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GENE THERAPY IN A SHEEP MODEL OF CNGA3 ACHROMATOPSIA

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Purpose: Mutations in the CNGA3 gene cause achromatopsia in humans. We identified a spontaneously-arising sheep model for this disease (Shamir et. al., Vet J. 185(2):130-7, 2010; Reicher et al., Genomics 95:101-4, 2010), and used this model to test the efficacy and safety of viral vector-mediated gene therapy.
Methods: Adeno-Associated Viral (AAV) vectors carrying the intact human or the intact mouse CNGA3 gene under control of the red-green opsin promoter were delivered to the subretinal space of affected day-blind sheep. Animals were electrophysiologically and behaviorally assessed preoperatively and up to 26 months postoperatively, and eyes were enucleated for molecular, histological and immunohistochemical (IHC) studies. Cone function was measured by electroretinography (ERG) under photopic conditions. Behavioral assessment included scotopic and photopic maze testing under standardized conditions. Passage times and number of collisions

were recorded. PCR was used to identify CNGA3 mRNA in retinas and IHC was performed to identify expression and localization at the protein level. Age-matched normal and day-blind sheep were similarly assessed as controls.

Results: Long-term (over two years) functional rescue was evident in day-blind animals treated by vectors carrying either the human or the mouse CNGA3 gene, with no evidence of adverse side effects. Passage time and number of collisions in the photopic maze improved dramatically, approaching the values of normal controls. Cone function as measured by ERG significantly improved, with increased amplitudes, shorter implicit times and higher flicker-fusion frequencies in treated eyes. PCR demonstrated the presence of the appropriate CNGA3 mRNA in the treated retinas. Histologically, retinal structure was well preserved, and IHC studies showed colocalization of the CNGA3 protein and red-green opsin in normal and day-blind treated eyes but not in day-blind control (untreated) retinas.

Conclusions: AAV-mediated gene therapy improves cone-mediated visual function in CNGA3 dayblind sheep, with a good safety profile. Long-term electrophysiological and behavioral improvement is evident, with expression of the CNGA3 gene at both the mRNA and protein levels in treated eyes. Our intent and hope is to ultimately apply similar treatment in human patients affected by achromatopsia.

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