Rescue of Cone ERG Function by Treatment with AAV-hCNGB3 Vectors in CNGB3 Knockout Mice

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ABSTRACT

- Restoration of cone function with recombinant adeno-associated virus (rAAV) vectors with different cone-specific promoters has been achieved in mouse and dog models of achromatopsia. However, not much information exists about their specificity and efficiency in transducing cones in primates.
- PR1.7 is a shorter version of the 2.1 kb human red cone opsin promoter (PR2.1). IRBP/GNAT2 is a hybrid promoter consisting of a 277-bp human GNAT2 promoter and a 214-bp IRBP enhancer. In dogs, PR2.1 directs specific expression of GFP in red/green (L/M) cones but not blue (S) cones (Komaromy et al., 2008), and IRBP/GNAT2 directs specific expression of GFP in both L/M cones and S cones (Yeh et al., 2013). In nonhuman primates (NHP) PR1.7 is more efficient than PR2.1 in directing specific expression of GFP in L, M and S cones, while IRBP/GNAT2 is unable to support expression of GFP in cones (Ye et al., 2014).
- In the present study, the efficiency of functional improvement in cone-mediated ERG by AAV vectors expressing hCNGB3 or codon-optimized hCNGB3, driven by PR1.7, PR2.1 or IRBP/GNAT2 promoters, packaged in an AAV2 capsid variant with Y444F, Y500F and Y730F mutations (AAV2tYF) or in AAV5 or AAV9 capsids and delivered by subretinal injection, was evaluated in CNGB3 KO mice. We demonstrated that the AAV-PR1.7-hCNGB3 vector containing the PR1.7 promoter, which is optimal for NHP, led to ERG functional rescue in CNGB3 KO mice. In addition, we confirmed that the vector containing the IRBP/GNAT2 promoter, which does not support reporter gene expression in NHP, achieved the best cone ERG functional rescue in CNGB3 KO mice.

METHODS

- AAV vectors were manufactured by plasmid transient transfection in HEK 293 cells, purified by double iodixanol step gradient centrifugation and formulated in balanced salt solution (BSS) containing 0.014% Tween 20.
- A total of 70 CNGB3 KO mice, age 30 days at the time of injection, were used in this study, with 10 animals assigned per group. Within each group mice received the appropriate AAV-CNGB3 vector by subretinal injection in one eye. Half of the contralateral eyes were treated with vehicle (BSS) and the other half remain untreated. The study design, including combinations of capsid, promoter and transgene, is summarized in **Table 1**.
- The primary outcome measure of this study was cone-mediated ERG at 3 months post injection. At 3 months post-injection groups of mice received full field scotopic (rod) and photopic (cone) ERGs. Maximum photopic b-wave amplitudes generated at 10 dB, from all treated, untreated, and control eyes within each cohort, were averaged and utilized in the analysis.

Table 1 Efficacy study design in CNGB3 KO mice

Group	Number of animals	Vector	Volume injected	Conc. (vg/mL)	Dose (vg/eye)
1	10	AAV5-PR2.1-hCNGB3	1 µL	5×10^{12}	5×10^{9}
2	10	AAV5-PR2.1-hCNGB3co	1 µL	5×10^{12}	5×10^{9}
3	10	AAV5-PR1.7-hCNGB3co	1 µL	5×10^{12}	5×10^{9}
4	10	AAV5-IRBP/GNAT2-hCNGB3co	1 µL	5×10^{12}	5×10^{9}
5	10	AAV2tYF-IRBP/GNAT2-hCNGB3co	1 µL	2.3×10^{12}	2.3×10^{9}
6	10	AAV9-IRBP/GNAT2-hCNGB3co	1 µL	5×10^{12}	5×10^{9}
7	10	AAV5-IRBP/GNAT2-hCNGB3	1 µL	5×10^{12}	5×10^{9}

RESULTS

- In nonhuman primates, PR1.7 is more efficient than PR2.1 in driving robust specific expression of GFP in L, M and S cones, while IRBP/GNAT2 is unable to drive expression of GFP in cones (**Figure 1** and **Figure 2**).
- The vector containing the PR1.7 promoter that supports high level gene expression in primate cones, rescued ERG cone responses in CNGB3 KO mice, but was less efficient than vectors containing the IRBP/GNAT2 promoter that does not support gene expression in primate cones (**Table 2** and **Figure 3**).

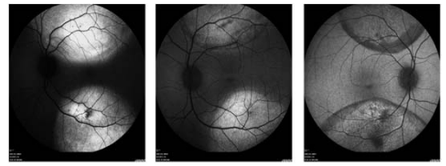

Figure 1 Fundus auto-fluorescence (FAF) images of primate retinas at 4 weeks post treatment. PR1.7 is a stronger promoter than PR2.1, and fluorescence with the IRBP/GNAT2 promoter is barely detectable even in overexposed images.

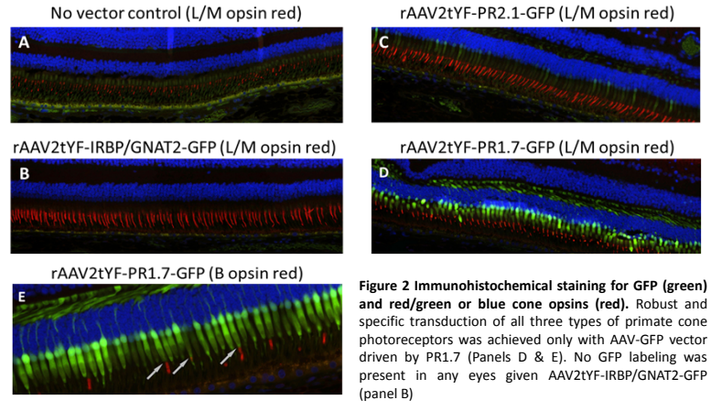

Figure 2 Immunohistochemical staining for GFP (green) and red/green or blue cone opsins (red). Robust and specific transduction of all three types of primate cone photoreceptors was achieved only with AAV-GFP vector driven by PR1.7 (Panels D & E). No GFP labeling was present in any eyes given AAV2tYF-IRBP/GNAT2-GFP (panel B)

Table 2 Cone ERG responses in CNGB3 KO mice 3 months after AAV-CNGB3 injection

Group	Vector	3 mo. Phot. b-wave ampl. (µV)			one-sided T-test (P-value)	
		un-inj. (n)	BSS (n)	AAV (n)	un-inj. vs treated	BSS vs treated
1	AAV5-PR2.1-hCNGB3	47.7 (4)	45.4 (6)	84.7 (10)	0.062	0.003
2	AAV5-PR2.1-hCNGB3co	36.6 (5)	47.7 (6)	39.8 (11)	0.381	0.245
3	AAV5-PR1.7-hCNGB3co	53.2 (7)	55.8 (5)	80.3 (10)	0.002	0.028
4	AAV5-IRBP/GNAT2-hCNGB3co	60.9 (5)	65.4 (7)	107.2 (10)	0.007	0.010
5	AAV2tYF-IRBP/GNAT2-hCNGB3co	83.9 (5)	63.7 (5)	83.4 (10)	0.487	0.131
6	AAV9-IRBP/GNAT2-hCNGB3co	47.5 (4)	65.0 (5)	110.8 (9)	0.0002	0.002
7	AAV5-IRBP/GNAT2-hCNGB3	50.8 (7)	61.5 (5)	117.9 (10)	0.00007	0.0002

RESULTS (CONTINUED)

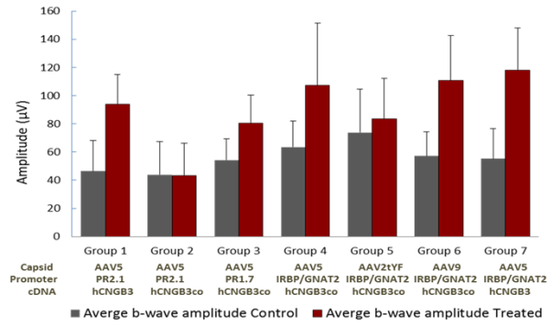

Figure 3 Cone ERG responses in CNGB3 KO mice 3 months after AAV-CNGB3 injection. B-wave amplitude elicited by 10-db stimulus was quantified and averaged for comparisons between AAV-treated eyes and control eyes (both vehicle injected and untreated eyes).

- Statistically significant improvements in cone ERG responses were observed in mice treated with AAV vectors containing non-codon-optimized hCNGB3 cDNA driven by PR2.1 or IRBP/GNAT2 (Groups 1 & 7), and with AAV vectors containing codon-optimized hCNGB3 cDNA (hCNGB3co) driven by PR1.7 or IRBP/GNAT2 promoters (Groups 3 to 6), but not with an AAV vector containing codon-optimized hCNGB3 cDNA driven by a PR2.1 promoter (Group 2). However, subretinal injection of the same batch of AAV5-PR2.1-hCNGB3co used in Group 2 achieved robust cone ERG rescue in CNGB3 mutant dogs (Komáromy et al, 2015).
- Between-group comparisons demonstrated greater cone ERG responses with non-codon optimized than codon-optimized hCNGB3 cDNA and with the IRBP/GNAT2 promoter than with PR2.1 or PR1.7 promoters.
- Vectors packaged in AAV5 and AAV9 capsids were equally effective at improving cone function in CNGB3 KO mice at 3 months post infection (Groups 4, 6 and 7). The vector packaged in AAV2tYF capsids also improved cone ERG rescue (Group 5) but, since a lower dose was used for the AAV2tYF vector, a direct comparison with vectors packaged in AAV5 or AAV9 capsids is not possible.

CONCLUSIONS

- This study confirms that a vector containing hCNGB3 cDNA that was optimized based on human codon usage, and the PR1.7 promoter that was optimized for robust expression in primate cones, are functional and able to improve cone ERG responses in this CNGB3 KO mouse model of ACHM, and emphasizes that an AAV vector with maximal efficiency in mice may not be optimally effective in primates

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