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Development and efficacy assessment of AAV2/8-hG1.7p.coCNGA3, a *CNGA3* gene therapy vector

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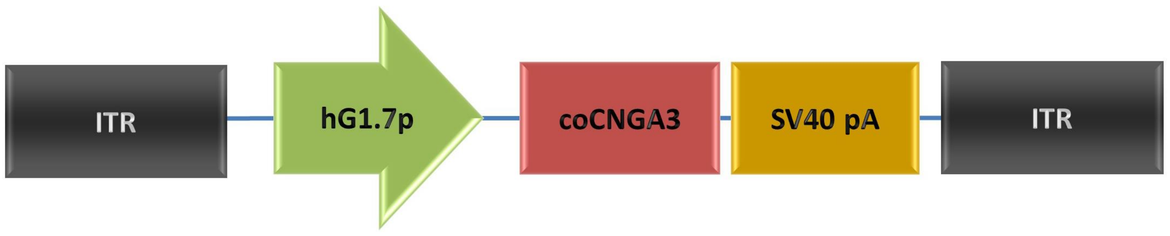
Introduction

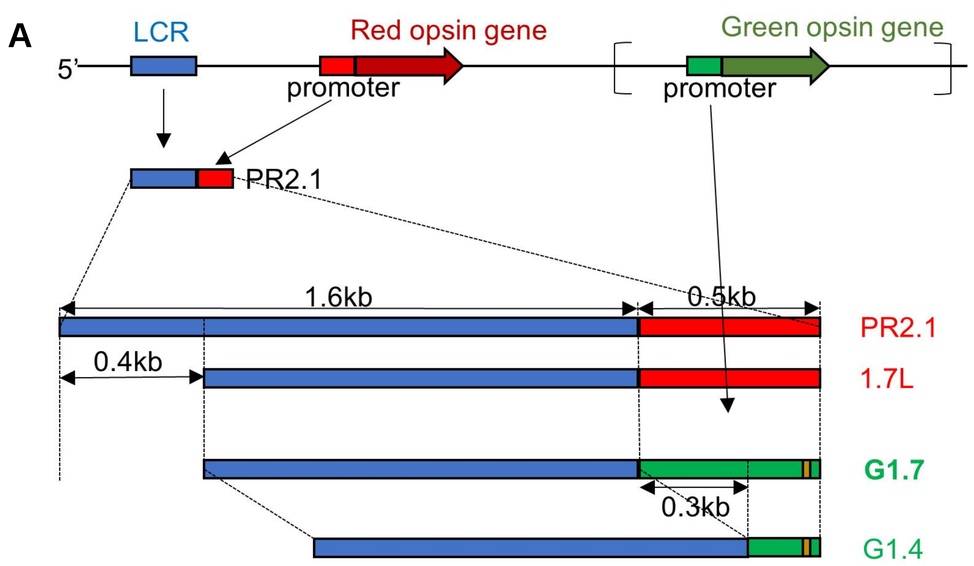
Achromatopsia is an inherited autosomal-recessive condition characterised by intact rod function and absent cone function. The cone cyclic nucleotide-gated (CNG) channel is the final critical effector in the phototransduction cascade (the biological conversion of light energy to electrical signalling) and mutations in the α subunit (CNGA3) are one of the leading causes of achromatopsia. We developed AAV2/8-hG1.7p.coCNGA3, an AAV2/8-based vector, carrying a human codon-optimised *CNGA3* gene driven by an engineered fragment based on the human green opsin promoter that leads to significant rescue of cone function in Cnga3-deficient mice.



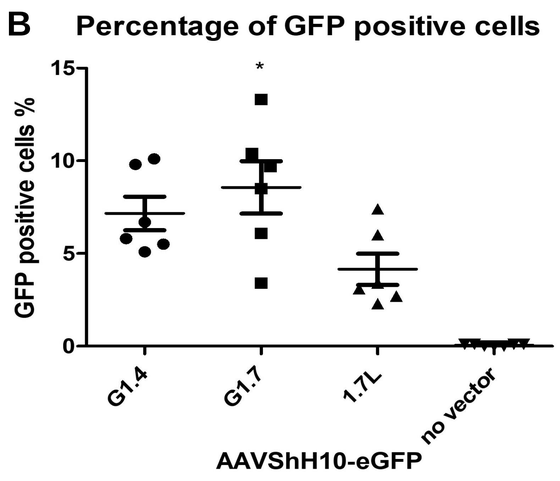
Promoter development

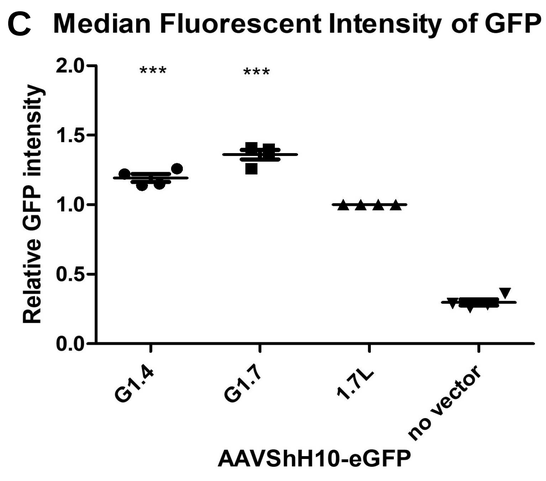
The most commonly used cone-specific promoters combine the Locus Control Region (LCR) with the red opsin core promoter to generate two red opsin-based promoters (PR2.1 and 1.7L) which are shown here indicatively (A).

In this study we combined a truncated LCR region fragment with either a longer or shorter fragment of the core green opsin promoter to generate the G1.7 and G1.4 engineered promoters, respectively. An additional mutation in the green core promoter fragment was also engineered (indicated as a yellow square).



A GFP reporter assay in iPSC-derived human retinal organoids was performed for G1.4, G1.7 and 1.7L promoters using AAVshh10 capsids (C). Cone-specific expression was confirmed by immunohistochemistry for all promoters (data not shown) and flow cytometry experiments (n=4-6) were performed to assess GFP transduction efficiency (B) and median fluorescence intensity within the transduced cells (C). Both G1.4 and G1.7 mediated a marked increase in percentage of GFP positive cells when compared to 1.7L ($p<0.5$ for G1.7) as well as a highly significant increase in MFI of transduced cells when compared to 1.7L ($p<0.001$ for both G1.4 and G1.7). Error bars; SEM.

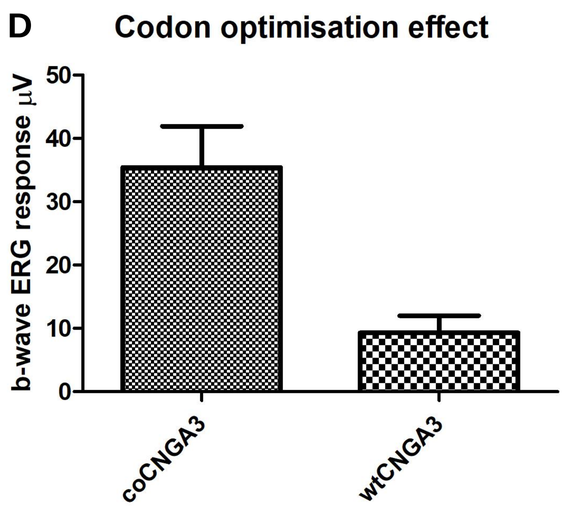




Codon optimisation of CNGA3

In silico codon optimisation of the human *CNGA3* gene was performed that led to the adjustment of 8 rare tRNAs, final Codon Adaptation Index (CAI) of 0.87 and removal of three destabilising sequences.

We next assessed the efficacy of either wild-type *CNGA3* (wtCNGA3) or codon-optimised *CNGA3* (coCNGA3) to improve photopic retinal sensitivity as measured by electroretinography (ERG) in the Cnga3-deficient mouse model. For this experiment we placed wtCNGA3 or coCNGA3 under the influence of the 1.7L promoter and produced AAV8 viral vectors.

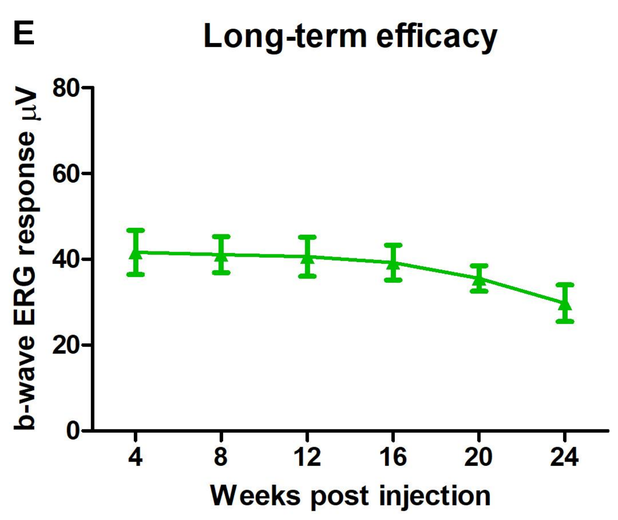


Cnga3-deficient mice were subretinally injected at 2 weeks of age with either vector [$1x10^{12}$ vg/mL] and photopic ERG responses were measured 4 weeks post-injection (n=14-16; $p<0.001$) (D). Error bars; SEM.

coCNGA3 provided more than 200% increased ERG responses in treated eyes compared to wtCNGA3. Untreated eyes exhibit no measurable ERG at the light intensity used ($10cdsm^{-2}$).

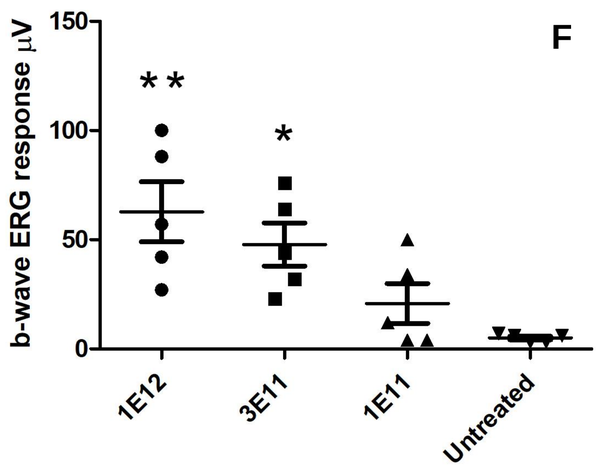
Long-term efficacy and dose escalation

We selected the G1.7 promoter and the coCNGA3 gene to generate an AAV2/8-G1.7p.coCNGA3 viral vector and assessed its long-term efficacy in rescuing photopic retinal sensitivity in Cnga3-deficient mice. In addition we tested whether a dose escalation using viral vector titres that could be used in a Phase I/II clinical trial for achromatopsia due to CNGA3 mutations, led to ERG improvements in the Cnga3-deficient mouse.



AAV2/8-G1.7p.coCNGA3 [$1x10^{12}$ vg/mL] was subretinally injected in Cnga3-deficient mice at 2 weeks of age and photopic ERG amplitudes were measured at 4, 8, 12, 16, 20 and 24 weeks after treatment (n=14) (E). A sustained improvement was observed in treated eyes with a slight trend of decreasing amplitudes at 6 months post-treatment. A similar trend was observed in wild-type mice at that age (data not shown). $10cdsm^{-2}$

AAV2/8-G1.7p.coCNGA3 was subretinally injected in Cnga3-deficient mice at 2 weeks of age and at three escalating titres. Photopic ERG amplitudes were measured at 4 weeks after treatment (n=5) (F). Significant improvements were observed for the high and middle titres ($p>0.01$ and $p<0.5$, respectively) and a trend of improving retinal sensitivity was seen for the lowest titre. $10cdsm^{-2}$



Conclusions

A potent cone-specific promoter (G1.7p) was designed and its efficacy was tested in human-derived retinal organoids. G1.7p in combination with a codon-optimised *CNGA3* gene and AAV8 capsid pseudotyping, led to long-term improvement of cone survival and retinal sensitivity *in vivo*.
Administration of AAV2/8-hG1.7p.coCNGA3 also resulted in a positive dose response at titres planned for a clinical trial to treat achromatopsia due to CNGA3 mutations.

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