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KCNV2 retinal organoid disease model for KCNV2 AAV gene therapy development

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1. Introduction

Mutations in *KCNV2* cause a form of inherited retinal dystrophy known as 'cone-dystrophy with supernormal rod response' (CDSSR) .The *KCNV2* gene product is a voltage gated potassium channel subunit, Kv8.2, which functions in the retina via its interaction with Kv2 type potassium channel Kv2.1. The Kv8.2/Kv2.1 heteromeric protein is found in rod and cone photoreceptor inner segments.

KCNV2 knockout (KO) retinal organoids and wild-type (WT) control organoids were generated from human iPSC using CRISPR/CAS9. The expression of the *KCNV2* gene product (Kv8.2) in wild type and KO organoids was assessed along with markers of mature outer and inner retinal cell types.

The KCNV2 KO retinal organoids were used to screen eight AAV vectors with either wildtype or codon optimised versions of *KCNV2*, driven by either constitutive (CAG) or photoreceptor specific rhodopsin kinase (RK) promoters. The vectors were assessed for their ability to restore Kv8.2 localisation to the inner segment region of rod and cone photoreceptor cells when packaged into either AAV5 or AAV7m8 vectors. Transduction and expression profiles were assessed by QPCR and single cell RNA seq. Sub-cellular localisation as well as native interactions were assessed by immunofluorescence and proximity ligation assay (PLA).

2. AAV Vector design

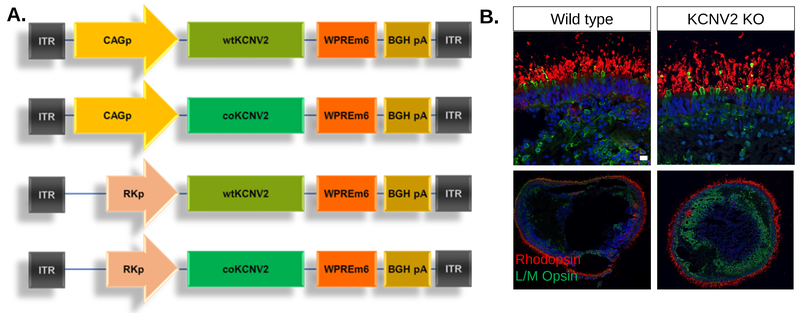


Figure 1. AVV vector design and retinal organoid model. A. KCNV2 wild type and codon optimized transgenes driven by constitutive CAG promoter (CAGp) and photoreceptor specific rhodopsin kinase (RKp) promoter were packaged in AAV5 and 7m8 capsids. **B.** Human iPSC were generated from healthy neonatal fibroblasts. KCNV2 KO iPSC were simultaneously generated using CRISPR/CAS9 and differentiated alongside unedited controls into mature retinal organoids (4 months +). Mature organoids expressed markers of rod (rhodopsin, red) and cone (L/M opsin, green). KCNV2 KO organoids displayed no gross morphological differences to WT. Scale bar = 10µm.

3. Transduction of KCNV2 knockdown retinal organoids

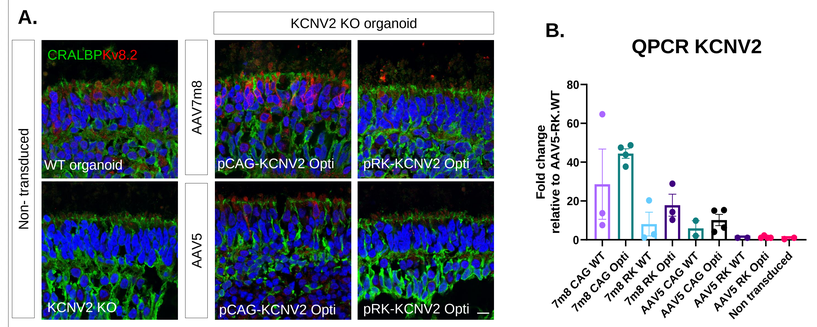


Figure 2. Relative Kv8.2/KCNV2 expression in transduced organoids. A. Immunofluorescence images showing KCNV2 protein product Kv8.2 (red) in KCNV2 knockdown organoids following transduction with KCNV2-Opti vectors in the photoreceptor cell layer. Kv8.2 was detected in photoreceptors with highest expression levels detected in AAV7m8 vectors and those with CAG promoters. Cells are co-stained with Muller Glia marker CRALBP (green). **B.** QPCR for KCNV2WT and KCNV2 Opti transcripts**.** mRNA from whole organoids following transduction with all 8 vectors was compared to AAV5-RK.KCNV2WT. Each data point represents one organoid.

4. Kv2.1/Kv8.2 co-localisation

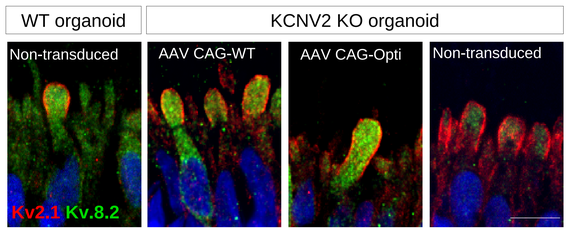


Figure 4. Kv8.2 localisation. *KCNV2* product Kv8.2 forms functional heteromers with potassium channel Kv2.1. High magnification view of WT organoid photoreceptor inner segments shows Kv2.1 (red) co-localizing with Kv8.2 (green) Scale bar = 10µm.

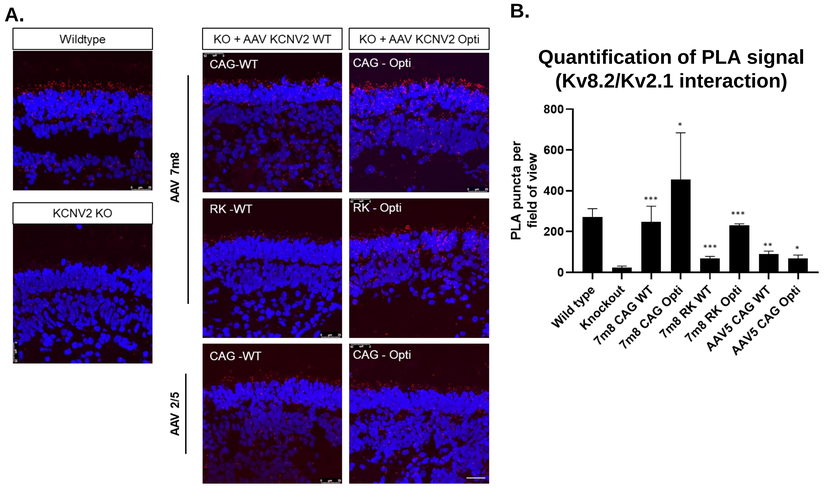
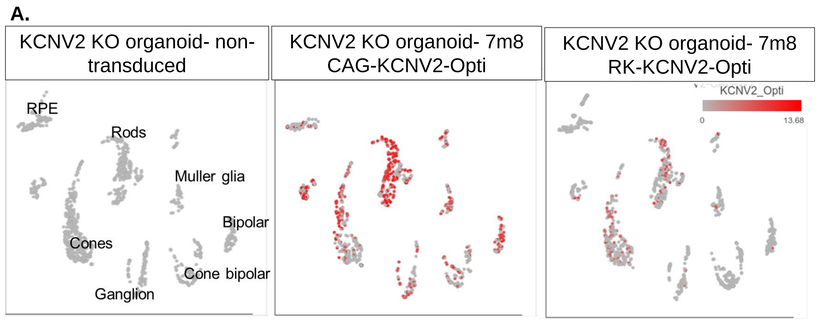


Figure 5. *In situ* quantification of protein-protein interactions by proximity ligation assay (PLA). A. Wild type, KCNV2 KO untreated and transduced KCNV2 KO organoids were probed for protein-protein interactions between Kv8.2 protein (endogenous and vector derived). Staining with Kv8.2 and Kv.2.1 antibodies and secondary PLA probes reveal interactions which appear as red dots (PLA puncta). **B.** Quantification of PLA puncta revealed a significant restoration of Kv2.1/Kv8.2 interaction following transduction with all AAV vectors (p<0.01, one way ANOVA). Transduction with AAV7m8-RK.KCNV2Opti produced a significantly higher signal than AAV7m8-RK.KCNV2WT, p<0.01, unpaired ttest.

5. Single cell RNAseq



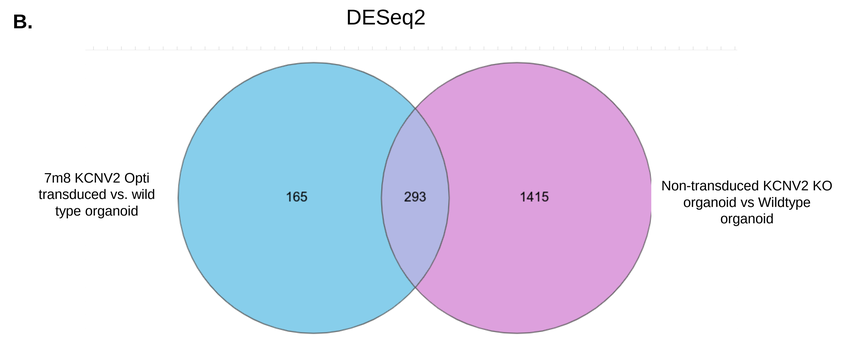


Figure 6. Single cell RNA seq. A. PCA was used to identify clusters and biomarker analysis was used to label Rod, cone, RPE, Muller glia ,bipolar and ganglion cells in wild type, KCNV2 KO and transduced organoids. Vector derived KCNV2 Opti transcripts (both CAG and RK promoter constructs) are overlaid into specific clusters (red dots). KCNV2opti transcripts derived from AAV7m8-CAG.KCNV2 opti vector were detected primarily in rod and cone clusters but also in muller glia,, bipolar and RPE cells. AAV7m8-RK.KCNV2Opti vector was also expressed in rods and cones but not in other cell populations. B. DESeq2 was used to analyse differentially expressed genes in WT vs. KCNV2 KO organoids of which 1415 genes were identified. Following transduction with AAV7m8-KCNV2Opti-expressing vectors this number was reduced to 165 indicating some restoration of global expression profile to WT.

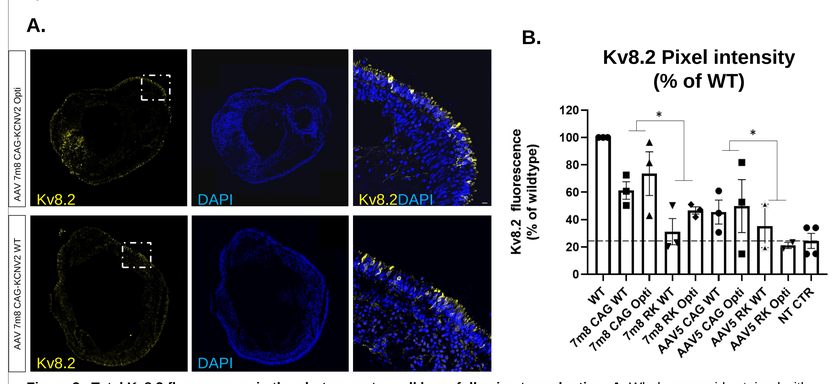


Figure 3. Total Kv8.2 fluorescence in the photoreceptor cell layer following transduction. A. Whole organoids stained with Kv8.2 antibody showing expression from both WT and Opti vectors in the outer nuclear layer (Photoreceptor cell layer). **B.** Quantification of total Kv8.2 fluorescence in the photoreceptor layer of organoids transduced with the 8 vectors, expressed as a % of wildtype. (integrated density/ONL area) $^{\text{transduced KO organoids}}$ / (integrated density/ONL area) $^{\text{WT organoid}} \times 100$. Error bars = +/- SEM. There was a significant difference in total fluorescence between CAG and RK promoters in both 7m8 and AAV5 capsids (p= 0.031 and 0.028 respectively, 2 tailed, paired student's t test). There was no significant difference between WT and Opti in vectors with CAG or RK promoters despite a trend towards increased fluorescence intensity in codon optimized (Opti) vectors.

6. Conclusions

All AAV vector designs delivered the *hKCNV2* gene to human photoreceptors (rods and cones) in our retinal organoid disease model. The protein product, Kv8.2, was produced and was correctly co-localised with endogenous Kv2.1 in the photoreceptor inner segments. The restoration of PLA (Kv2.1-Kv8.2) signal in transduced organoids indicates the formation of functional heteromers with potassium channel Kv2.1. The CAG promoter increased total RNA and protein levels relative to the RK promoter. Condon optimisation of *KCNV2* increased Kv8.2 protein expression levels relative to the WT sequence. Transcriptomic analyses following restoration of Kv8.2 in human photoreceptors, reverted to a more WT-like profile indicating disease correction at a deep transcriptional level.

AAV-mediated *KCNV2* gene supplementation might be beneficial in patients with CDSSR due to *KCNV2* mutations.

References:

Gayet-Primo J, Yaeger DB, Khanjian RA, Puthussery T. Heteromeric $K_V2/K_V8.2$ Channels Mediate Delayed Rectifier Potassium Currents in Primate Photoreceptors. J Neurosci. 2018 Apr 4;38(14):3414-3427