Genetic therapy for *USH2A*-associated retinal dystrophy: future perspective or…?

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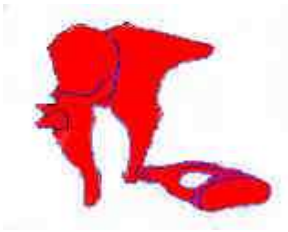
Human Genetics Nijmegen



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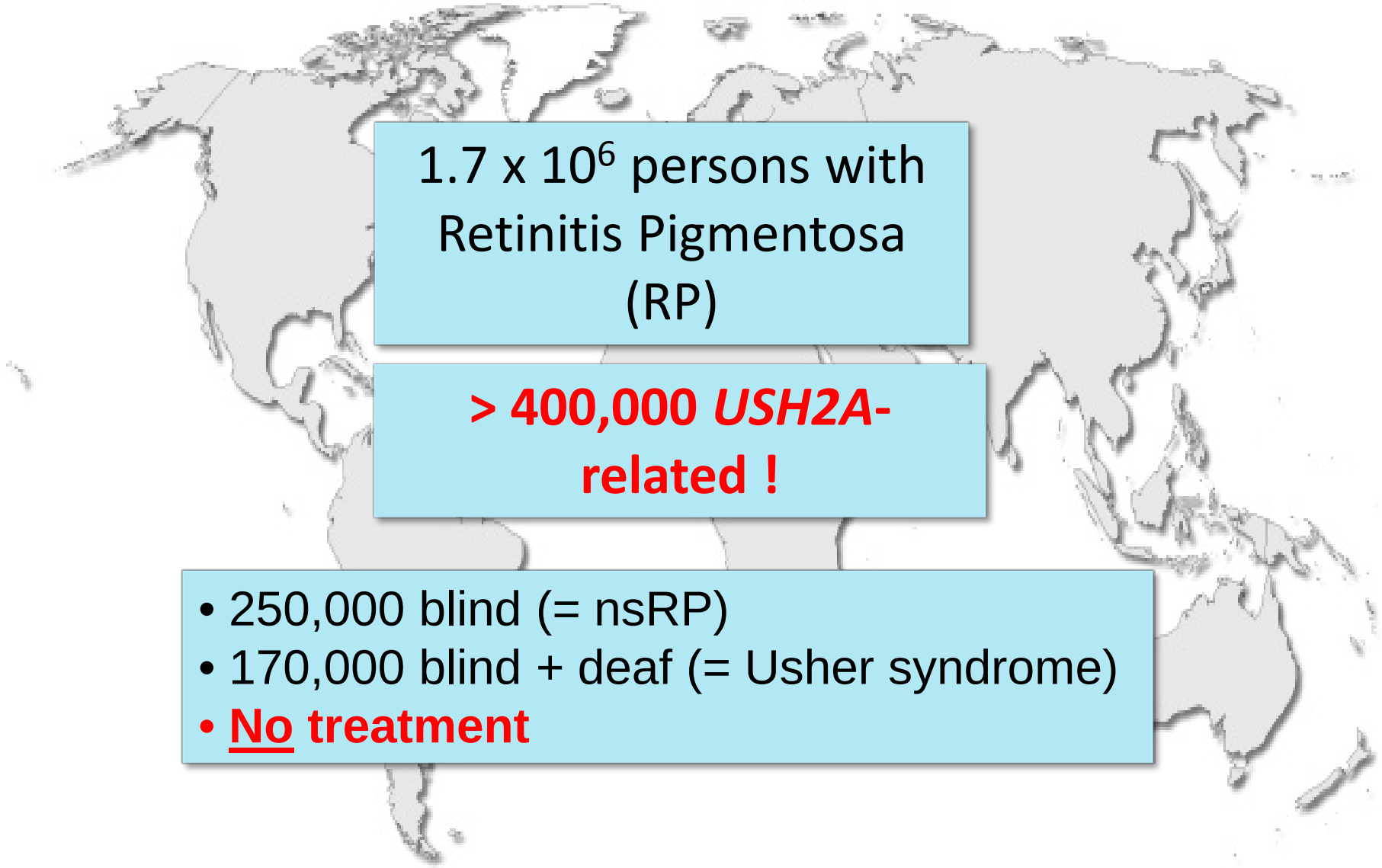
Retinitis pigmentosa worldwide



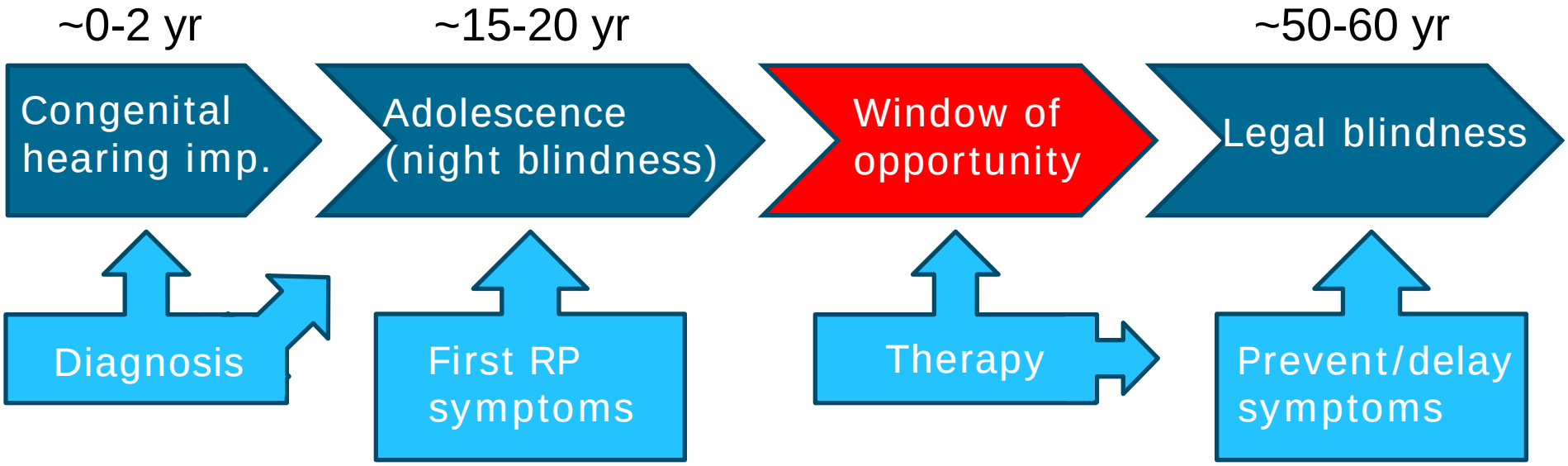
1.7 x 10^6 persons with Retinitis Pigmentosa (RP)

> 400,000 *USH2A*-related !

- 250,000 blind (= nsRP)
- 170,000 blind + deaf (= Usher syndrome)
- **No treatment**

Clinical timeline…

- USH2A most frequently mutated gene:
  ~50% of USH2 and ~4-20% of nsRP cases



What is needed for therapeutic development ?

1.Strategy → “Classical” USH2A-gene augmentation?

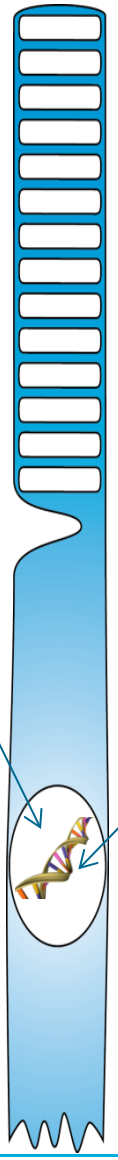
Challenges in potential USH2A therapies

Augment wildtype USH2A gene

But,
*Gene size +++ (15,606 bp cDNA)
*USH2A isoforms

Ideally, interfere on **functional** or **transcript** level

*not alter isoforms
*not alter expression levels



Gene editing

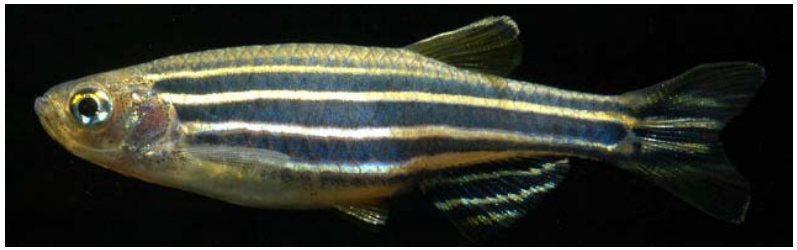
But,
*low efficiency
*off-target effects?
*not ready for clinical application

What is needed for therapeutic development (2)?

1. Strategy → "Classical" USH2A-gene augmentation? ✗

2. Animal model → Ush2a-mouse model ? ✗

Model



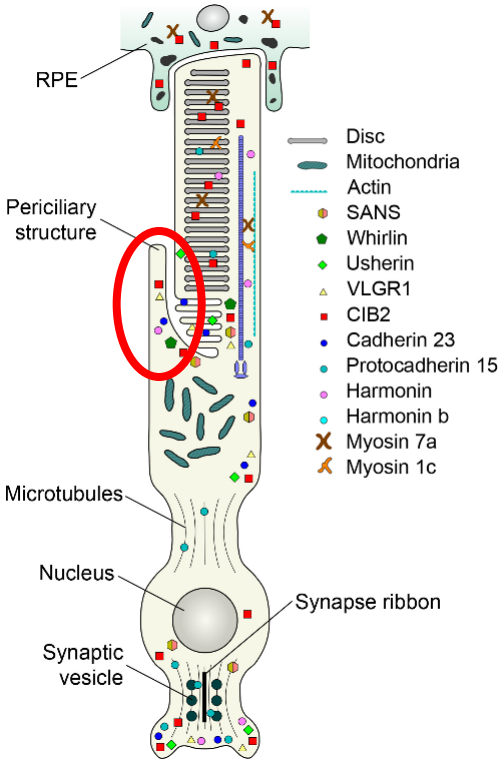
Zebrafish ush2a knockout model:

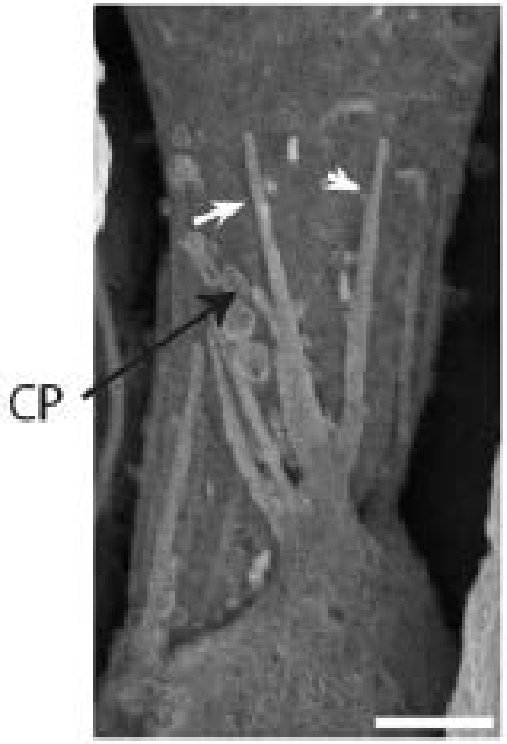
Early-onset retinal degeneration and impaired visual function !

-All known human USH-genes are present in zebrafish

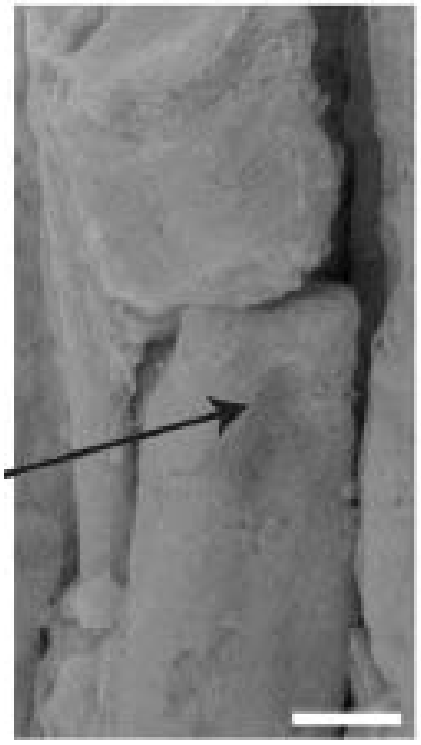
-Human vs. zebrafish USH2A: gene and protein are highly similar

Anatomy of photoreceptor cells

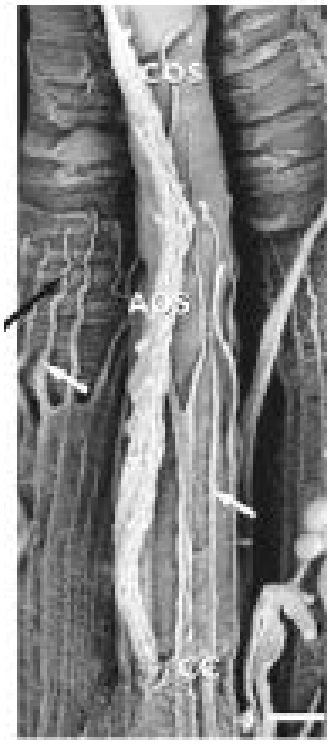




human

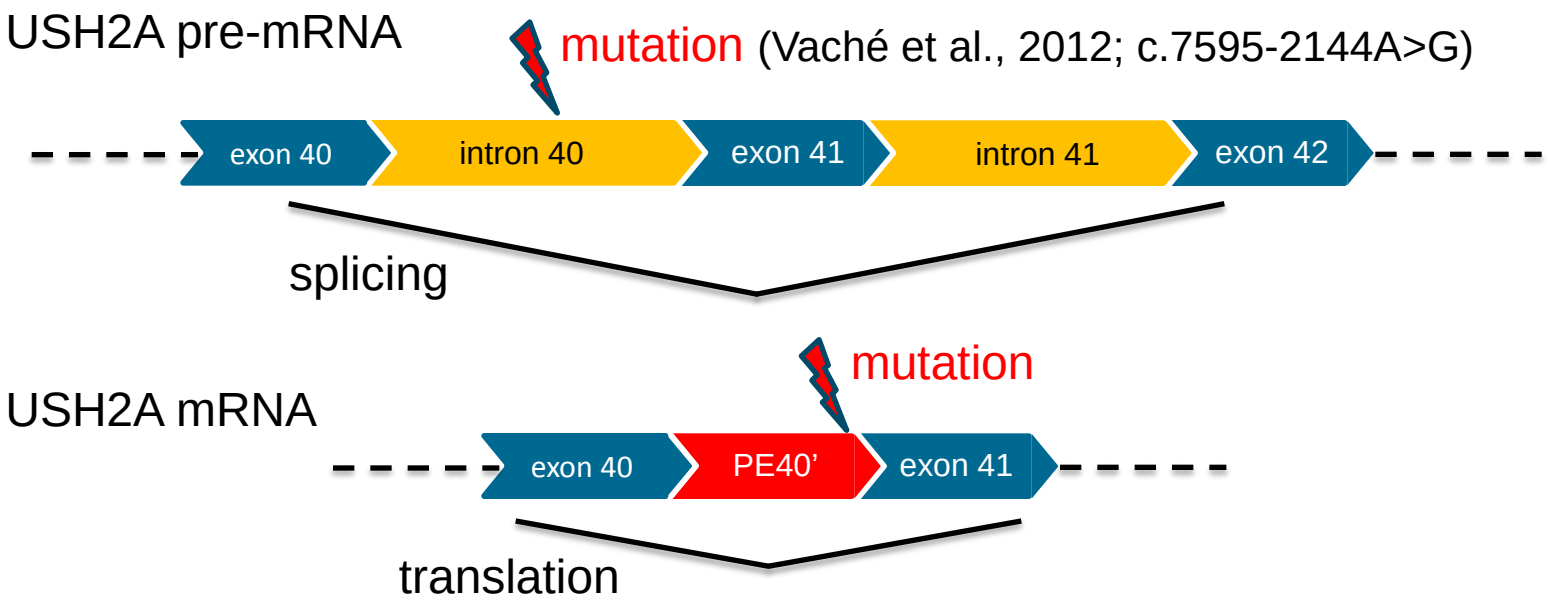


mouse

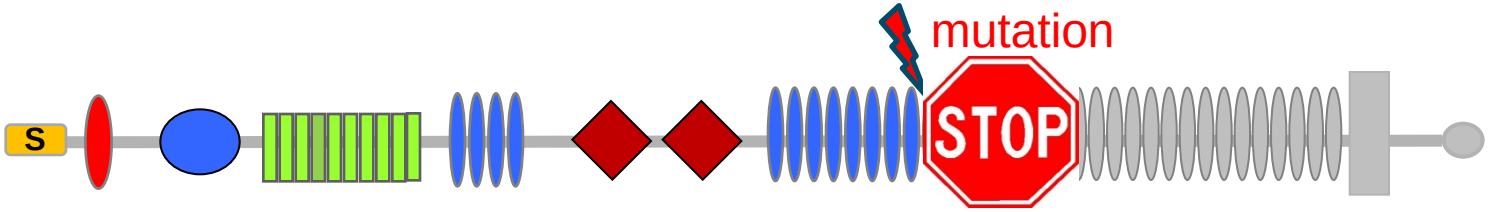


zebrafish

Important genetic cause: pseudoexon 40



Incorrect, non-functional USH2A protein !



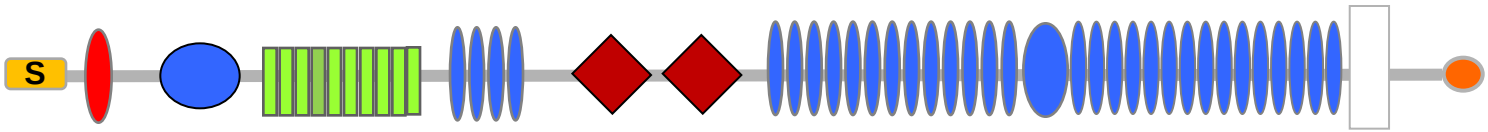
Strategy: splice correction!

USH2A pre-mRNA

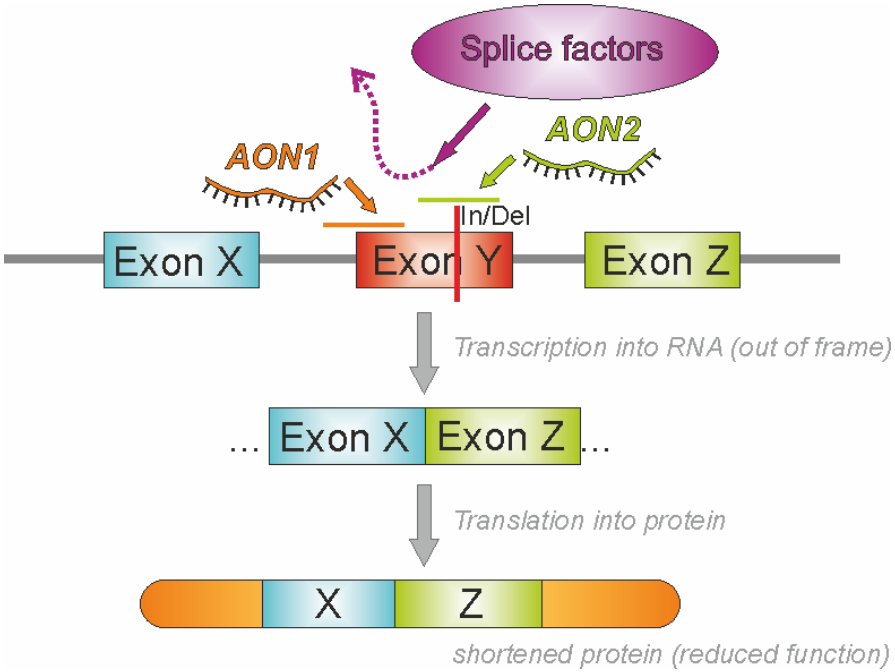
tion (Vaché et al., 2011; c.7595-2144A>G)

USH2A mRNA

Skipping of pseudo-exon, result: normal, fully functional USH2A protein !



“Genetic tape”: antisense oligonucleotides (AON)



Prevents binding of splice factors → induces exon-skipping during splicing!

Strategy against USH2A pseudoexon 40

Strategy: Use AONs to mask PE40 during splicing

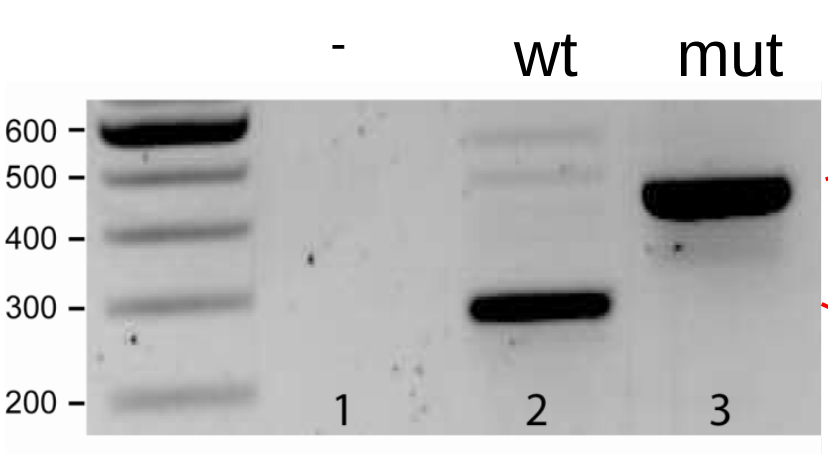
[1] Study the effect of the mutation in patient-derived cells

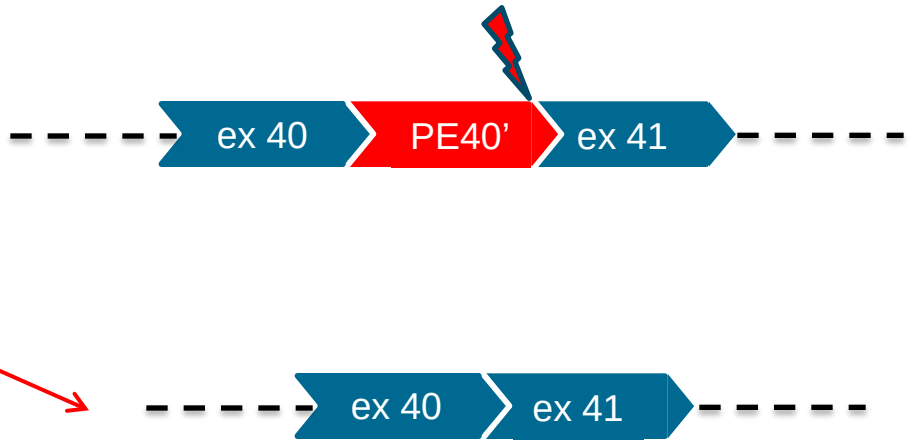
[2] Design AONs to redirect USH2A splicing

[3] Use patient-derived cells to confirm AON potential

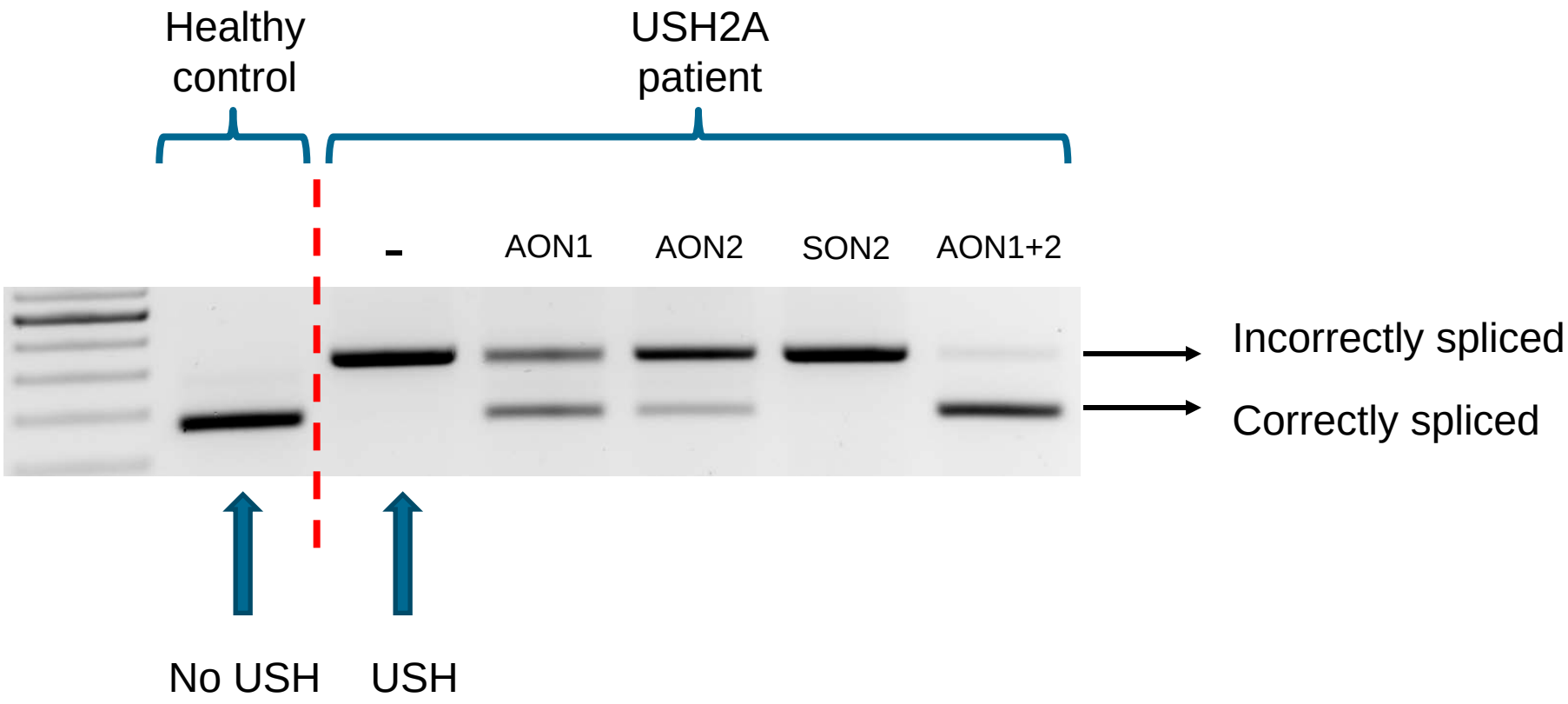
[4] Generate zebrafish knockin model to study effect on visual function

Effect of the mutation…





Splice correction ?



(Slijkerman *et al*., *Mol Ther Nucl Acids*, 2016)

Future delivery of “genetic tape”?

1) “**Naked**”;

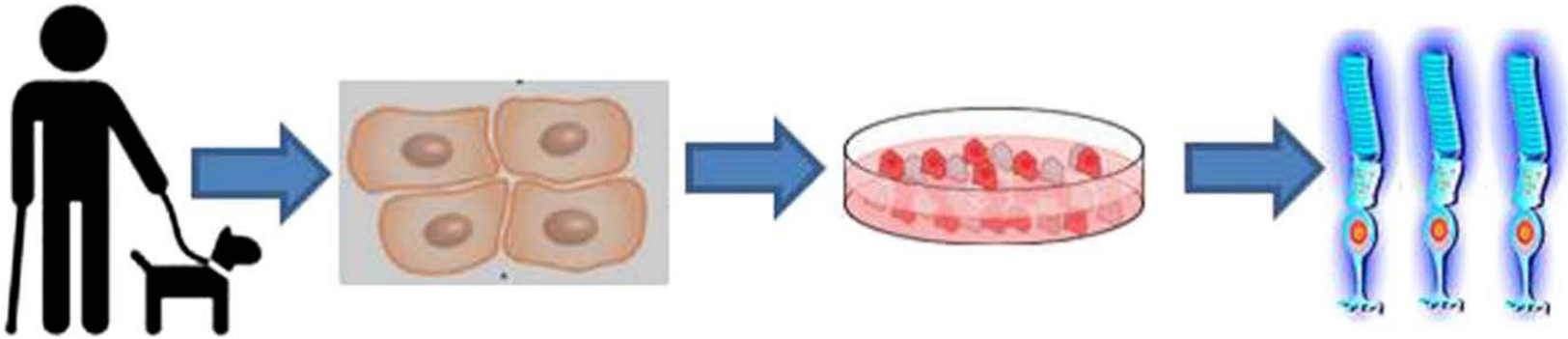
* **Repetitive** intraocular injections (~ 3-4 times a year)

2) Packaged into an **Adeno-Associated Virus (AAV)** or **Lentivirus**

* Presumably a **single** subretinal injection

Future research will determine the best and safest route of delivery

Translation to the proper context…



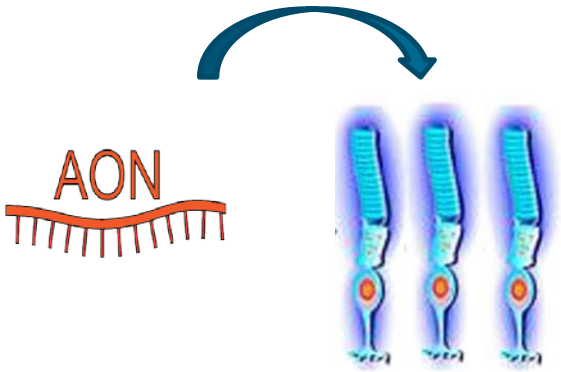
USH2A patient

Fibroblasts from skin biopsy

Stem cells [iPSC]

Photoreceptor-like cells (containing mutation!)

Follow-up



Photoreceptor-like cells (containing mutation!)

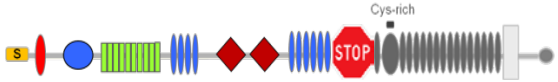
1) **Functional ?**
   (splice correction/zebrafish)
2) **Mode of delivery ?**
   ("naked"/AAV-based)
3) **Specific ?**
   (off target effects)
4) **Safe ?**
   (toxicity)

Phase I/II clinical trials !

In summary…

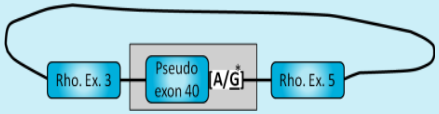
USH2A patients

Mutation in USH2A > non-functional protein



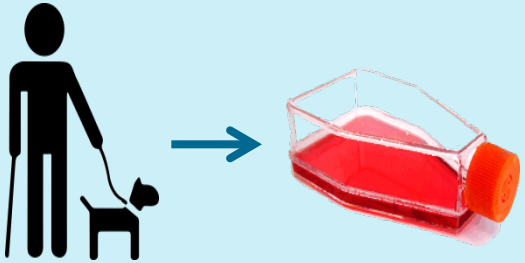
Interfere with splicing using AONs

Model PE40 splicing:



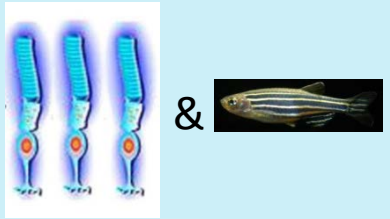
Minigene splice assay
+/- AONs

Confirmed in patient material:



Patient derived fibroblasts
+/- AONs

Preclinical efficacy & safety:



iPSC-P and zebrafish
+/- AONs

Clinical validation

Radboudumc
university medical center

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USHERSYNDROOM
reaching out for sight and sound

Foundation Fighting Blindness℠
Driving research to save and restore sight

NWO

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