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Safety & Efficacy of AAV2tYF-PR1.7-CNGA3 in CNGA3-Deficient Sheep

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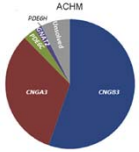
INTRODUCTION

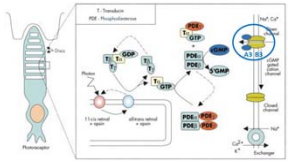
Congenital achromatopsia (ACHM) is an inherited autosomal recessive retinal disorder of cone photoreceptors characterized by markedly reduced visual acuity, extreme light sensitivity and absence of color discrimination [1]. Current management of patients with ACHM consists of the use of heavily tinted lenses, which reduces the photophobia that occurs outdoors and in normally illuminated public spaces, but does not address the underlying defects in visual acuity, color discrimination or daytime blindness. There is currently no specific therapy for this disease.

In Western populations, approximately 25% and 50% of ACHM cases are caused by mutations in the cone photoreceptor-specific cyclic nucleotide gated channel alpha subunit (CNGA3) or beta subunit (CNGB3) gene, respectively [2, 3]. In other populations, CNGA3-associated ACHM seems to be more prevalent [4, 5]. CNGA3 and CNGB3 are expressed in the cone outer segment disc membranes, and mutations in the homologous genes result in a loss of photoreceptor function in humans and in animals [6-9].

AGTC is developing AAV2tYF-PR1.7-CNGA3, a recombinant adeno-associated virus (AAV) vector expressing *CNGA3*, for treatment of CNGA3-related ACHM. The AAV vector contains a cone-specific promotor (PR1.7), a codon-optimized human CNGA3 cDNA, and an SV40 polyadenylation sequence packaged in an AAV2 capsid containing three tyrosine to phenylalanine (YF) mutations. Here we report results of a toxicology and efficacy study of this vector administered by subretinal injection in CNGA3-deficient sheep.



Genetic mutations causing achromatopsia



CNGA3 role in cone phototransduction

METHODS

Thirteen day-blind sheep divided into three groups of 4 or 5 animals each received a 0.5 mL subretinal injection in the right eye of AAV2tYF-PR1.7-CNGA3 at one of two dose levels or AAV5-PR2.1-hCNGA3, a vector previously shown to rescue cone photoreceptor responses [10]. The left eye received a 0.5 mL subretinal injection of vehicle (4 animals) or was untreated (9 animals). Toxicity assessment was based on mortality, clinical observations, ophthalmic examinations, electroretinogram (ERG), and clinical and anatomic pathology. CNGA3 expression was assessed by immunohistochemistry. Efficacy was assessed by cone ERG responses and maze navigation testing performed before, then 6 and 12 weeks after treatment.

Group	Number of animals	Vector	Dose level (vg/mL)	Volume	(vg/eye)
1	4	AAV5-PR2.1-hCNGA3	1.2×10^{12}	0.5 mL	6.0×10^{11}
2	4	AAV2tYF-PR1.7-hCNGA3	3.6×10^{11}	0.5 mL	1.8×10^{11}
3	5	AAV2tYF-PR1.7-hCNGA3	3.0×10^{12}	0.5 mL	1.5×10^{12}

RESULTS

Subretinal injections were generally well tolerated and not associated with systemic toxicity. Most animals had mild to moderate conjunctival hyperemia, chemosis and subconjunctival hemorrhage immediately after surgery that generally resolved by post-operative Day 7. Two animals treated with the higher dose of AAV2tYF-PR1.7-CNGA3 and three of the efficacy control group animals treated with AAV5-PR2.1-CNGA3 had microscopic findings of outer retinal atrophy with or without inflammatory cells in the retina and choroid, that were procedural- and/or test article–related. All vector-treated eyes showed improved cone-mediated ERG responses with no change in rod-mediated ERG responses (Figure 1). Behavioral maze testing under photopic conditions showed significantly improved navigation times and reduced numbers of obstacle collisions in all vector-treated eyes compared to their contralateral control eyes or pre-dose results in the treated eyes (Figure 2). hCNGA3 protein expression was detected by IHC in eyes treated with AAV2tYF-PR1.7-hCNGA3 (Figure 3).

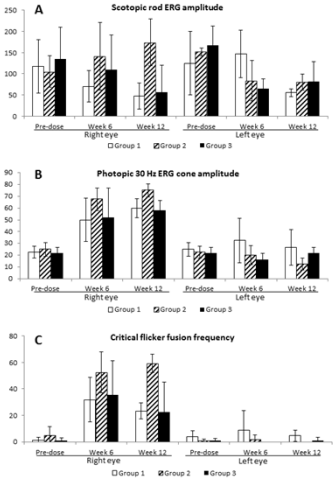


Figure 1. ERG responses in CNGA3-deficient sheep before treatment, and 6 and 12 weeks after treatment with an AAV vector expressing CNGA3. (A) Mean ∝ SD dark-adapted ERG b-wave amplitude (μV). (B) Mean ∝ SD light-adapted 30 Hz cone ERG cone amplitude (μV). (C) Mean ∝ SD light-adapted critical flicker fusion frequency (Hz).

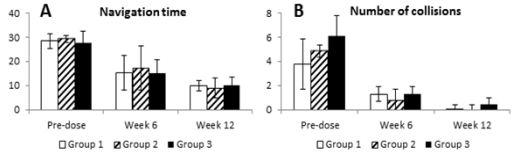


Figure 2. Maze navigation test results in CNGA3-deficient sheep before treatment, and 6 and 12 weeks after treatment with an AAV vector expressing CNGA3. (A) Mean ± SD time in seconds taken to move through a maze containing two obstacles. (B) Mean ± SD number of collisions during navigation through the maze. Each animal received a 0.5 mL subretinal injection to the right eye. All results were evaluated both by the investigator and an independent masked observer.

RESULTS (CONTINUED)

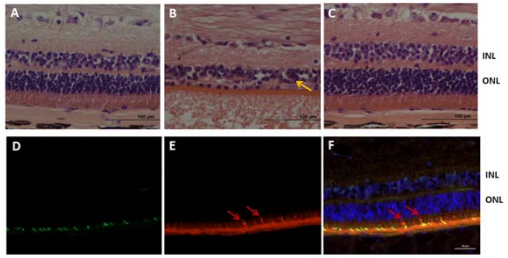


Figure 3. Representative images of histopathologic assessment and immunohistochemical staining. Severe retinal atrophy is shown in the vector treated area of the right eye of Animal ID #6404, Group 3 (Panel B) when compared to the control left eyes at the same location (mid dorsal) (Panel A). No retinal atrophy was observed in the untreated retinal area (outside of bleb) of the vector treated right eye (Panel C). The arrow in Panel B indicates disruption or thinning of inner and outer nuclear layers. L/M cone opsin (in green) and hCNGA3 protein expression (in red) was detected by IHC in eyes treated with AAV2tYF-PR1.7-hCNGA3 (Panels D, E, and F, Animal #5600, Group 2). Arrows indicate examples of hCNGA3 protein positively stained in cone photoreceptor inner segments (in red, Panel E), and alignment to the cone opsin positively stained (in green, Panel D) in the outer segment when double stained for cone opsin and hCNGA3 protein (yellow with merge, Panel F).

CONCLUSIONS

Subretinal injection of AAV2tYF-PR1.7-CNGA3 in CNGA3-deficient sheep was well tolerated. The outer retinal atrophy and retinal and choroidal inflammation observed in two animals treated with AAV2tYF-PR1.7-CNGA3 could be procedure- and/or test article-related. ERG and behavioral testing showed that a single injection restored photopic vision in all vector-treated eyes. These results support the use of AAV2tYF-PR1.7-CNGA3 in clinical studies in patients with ACHM caused by CNGA3 mutations. A Phase 1/2 clinical trial evaluating AAV2tYF-PR1.7-hCNGA3 is scheduled to begin in 2017.

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