CRISPR-Based Allele-Specific Editing for the Treatment of Autosomal Dominant Retinitis Pigmentosa (ADRP)

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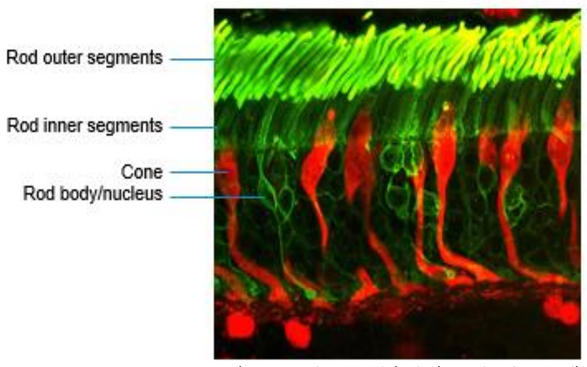
Introduction

- Retinitis pigmentosa (RP) is a group of inherited diseases causing retinal degeneration. RP results from mutations in over 60 genes, and is inherited in an autosomal recessive, autosomal dominant, or in an X-linked manner. RP affects 1:4000 individuals.
- The most common autosomal dominant mutation in Rhodopsin (g.129528801C>A) leads to an amino acid substitution in codon 23 (Proline 23 to Histidine) in the Rhodopsin protein.
- Rhodopsin is a G-protein receptor and a pigment found in the outer segments of rod photoreceptor cells. It is essential for phototransduction.
- RP evolves over decades, starting with night blindness, followed by progressive loss in the peripheral visual and blindness.



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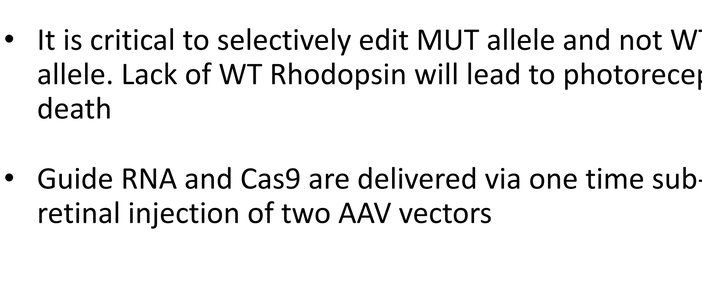
Therapeutic Strategy

- Develop a guide to selectively target the RhodopsinP23H mutant allele to cause INDEL-induced frame shift and early termination of translation.
- It is critical to selectively edit MUT allele and not WT allele. Lack of WT Rhodopsin will lead to photoreceptor death
- Guide RNA and Cas9 are delivered via one time sub-retinal injection of two AAV vectors

In Vitro Allele-Specific Editing of RHO-P23H

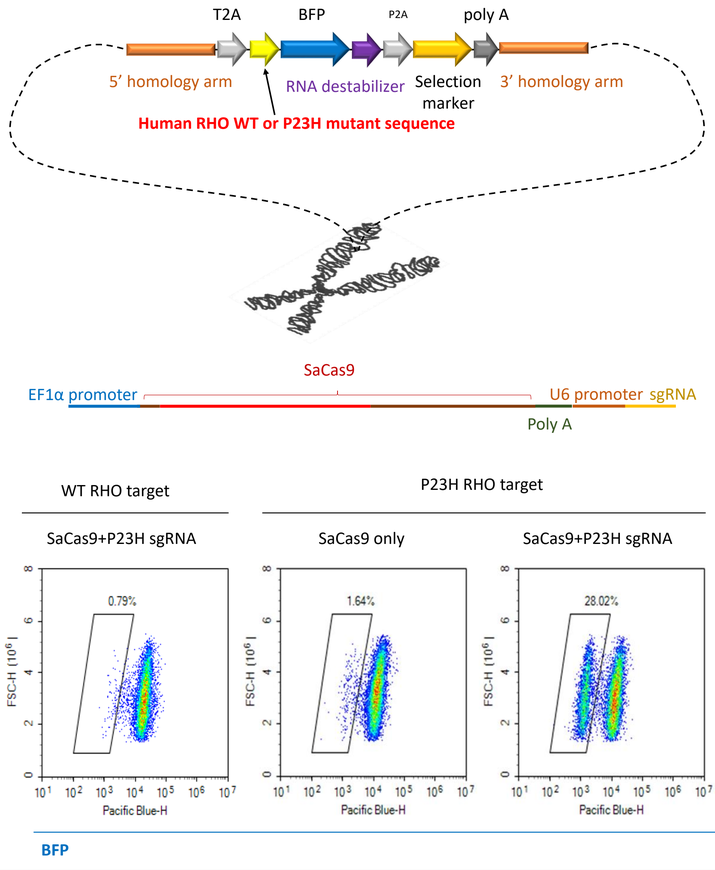
A K562 cell line homozygote for the P23H mutation was generated from K562 cells stably expressing SaCas9 under an inducible promoter. The WT K562 cells were used for allelic off-target analysis



K562 cells were doxycyclin-induced to express SaCas9, and then transfected with plasmid-encoded sgRNA with various spacer length of sgRNA

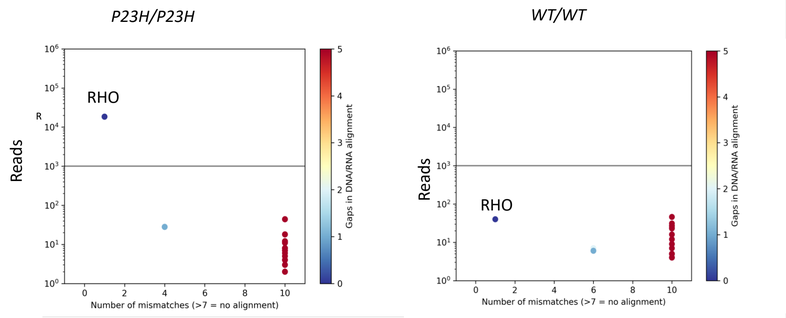
In Vitro Allele-Specific Editing of RHO-P23H

- Two 293 HEK reporter cell lines carrying the human RHO sequence upstream of a Blue Fluorescent Protein (BFP) sequence were developed. The RHO sequence corresponds either to WT or P23H protospacer target and PAM. The WT sequence evaluates the off-target editing of the sgRNA, while the P23H sequence estimates the on-target editing.
- Gene editing at the target site for SaCas9/P23H sgRNA can lead to inactivation of BFP by frame-shift mutations, which was assayed by flow cytometry.
- Plasmids carrying both SaCas9 and RHO P23H targeting sgRNA were used for this assay.



Off-Target analysis

- GUIDE-seq results for both P23H/P23H and WT/WT cell lines showed no significant off-targets

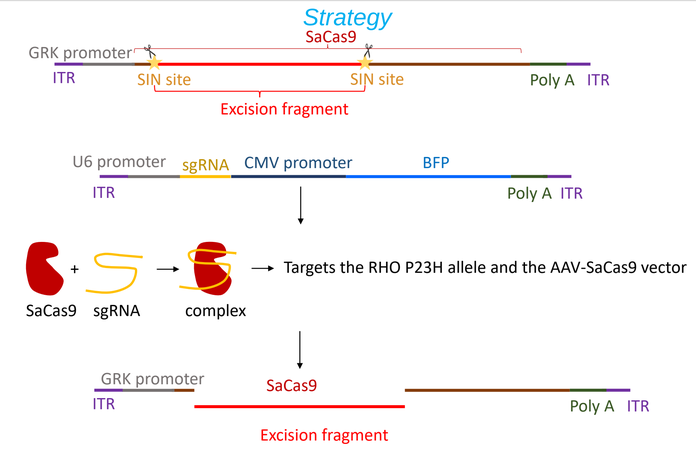


Amplicon-sequencing of in silico predicted targets showed no off-target activity

	WT		P23H		Untreated	
Site	Total reads	% indel	Total reads	% indel	Total reads	% indel
1	55042	0.08	74683	0.07	69362	0.07
2	94321	0.04	131752	0.02	141151	0.02
3	134440	0.14	181976	0.14	189987	0.17
4	142219	0.08	186730	0.10	184185	0.09
5	30186	0.04	29296	0.06	21214	0.03
6	42725	0.04	58933	0.05	54870	0.05
7	159620	0.09	220247	0.10	213870	0.08
8*	**215595**	**0.55**	**248451**	**94.84**	**351356**	**0.04**
9	4987	0.02	5537	0.05	5038	0.00
10	122972	0.06	159445	0.06	154543	0.05
11	232487	0.02	308479	0.02	334759	0.02
12	179571	0.02	255757	0.02	241768	0.03
13	81705	0.10	113649	0.09	110970	0.09

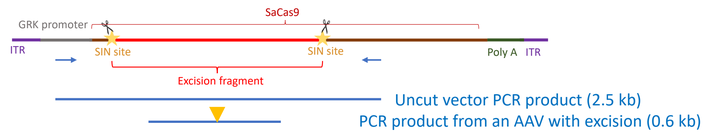
*Site 8 is RHO (on-target)

Vector Self-inactivation (SIN)

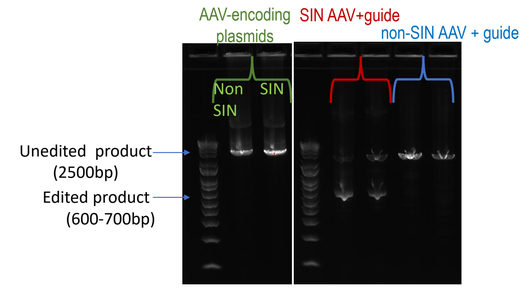


AAV-SaCas9 in a non-SIN version was used to compare maximum editing efficiency of a dual AAV vector system

In vivo DNA analysis

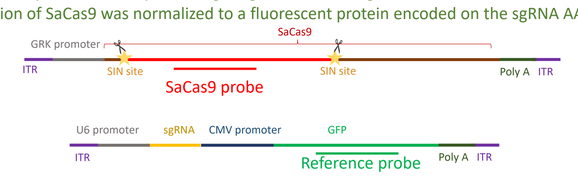


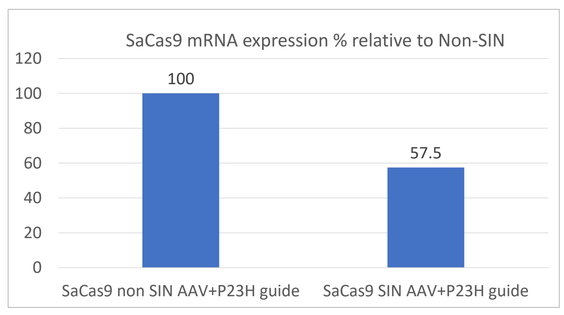
PCR of retina samples from dual vector injected in WT C57BL/6j animals at 4 weeks post-treatment



In vivo RNA analysis strategy

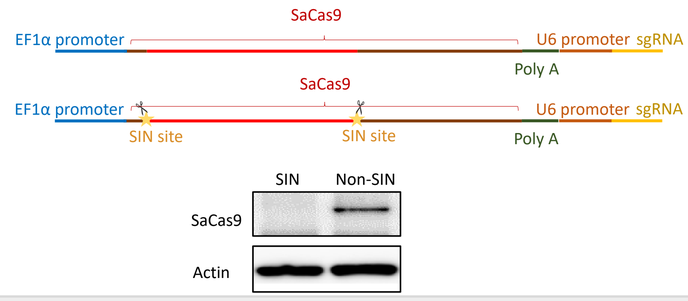
ddPCR was set up with SaCas9 probe targeting the excision region of the nuclease-encoding AAV, and the expression of SaCas9 was normalized to a fluorescent protein encoded on the sgRNA AAV vector.





In vitro protein analysis strategy

293 HEK reporter cell line was transfected with SIN or Non-SIN plasmids expressing both SaCas9 and RHO P23H targeting sgRNA. SaCas9 protein expression was analyzed by Western blot



Conclusions

- SaCas9 guide RNAs targeting the P23H allele were identified
- Cell based assays indicated high level of editing and allelic discrimination
- Guides with 20 and 23mer length spacer region showed highest levels of indels
- Indel distribution indicated that the vast majority of edits lead to a frameshift of the RHO reading frame
- Off-target analysis indicated very low indel levels in the RHO-WT allele and other genome loci of the K562 human cell line
- Self-inactivation of the AAV vector was confirmed on RNA, DNA and protein levels.
- AAV vectors are tested *in vivo* in animal models of ADRP.

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