Genetic therapy for

**USH2A-associated retinal dystrophy:**

future perspective or...?
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 (X)
  - *Gene size +++ (15,606 bp cDNA)
  - *USH2A isoforms

But,
- Ideally, interfere on **functional** or **transcript** level
  - *not alter isoforms
  - *not alter expression levels

- Gene editing (X)
  - *low efficiency
  - *off-target effects?
  - *not ready for clinical application

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Radboudumc

University Medical Center
What is needed for therapeutic development (2)?


2. Animal model → Ush2a-mouse model? [X]
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

USH2A pre-mRNA

- exon 40
- intron 40
- exon 41
- intron 41
- exon 42

Mutation (Vaché et al., 2012; c.7595-2144A>G)

Splicing

USH2A mRNA

- exon 40
- PE40'
- exon 41

Mutation

Incorrect, non-functional USH2A protein!
Strategy: splice correction!

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

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Effect of the mutation...
Splice correction?

Healthy control

USH2A patient

- AON1       AON2       AON1+2       SON2

Incorrectly spliced

Correctly spliced

No USH       USH

(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

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...

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
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