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Development and Evaluation of Cone-Specific Promoters in Non-human Primates for Gene Therapy of Congenital Cone Diseases Including Achromatopsia

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Purpose: To select an optimal cone-specific promoter for gene therapy of congenital cone diseases by constructing a series of novel small cone-specific promoters and testing their function in mice and non-human primates (NHP).

Methods: PR1.1, PR1.5, and PR1.7 designed at AGTC are smaller versions of the human red cone opsin promoter (PR2.1). In dogs, PR2.1 has been shown to direct specific expression of GFP in L/M cones but not S cones (Komaromy et al., 2008). IRBP/GNAT2 is a small hybrid promoter shown to direct specific expression of GFP in L/M and S cones (Komaromy et al., 2013). The ability of PR1.1, PR1.5 and PR1.7 to target cones was initially evaluated by subretinal injection of AAV5-GFP vectors in mice, with the PR2.1 promoter serving as a control. PR1.7, PR2.1 and IRBP/GNAT2 were further evaluated in NHP. Six cynomolgus macaques received bilateral subretinal injections of AAV2tYF-GFP vectors containing the PR1.7, IRBP/GNAT2 or PR2.1 promoter, at a concentration of 5 x 10^11 vg/mL (2 blebs/eye, 1 x 10^11 vg/eye). At 12 weeks post injection retinal tissue was obtained for quantitative reverse transcriptase PCR (qRT-PCR) and immunohistochemistry (IHC). GFP expression was evaluated by fluorescence photography (in-life) and by qRT-PCR and IHC (post-life).

Results: In mice, vectors with PR1.7 and PR2.1 promoters achieved strong GFP expression in cones and relatively weaker expression in rods, the PR1.5 promoter achieved relative weak expression in both cones and rods, and the PR1.1 promoter achieved strong GFP expression in both cones and rods and weak expression in RPE cells. In NHP, the vector with the PR1.7 promoter achieved robust and specific targeting of GFP expression (Grade 3) in L/M and S cones in the subretinal bleb areas in all eyes but the PR2.1 promoter achieved variable staining of GFP (Grades 0, 1 or 2) in the subretinal bleb areas. In contrast to the observation from published studies in mice and dogs, the subretinal bleb areas in all NHP eyes receiving the vector with the IRBP/GNAT2-GFP promoter had no GFP labeling (Grade 0).

Conclusions: The PR1.7 promoter was significantly more efficient at directing expression in primate cones and is therefore preferred for gene therapy of congenital cone diseases. Results obtained from mice and dogs did not predict the results in NHP.

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