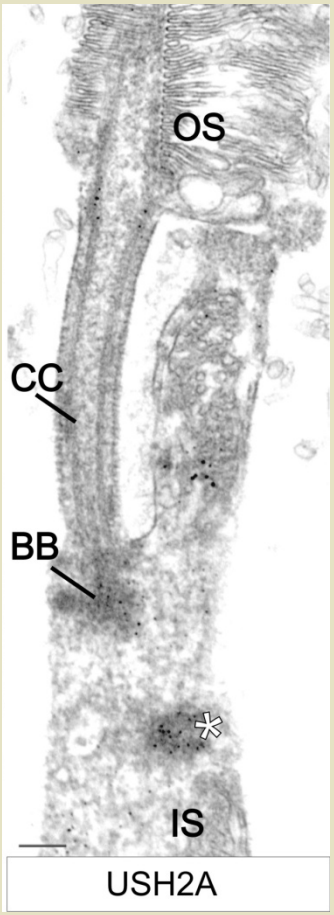
Outer Hair cells - Synapse



Adato et al. 2005
Goodyear et al 2005
Van Wijk et al 2006
Kazmierczak et al 2007

USH2A protein in the retina

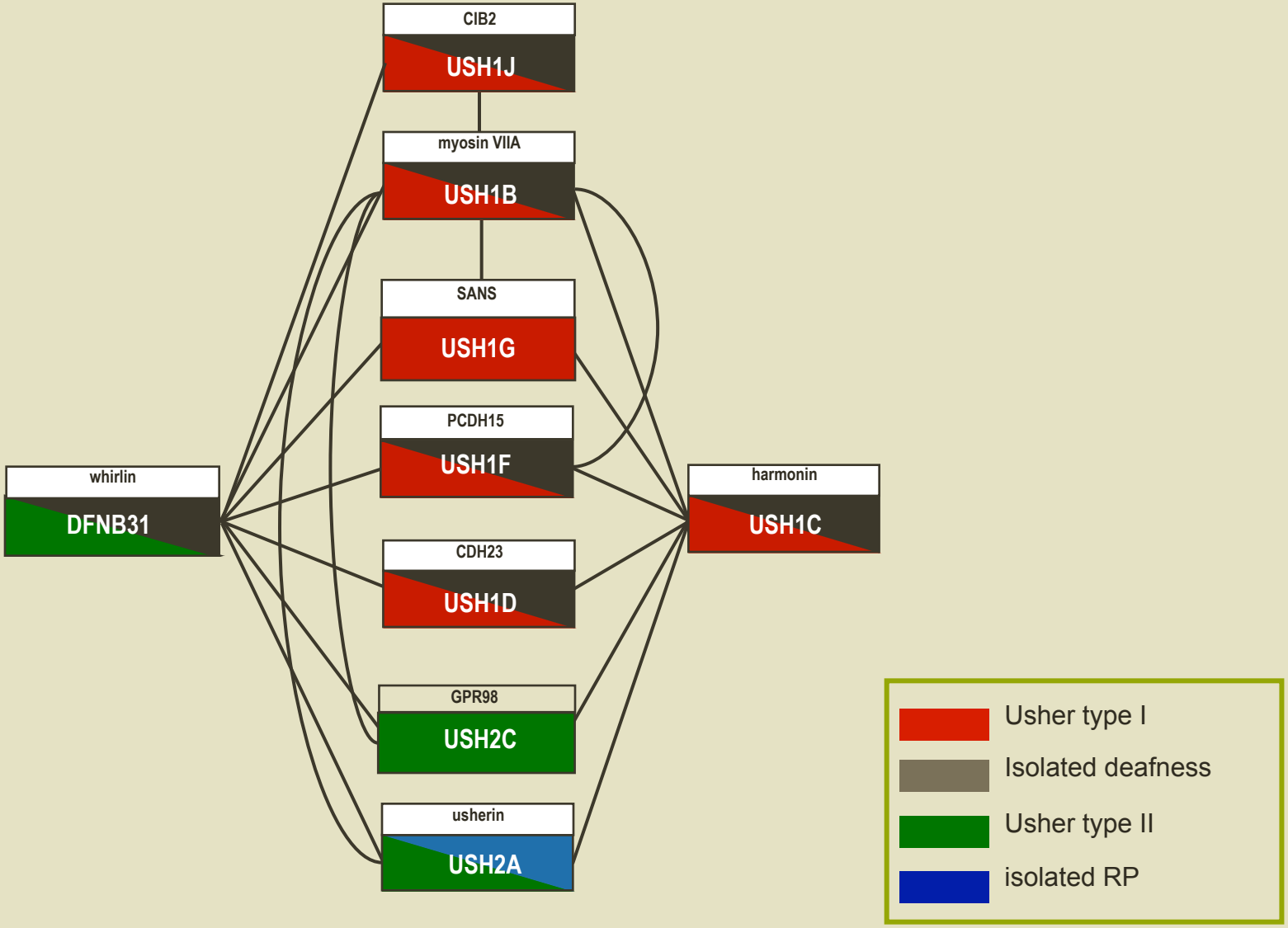




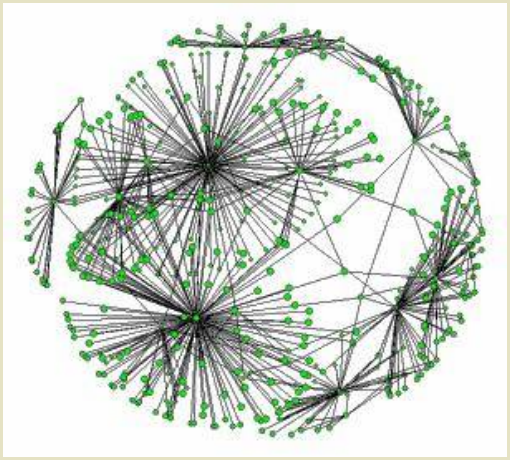
Usher Research in Nijmegen - USH2A

- Protein Function
- Strategies for retinal therapy

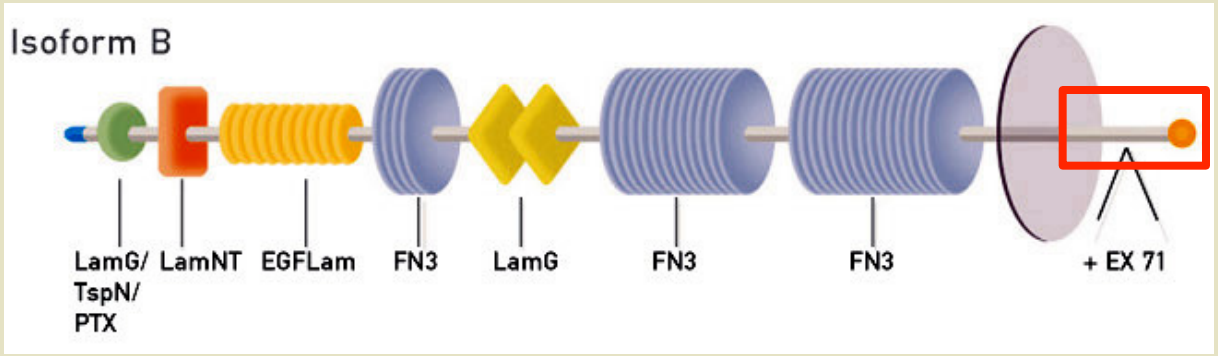
USH2A: Usher Protein Network



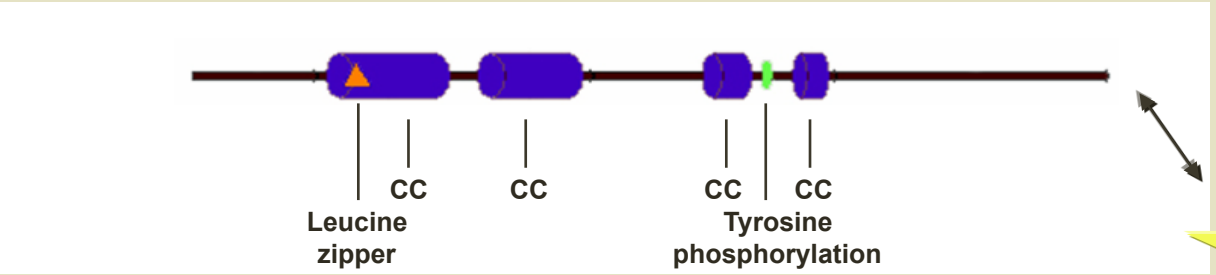
USH2A: Protein-Protein Interactions

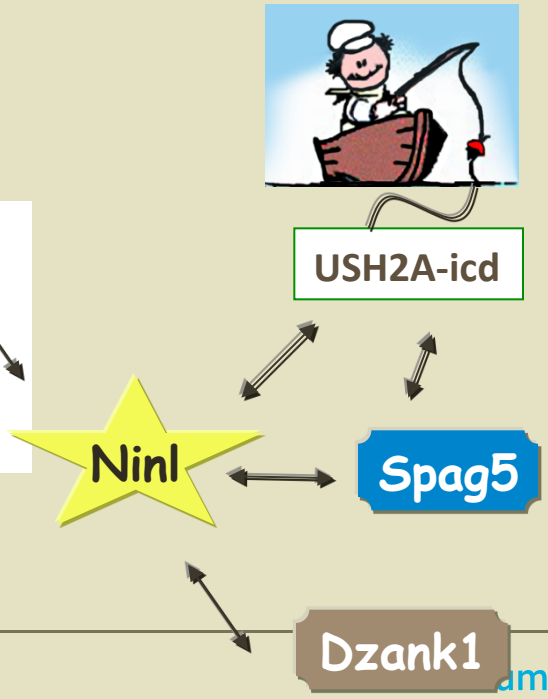


USH2A



Lebercilin (LCA5)





The *USH2A* patient…

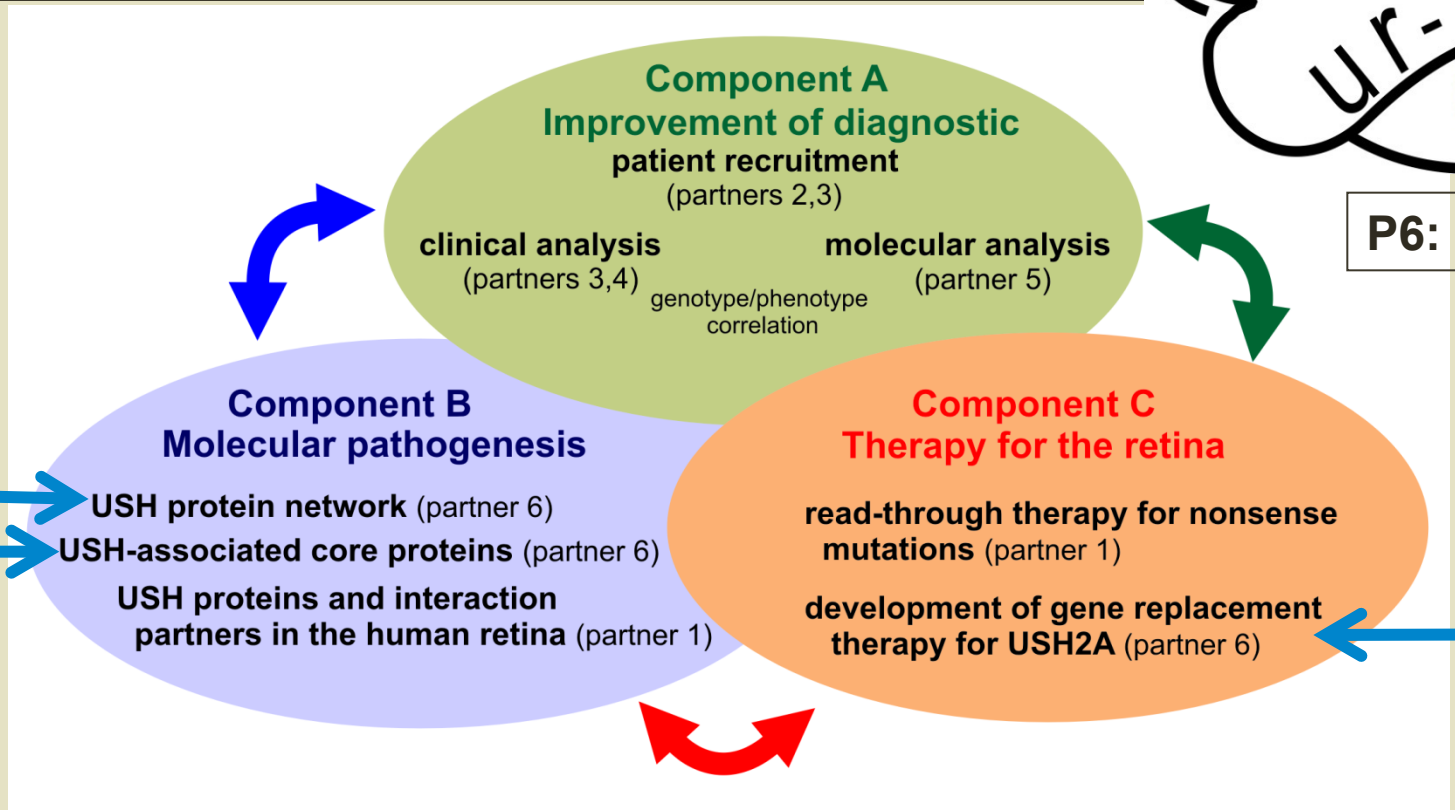
- Clinically:
  - (Slightly progressive) hearing loss
  - Progressive Retinitis pigmentosa (Rods to cones)

What´s needed for therapeutic development ?

1. Strategy ⟹ "Classical" *USH2A*-gene augmentation ? X

2. Animal model ⟹ Representative *Ush2a* knockout mouse ? X

The EUR-USH project

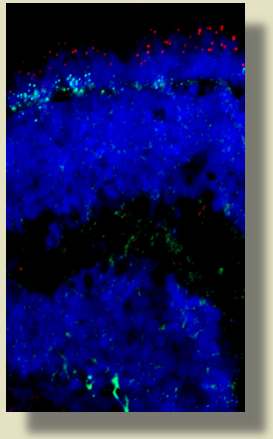


P6: Nijmegen

- **1. Tagged USH-proteins**
  - unravel Usher proteome function
- **2. Therapy**
  - gene augmentation (and exon-skipping)

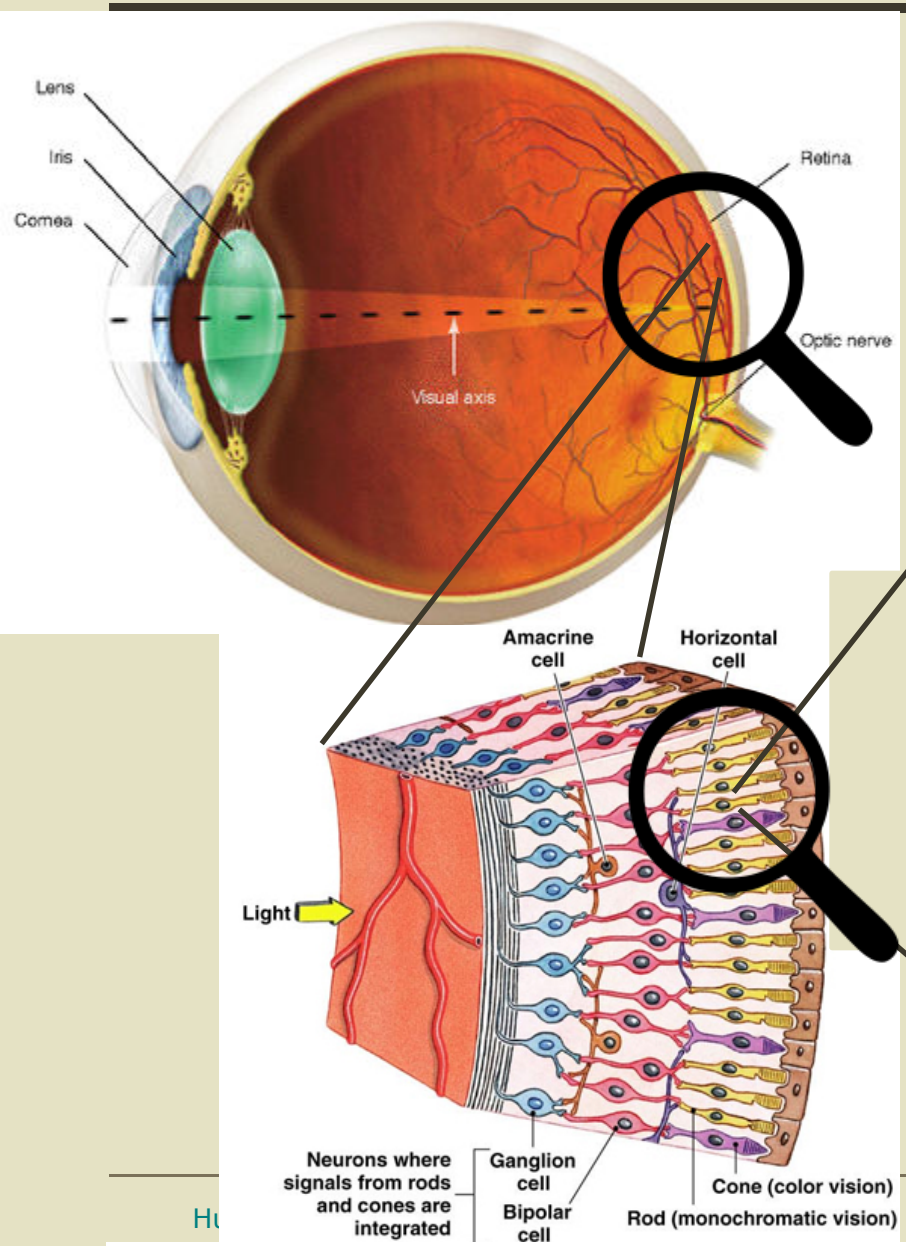
Our solutions for the *USH2A* patients

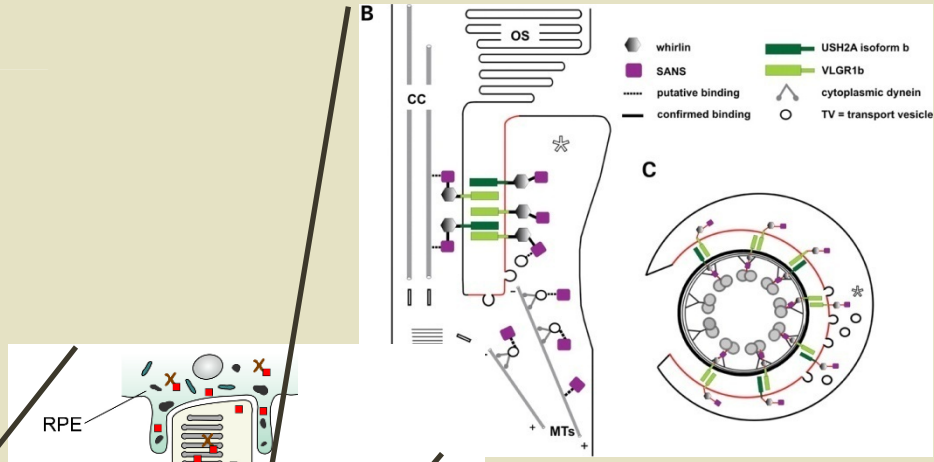
SANSB

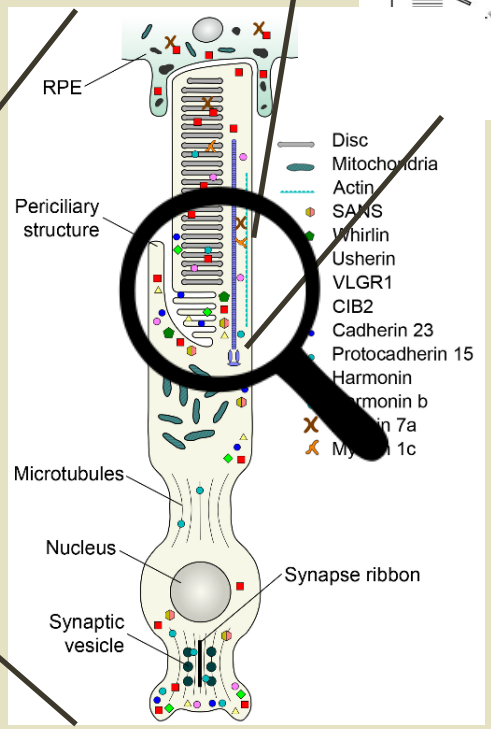


- 1] Interfere with dysregulated function
  - ✔ Unraveling link between Usher protein interactome and cellular function
- 2] Augment *USH2A* gene
  - ✘ Coding sequence = 15.6 kb…
  - ✔ >mini-genes (compress *USH2A* gene; ongoing project)
- 3] Skip mutation
  - ✔ Interfere with mRNA splicing by using AONs

USH2A and photoreceptor localization







Saima Riazuddin et al.; Nature Genetics, 2012, vol 44, pages 1265–1271
Tina Maerker et al.; Human Molecular Genetics, 2008, Vol. 17, No. 1, pages 71–86

To study the eye, we need a model

- Human eye is difficult to obtain, alternatives:
- Mouse model for *Ush2a*: Deafness, but no retinal degeneration…
- Zebrafish model for *ush2a*:

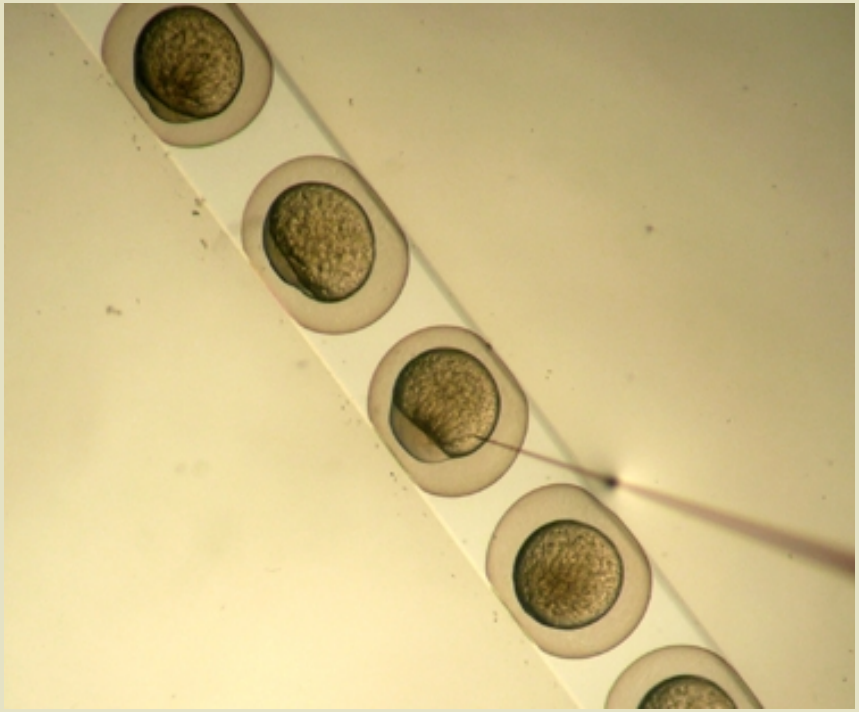
Zebrafish *ush2a* model:
- Retina degeneration
- Hearing impairment

  - All known USH-genes are conserved between human and zebrafish
  - Human vs. zebrafish USH2A: both gene and protein structure is conserved

Kerstin Howe et al.; Nature 496, April 2013, pages 498–503

Our model system: Zebrafish

- ~70% genomic similarity
- Advantages:
  - Genome (well) known
  - Genomic manipulations
  - Relatively cheap
  - Fast reproductive cycle
  - Much offspring, many times
  - Development *ex utero*
  - etc…
- → 84 % of genes known to be associated with human disease have a zebrafish ortholog.

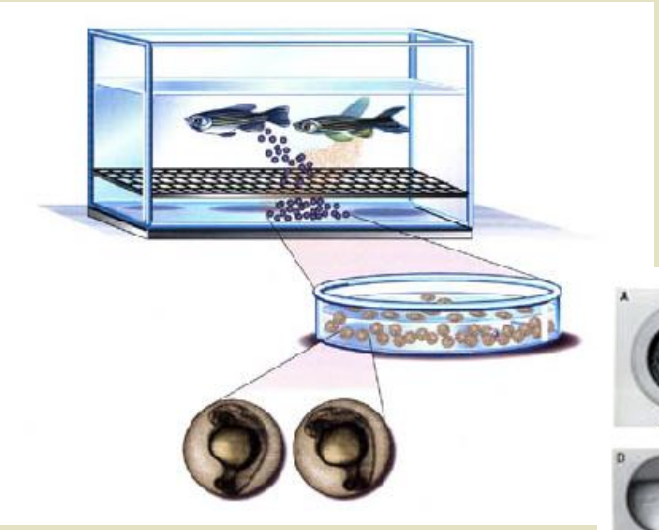


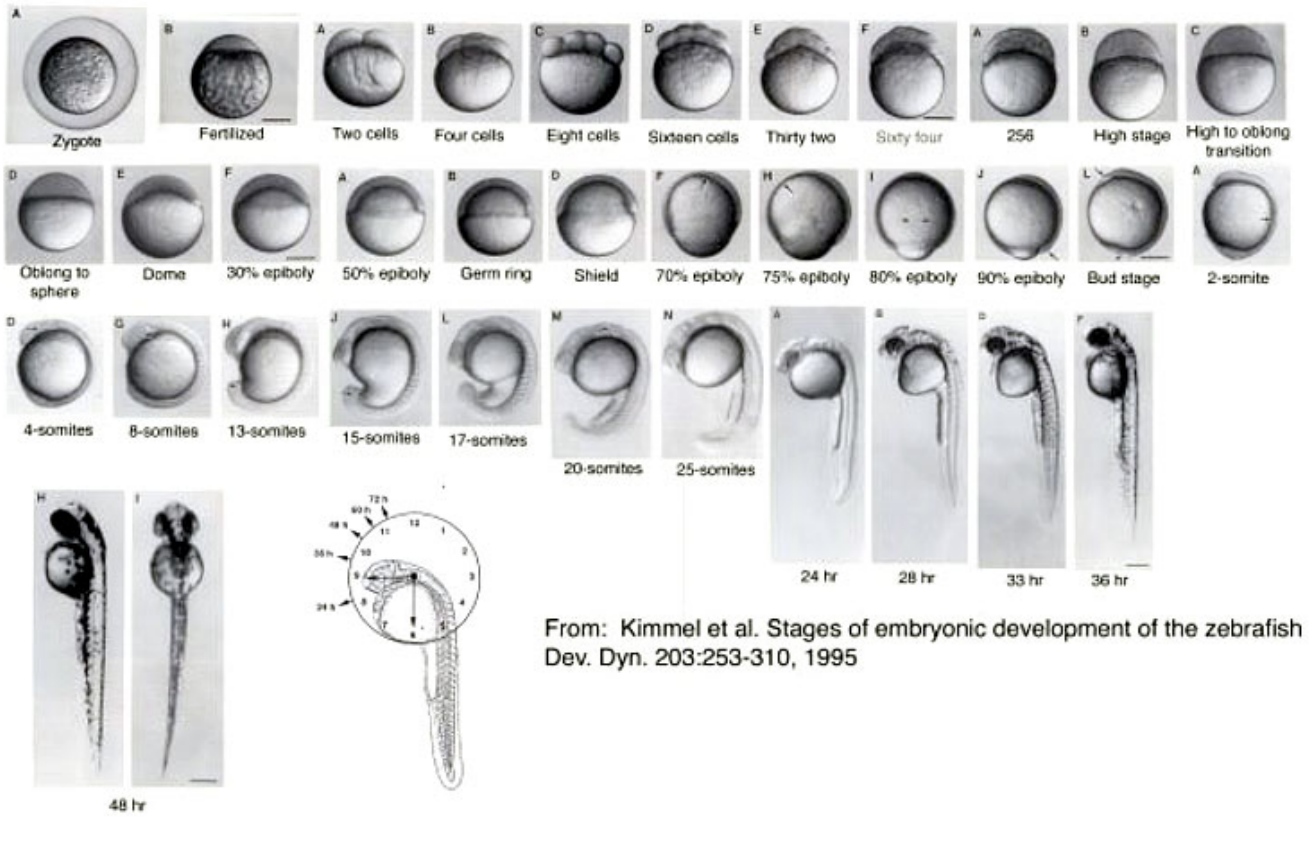
Kerstin Howe et al.; Nature 496, April 2013, pages 498–503

Zebrafish in Nijmegen

- ‘our’ facility:

Zebrafish reproduction and development





From: Kimmel et al. Stages of embryonic development of the zebrafish Dev. Dyn. 203:253-310, 1995

Using Zebrafish as a model system for therapeutic development

- Use specific zebrafish *ush2a* mutant for functional studies:

1] Retinal function

Can we see a difference before and after treatment..?!

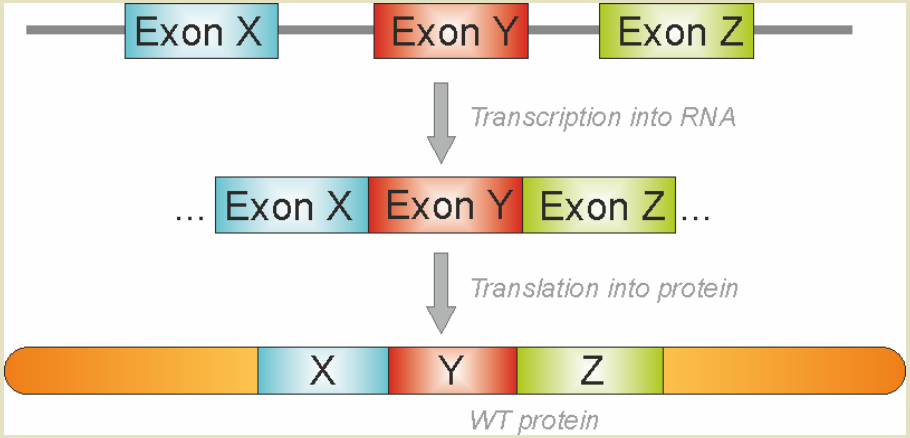
2] Morphology

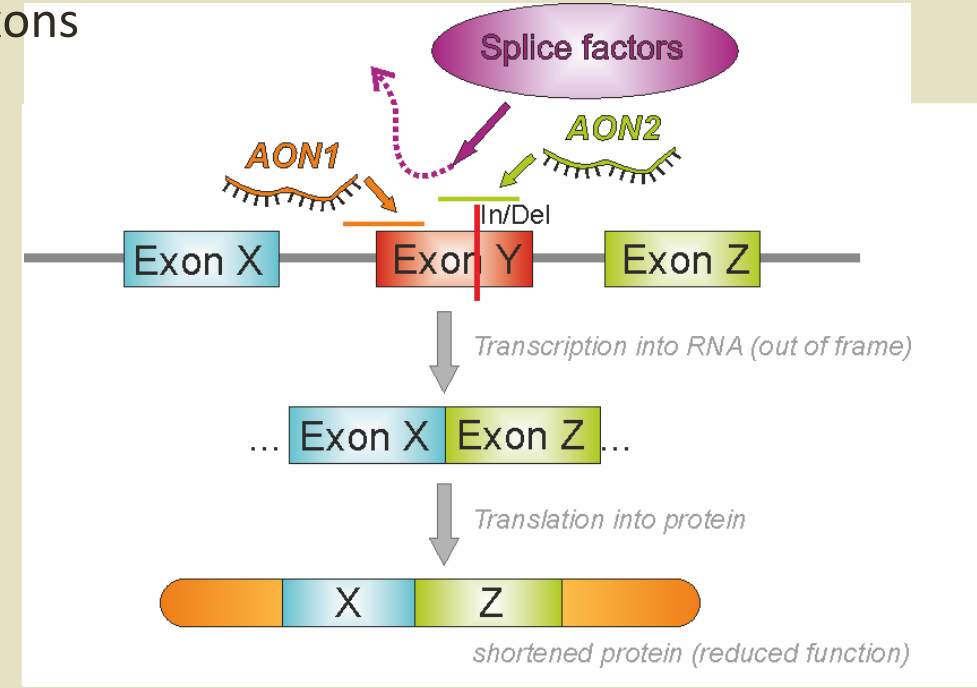
- (Transmission)Electromicroscopy
- Immuno histochemistry
- Apoptosis stainings photoreceptors

Exon skipping background

- How to successfully skip an exon?
  → Resulting transcript is NOT out-of-frame!

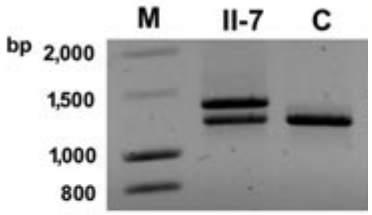
- Used for: 1] pseudo exons
  2] mutated native exons





USH2A PE40 intronic mutation

Mutation: **c.7595-2144A>G** *(Expected 3-5% of all USH2A alleles – unpublished data A.F. Roux, Montpellier)*



- 152 bp insertion > out-of-frame > STOP codon (75bp downstream) (=pseudo-exon)

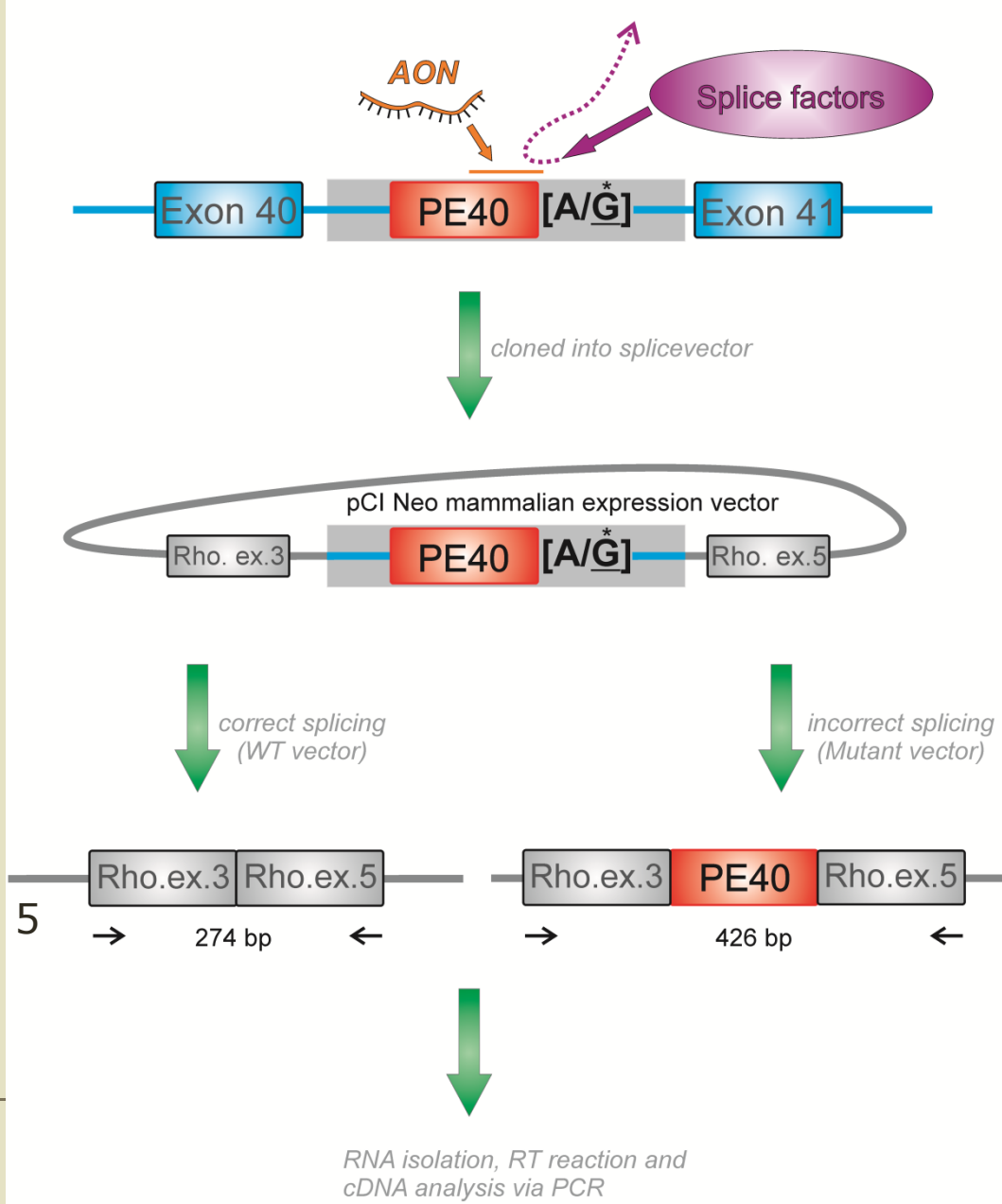
Skipping PE40 = WT mRNA !

Start PE40 model: minigene splice assay

Two minigene splice assays:

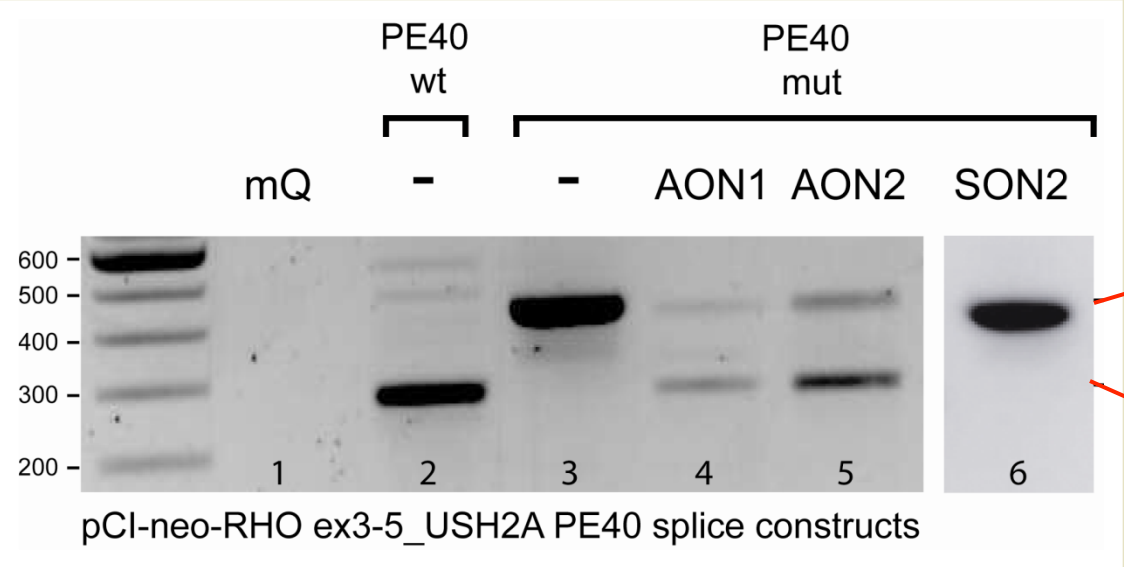
WT vs. Mut

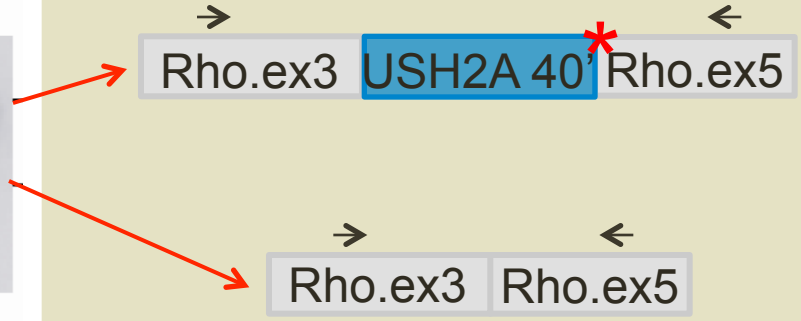
- PE40 intronic sequence +/- c.7595-2144A>G mutation
- 500bp flanking sequence both up- and downstream PE40
- between *rhodopsin* exons 3 and 5



H.s. PE40 minigene splice assay: data (2)

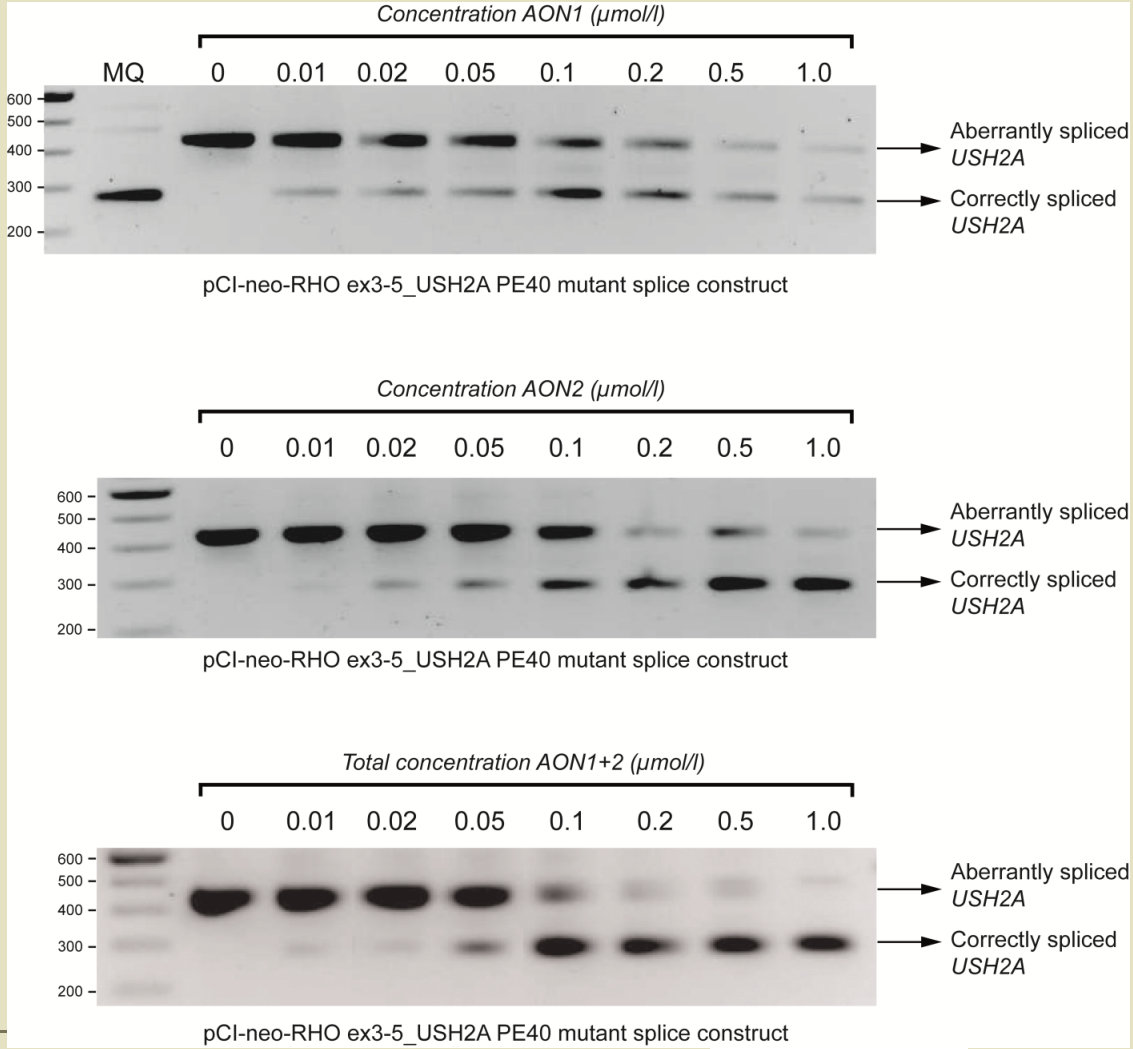
- Testing AON 1 and 2 in HEK293T cells





PE40 AONs titration curve on PE40 minigene splice assay

- AON 1 and 2
  in HEK293T cells

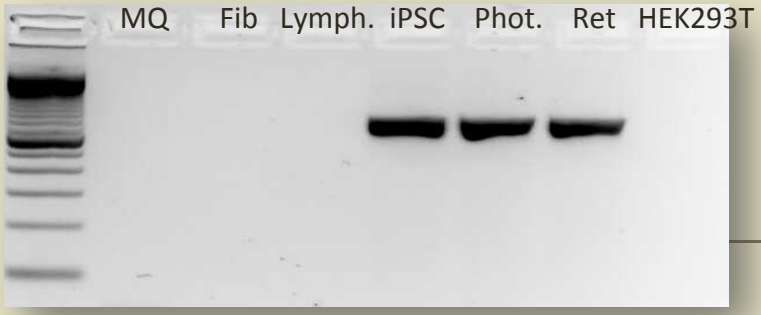


AONs for *USH2A* PE40

- 1] Splice construct is working!
- 2] AONs 1 and 2 are successful in skipping PE40
- → Test AONs 1 and 2 on *USH2A* PE40 in the right genetic context:

Patient derived iPS cells
(AONs also functional right genetic context?)
→ *AAV cloning/iPS diff. ongoing*

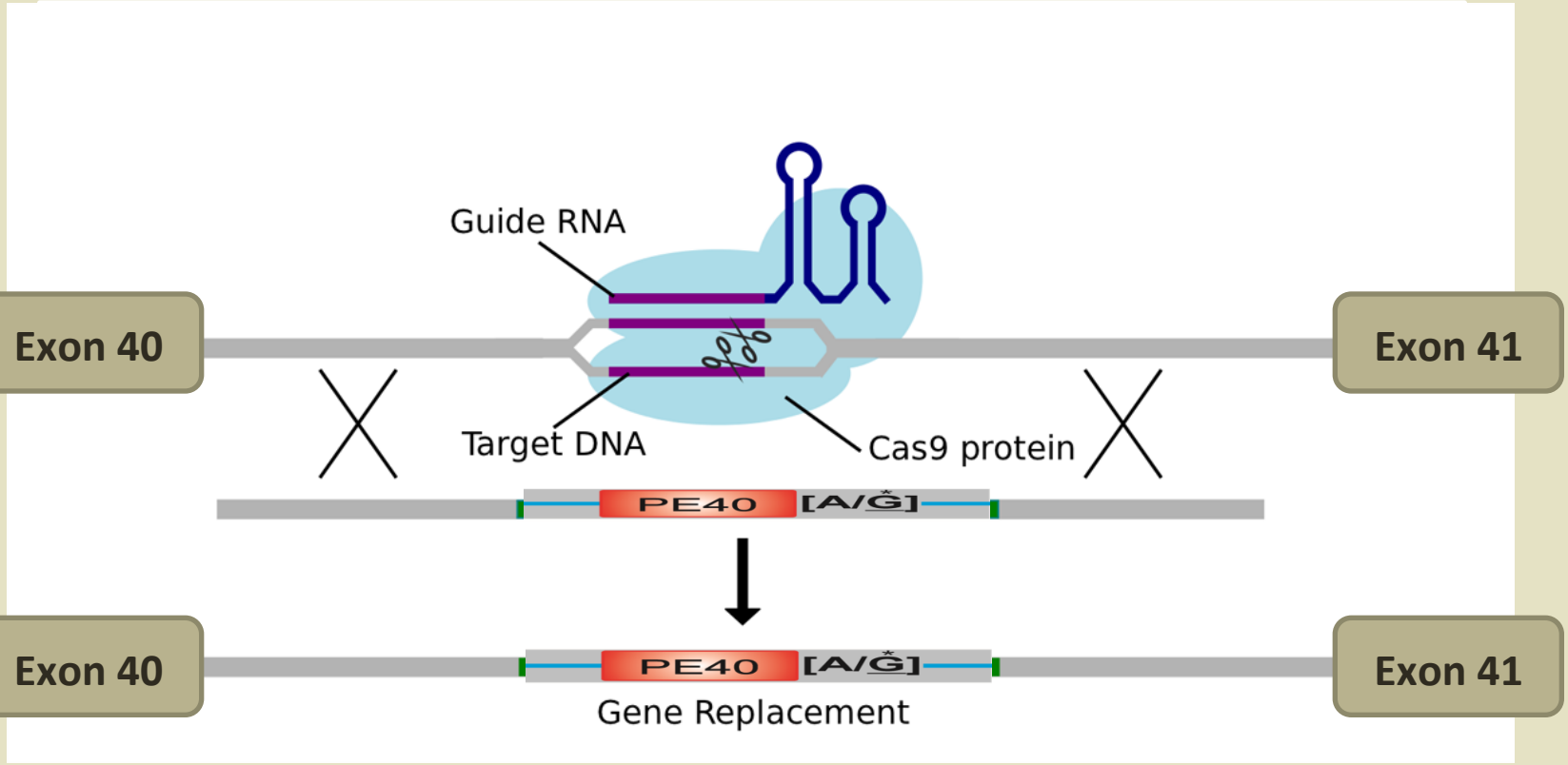
Zebrafish knock-in model



Radboudumc
university medical center

Relevant *USH2A* PE40 model (zebrafish)

- Generate a humanized *ush2a* PE40 model:
- Knock-in via Crispr/Cas9 system



How to test our AONs

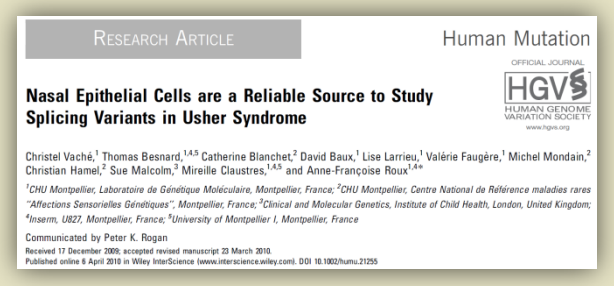
- A lot of promising AONs, how to evaluate the effect in a patient-derived context?

  → need: any cell type expressing *USH2A*

  - Photoreceptor cell
  - Hair cell (organ of corti)

  } Sampling difficulties…

  - Nasal epithelial cells → - ~1/3 sampling efficiency
    - Need many samplings
    - Non-immortalized cells… (~max p5)



RESEARCH ARTICLE

Human Mutation

OFFICIAL JOURNAL HGVS HUMAN GENOME VARIATION SOCIETY www.hgvs.org

Nasal Epithelial Cells are a Reliable Source to Study Splicing Variants in Usher Syndrome

Christel Vaché,[1] Thomas Besnard,[1,4,5] Catherine Blanchet,[2] David Baux,[1] Lise Larrieu,[1] Valérie Faugère,[1] Michel Mondain,[2] Christian Hamel,[2] Sue Malcolm,[3] Mireille Claustres,[1,4,5] and Anne-Françoise Roux[1,4]*

[1]*CHU Montpellier, Laboratoire de Génétique Moléculaire, Montpellier, France;* [2]*CHU Montpellier, Centre National de Référence maladies rares "Affections Sensorielles Génétiques", Montpellier, France;* [3]*Clinical and Molecular Genetics, Institute of Child Health, London, United Kingdom;* [4]*Inserm, U827, Montpellier, France;* [5]*University of Montpellier I, Montpellier, France*

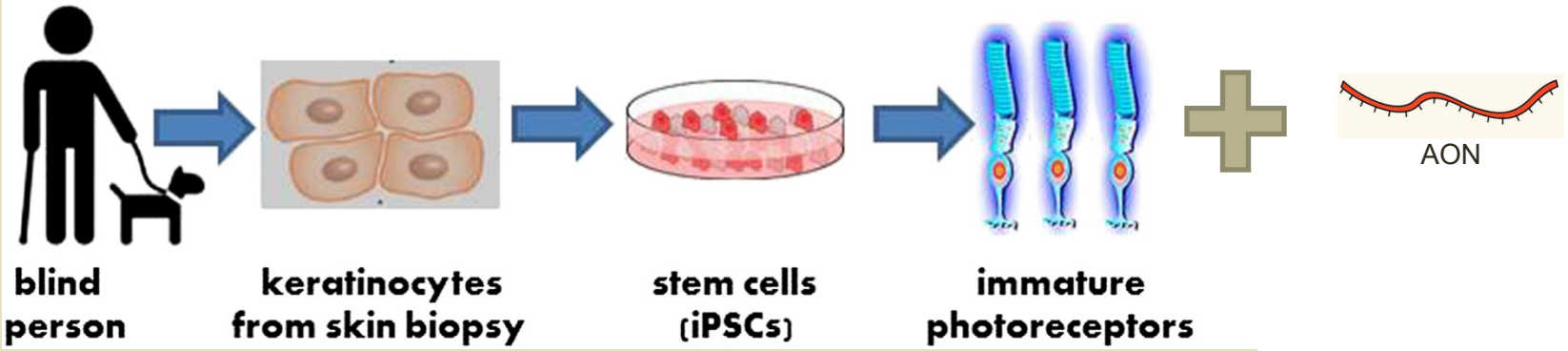
Communicated by Peter K. Rogan

Received 17 December 2009; accepted revised manuscript 23 March 2010.
Published online 6 April 2010 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/humu.21255

- **What's next..?**

iPSCs → photoreceptor like cells

- Isolate patients' fibroblast/keratinocyte cells:



1] Splicing restoration in patient material..?!
2] Off target effects (transcriptome seq.)?

(Performed in collaboration with Dr. Budd Tucker, Iowa City)

Concluding our *USH2A* research

~~*USH2A* patients~~

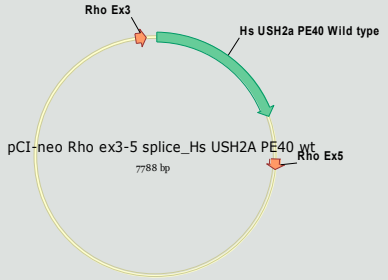
USH2A ex-patients

Mutation in USH2A > truncated protein

Interfere splicing with AONs

AON

Proof of concept:

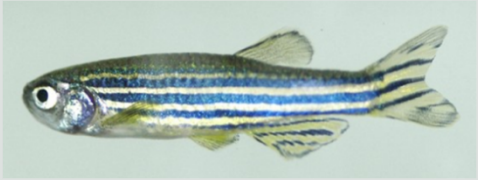


1. Splice constructs (positive results!)

Patient material:

2. Fibroblast RNA Photoreceptor cells

Functional read-out:



3. Zebrafish lines (Crispr/Cas9 system)

Clinical testing

AON

AONs testing

Acknowledgements

- <u>The Usher syndrome researchteam:</u>
- Ralph Slijkerman
- Alex Goloborodko
- Margo Dona
- Lisette Hetterschijt
- Erik de Vrieze
- Theo Peters
- Erwin van Wijk
- Hannie Kremer

Radboud Universiteit Nijmegen

<u>Zebrafish caretaking:</u>
- Gert Flik
- Tom Spanings

<u>Montpellier:</u>
- Anne-Francoise Roux
- Christel Vaché

Human Genetics Nijmegen

www.eur-ush.eu

<u>Collaborators:</u>
- Rob Collin (Nijmegen)
- Budd Tucker (Iowa)
- Monte Westerfield (Eugene)

Foundation Fighting Blindness
Driving research to save and restore sight