Developing a CRISPR/Cas9 Editing Approach for the Treatment of *USH2A*-Related Inherited Retinal Degeneration

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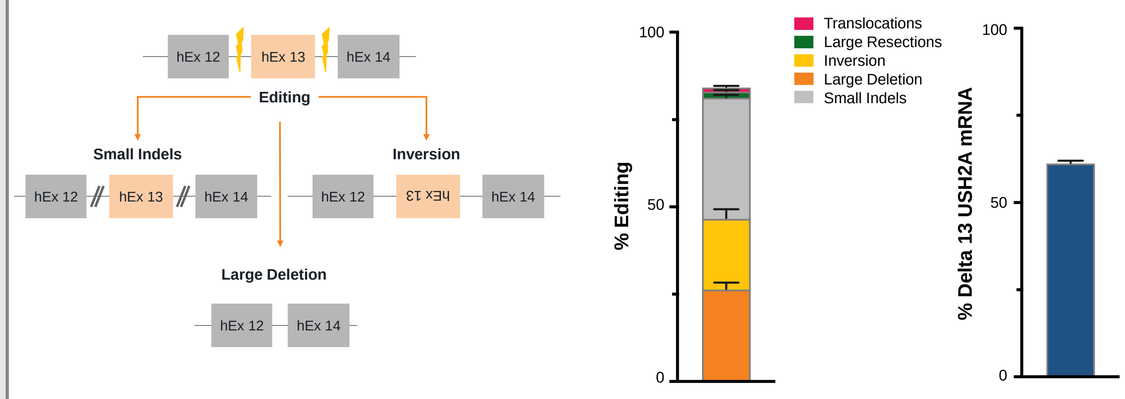
Abstract

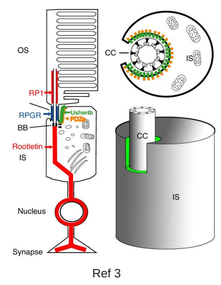
The most common cause of both Usher syndrome type II (USH2) and nonsyndromic autosomal recessive retinitis pigmentosa (arRP) are mutations in the *USH2A* gene; these diseases are also known as *USH2A*-related inherited retinal degeneration (IRD). A single nucleotide deletion in exon 13 (c.2299delG, p.Glu767fs) is the most common mutation in the *USH2A* gene in the United States. This mutation causes a premature termination codon and a truncated Usherin protein, resulting in loss of protein function. Previous research showed that removal of exon 13 of *USH2A* results in a functional in-frame protein. Since the size of the *USH2A* cDNA is too large for AAV-mediated delivery, we are developing an editing approach to treat *USH2A*-related IRD through CRISPR/Cas9-mediated excision of *USH2A* exon 13. The optimal guide RNA pair cutting within introns 12 and 13 of *USH2A* was chosen based on excision rates of exon 13 and on specificity, which was evaluated utilizing three orthogonal methods. Editing with our lead gRNA pair in a cell line that expressed *USH2A* resulted in up to 60% expression of *USH2A* Δ13. Moreover, DNA editing and expression of *USH2A* Δ13 mRNA was observed in human retinal explants, a relevant target tissue, after AAV-mediated delivery of CRISPR/Cas9. These results support the further preclinical development of CRISPR/Cas9 therapies for c.2299delG-associated *USH2A*-related IRDs.

In Vitro Proof of Concept Editing Causes Formation of Delta Exon 13 *USH2A* mRNA



Disease Background and Editing Approach

- Patients with USH2A syndrome experience deafness and retinal degeneration
- c.2299delG in exon 13 is the most common mutation in USH2A gene, resulting in a truncated and non-functional Usherin protein[1]
- Estimated 2,000-4,000 patients in US with c.2299delG mutation
- Usherin localizes to connecting cilia of mammalian photoreceptors and its absence leads to a ciliary defect and cell death
- Removal of exon 13 from the USH2A locus with CRISPR/Cas9 will restore an in-frame protein that has been shown to be functional in a mouse model[2]



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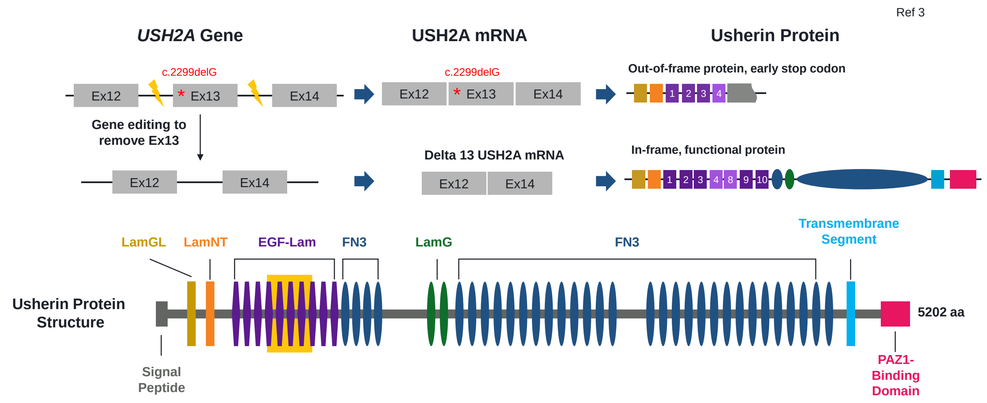
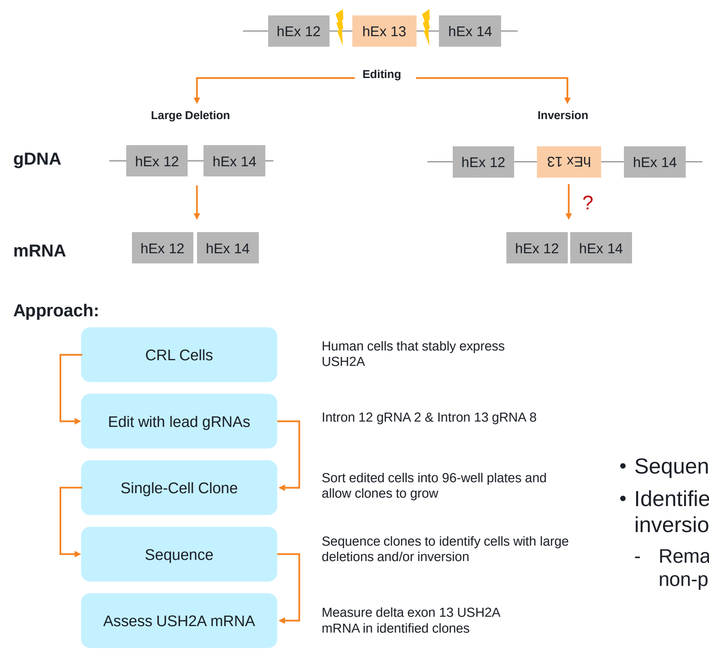
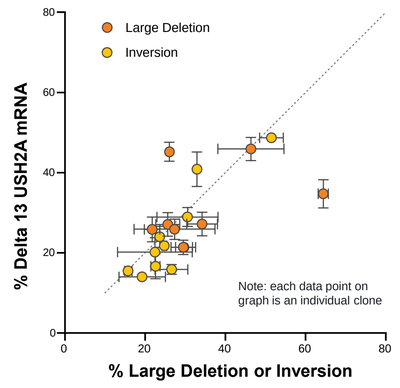


Large Deletions and Inversions are Productive Edits that Cause Formation of Delta Exon 13 *USH2A* mRNA





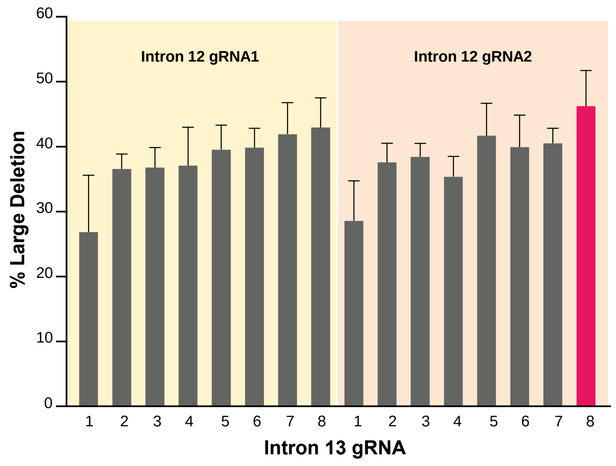
- Sequencing showed that most clones had >1 cell
- Identified clones that had either large deletions or inversions
  - Remaining alleles were either unedited or had small non-productive indels

Identification of Top Editing gRNA Pair

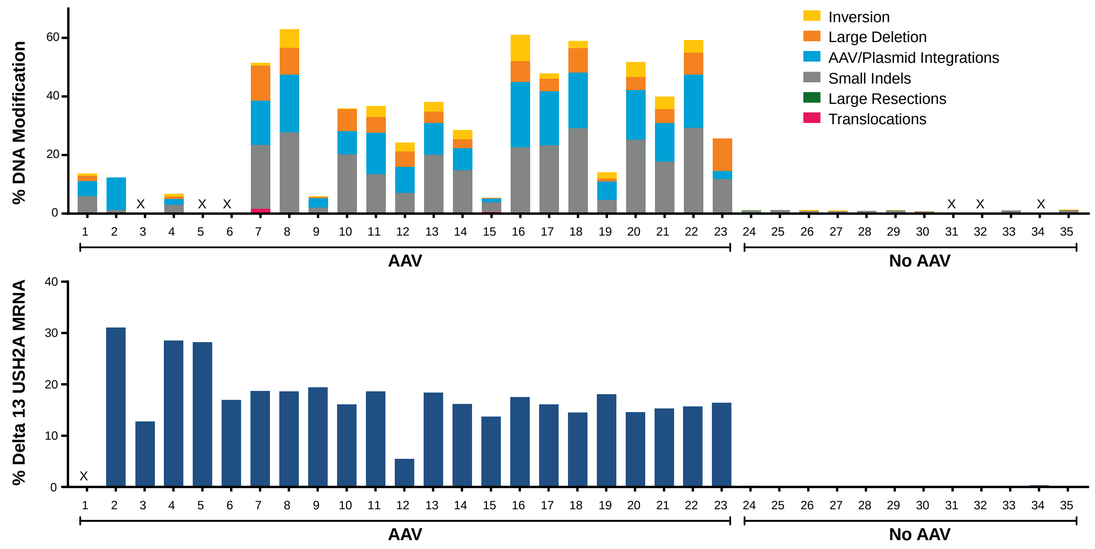
Identification of top performing single gRNA

	Number of gRNA Screened	Number of gRNA Selected Based on Editing
***USH2A* Intron 12**	41	2
***USH2A* Intron 13**	72	8

Identification of top gRNA pair for excision of USH2A exon 13

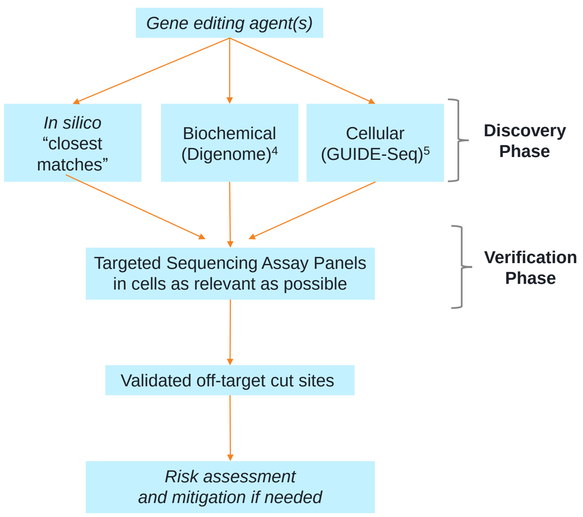


AAV-Mediated Editing in Human Retinal Explants Leads to Formation of Delta 13 *USH2A* mRNA



No off-target Editing Observed at Candidate Sites for Top gRNA Pair

Editas' approach to molecular specificity



gRNA	# Predicted *In Silico* Off-Targets	# Digenome Off-Targets	# GUIDE-Seq Off-Targets
Intron 12 gRNA 2	14	1	0
Intron 13 gRNA 8	62	1	0

- Candidate off-target sites were assayed for editing in primary T cells using high-throughput sequencing at each site
- No editing was observed at all candidate off-target sites

Conclusions

- Optimal gRNA pairs were identified in USH2A intron 12 and 13 that efficiently remove exon 13.
- No off-target editing was observed with top gRNA pair at identified candidate sites.
- Editing with top gRNA pair in cell line expressing USH2A results in delta 13 USH2A mRNA.
- Productive edits that will form delta 13 USH2A mRNA include large deletions and inversions.
- AAV-expressing Cas9 and gRNA in human retinal explants resulted in 5-60% editing and 5-31% delta exon 13 USH2A mRNA.
- Taken together, we have efficiently edited the *USH2A* gene to yield a transcript that has been shown to form a functional and in-frame Usherin protein.

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References:
[1]Hartong, et al., Lancet 368: 1795-1809 (2006)
[2]Pendse et al. Molecular Therapy 27:4S1 Apr 2019 Abstract 675)
[3]Liu et al. PNAS 104:11, 4413-4418 (2007)
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