Poster ID
#P219

AAV delivery of G protein gated K+ channel increases cone-mediated vision in the rd10 mouse model of Retinitis Pigmentosa

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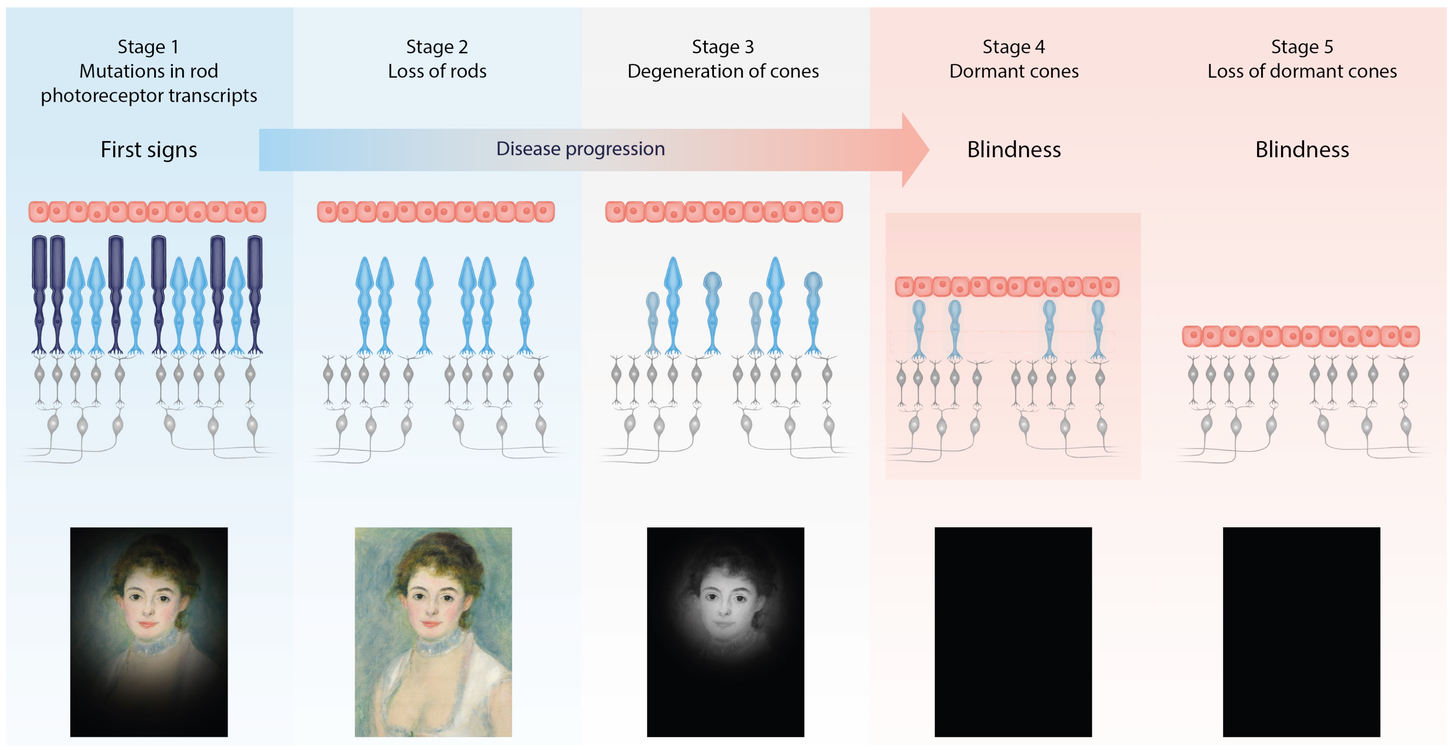
Disease Background

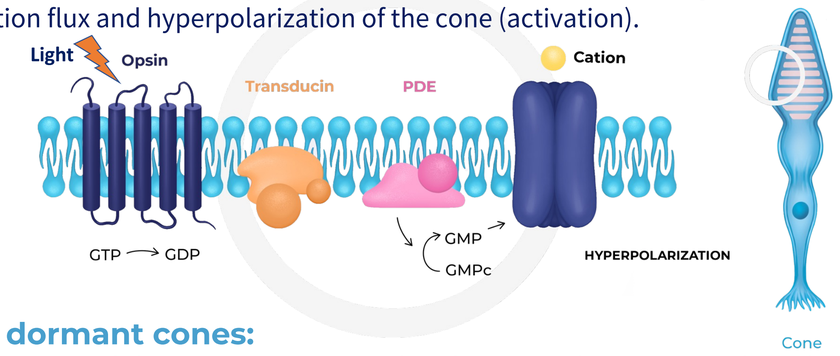
Retinitis Pigmentosa (RP) is a debilitating disease causing progressive, irreversible vision loss.
This heterogeneous group of inherited retinal degenerations can be caused by mutations in over 70 genes.



SPVN20: Mechanism of action

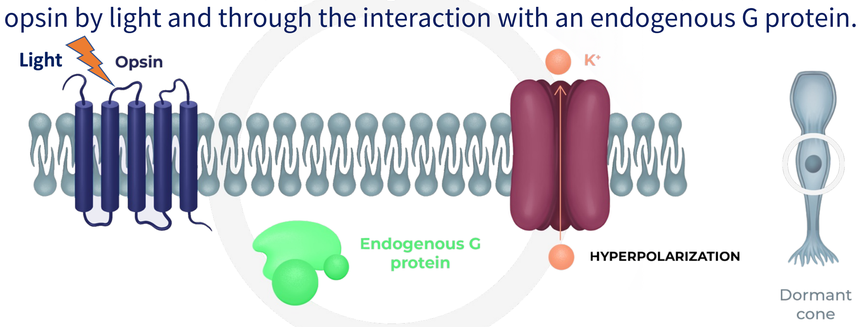
In healthy cones:

The phototransduction cascade converts light signal into an electrical signal. This happens in stacked membranes of the cone photoreceptor outer segment. The activation of a series of proteins in cascade controls the activity of CNG channels, cation flux and hyperpolarization of the cone (activation).



In dormant cones:

In cones with lost outer segments, the phototransduction cascade is disrupted. This is due to the loss of several cascade proteins' expression. Our approach aims at **restoring cone cell bodies' light sensitivity** through the expression of a potassium channel, GIRK (G protein gated Inward Rectifier K+), after activation of the opsin by light and through the interaction with an endogenous G protein.

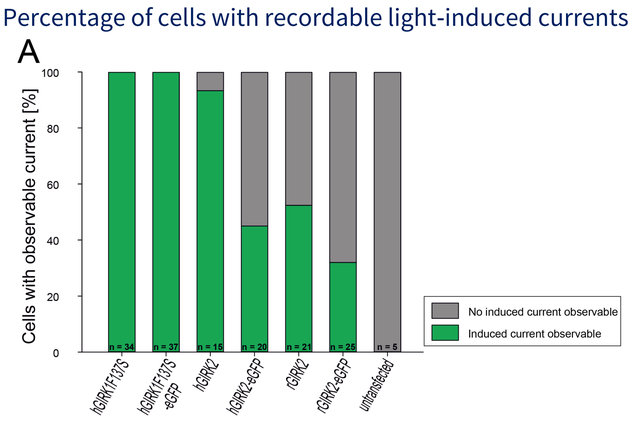


In vitro characterization of the candidate

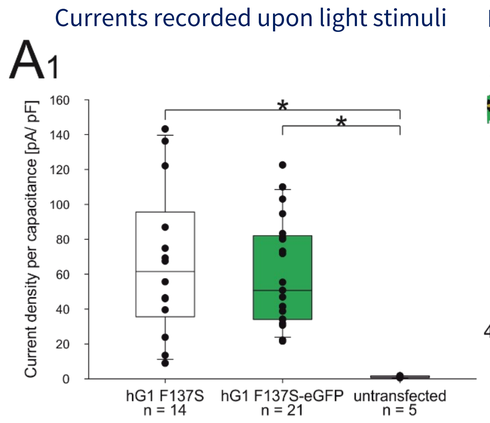
HEK cells rendered light sensitive upon co-expression of opsin and hGIRK1 F137S

- → Upon transfection of CMV-hGIRK F137S in HEK cells stably expressing opsin, light stimuli were applied to test their light responsiveness
- → Single cell patch clamp recordings were performed to record GIRK currents
- → Cells expressing GIRK1 F137S reliably respond to light stimuli
- → hGIRK1 F137S outperforms the other variants tested (ratGIRK2, hGIRK2 and their GFP variants)

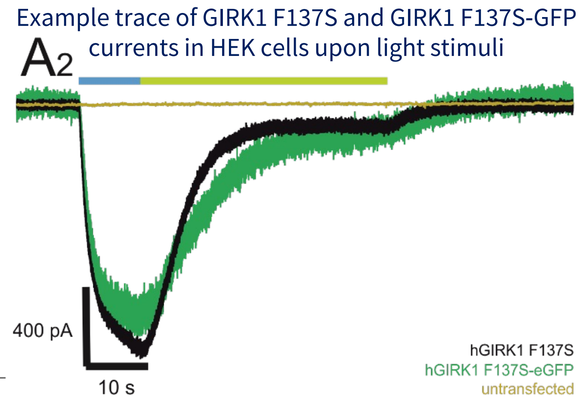
Percentage of cells with recordable light-induced currents



Currents recorded upon light stimuli



Example trace of GIRK1 F137S and GIRK1 F137S-GFP currents in HEK cells upon light stimuli

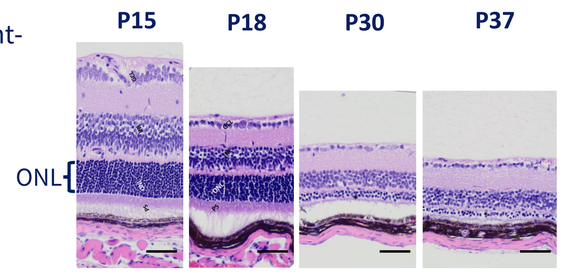


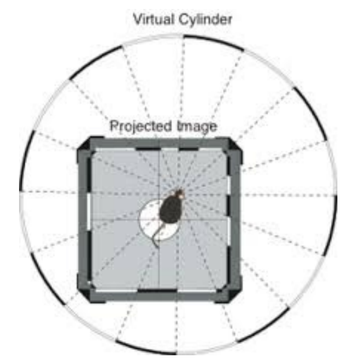
In vivo validation of the candidate

rd10 mouse model for RP: course of retinal degeneration and functional defects

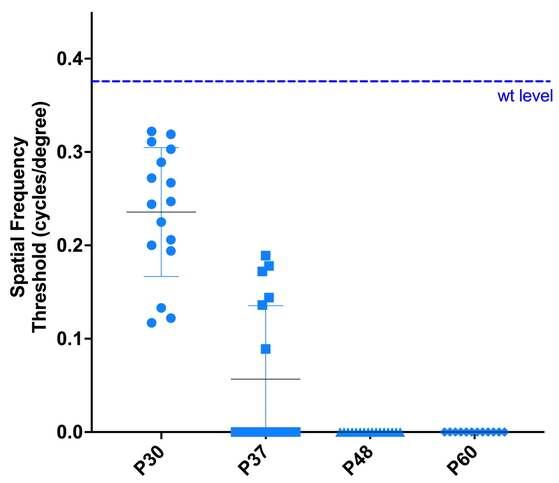
- → rd10/rd10 mice carry a missense mutation R560C in exon 13 of the Phosphodiesterase β gene (*Pde6b*)
- → Progressive retinal degeneration in light-reared animals in a 12h/12h dark-light cycle
- → Photoreceptor dysfunction and degeneration, starting with rod and followed by cone loss
- → All mice are blind by P48, i.e. outer segments are lost, but dysfunctional (dormant) cones persist
- → Evaluation of the optokinetic reflex allows evaluation of treatment effect on the retina and cortical integration

H&E staining - Naive mice





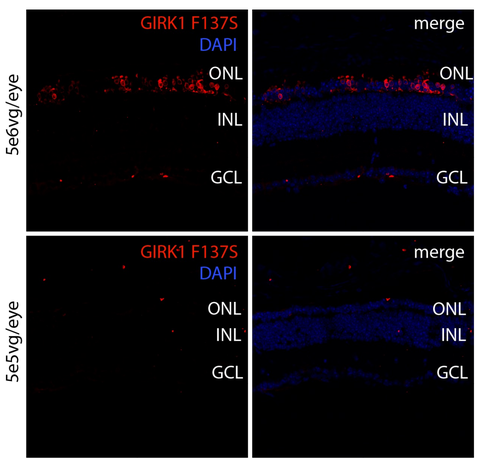
***In vivo* evaluation of the optokinetic reflex**



AAV injections and evaluation of therapeutic benefits

→ Bilateral subretinal AAV-hGIRK1 F137S injections in rd10 mice at P15.

Confocal imaging (40x) of GIRK1 F137S positive cones detected at 5e6vg/eye, but not at 5e5vg/eye, at P60



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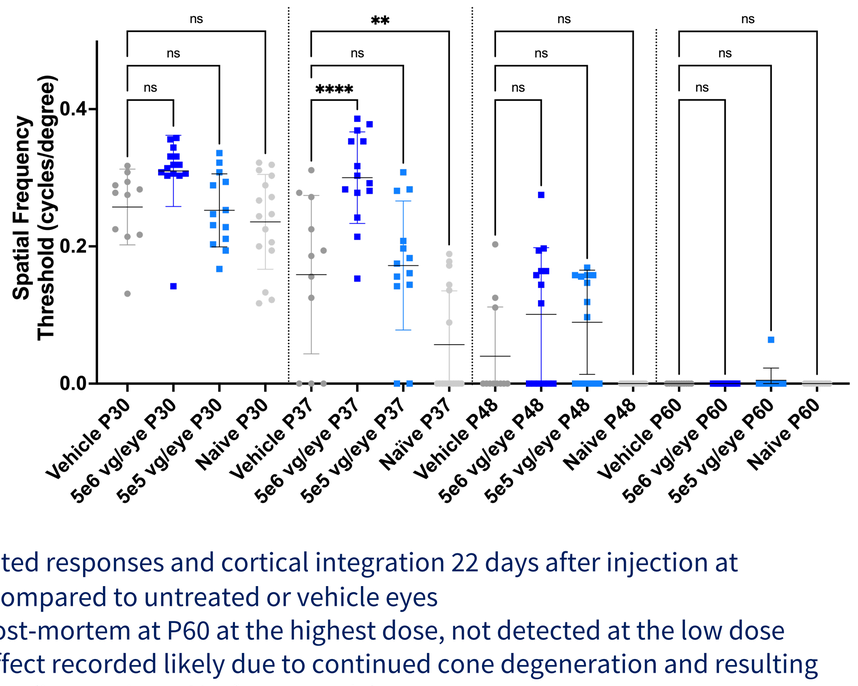

- → Significant restoration of cone-mediated responses and cortical integration 22 days after injection at 5e6vg/eye, but not at 5e5 vg/eye, as compared to untreated or vehicle eyes
- → Persistence of GIRK1 F137S+ cones post-mortem at P60 at the highest dose, not detected at the low dose
- → No effect at P48 and P60. Transient effect recorded likely due to continued cone degeneration and resulting low number of cones remaining in mice (in wt mice, 3% of cones only)

Conclusions

- Co expression of opsin together with the G-protein sensitive potassium channel GIRK1 F137S, confers light sensitivity to HEK 293 cells as observed in *in vitro* electrophysiology assays
- In the rd10 mouse retina, the exogenous expression of GIRK1 F137S in degenerating cones leads to improved cone function as measured *in vivo*
- These encouraging in vivo data support exploring the potential of AAV-hGIRK1 F137S in other models with the aim of developing a vision restoration gene therapy product for RP patients

References

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Disclosures / SparingVision employees: HK, FL, MG / SparingVision personal interest: HK, FL, MG, DD

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