Product Development for Gene Therapy of XLRP: Evaluation of AAV Capsids, Promoters in Non-human Primates and Rational Design of RPGR ORF15 cDNA

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INTRODUCTION

X-linked retinitis pigmentosa (XLRP) caused by mutations in the retinitis pigmentosa GTPase regulator (RPGR) gene is a primary rod and cone disease affecting ~20,000 patients in the US and EU [1, 2]. Gene replacement therapy using adeno-associated virus (AAV) vectors for gene delivery is effective in preventing photoreceptor degeneration and preserving retinal structure and function in disease animal models [3, 4].

Important considerations in the design and evaluation of an AAV vector include the capsid, promoter, cDNA, and the species of animals [5]. AAV5-RPGR vectors containing either IRBP or GRK1 promoters provide structural and functional rescue in XLPR2 dogs [3], and AAV-GRK1-RPGR vectors packaged in capsids of various serotypes provide structural and functional rescue in RPGR knockout mice [4], but comparison of the efficiency and specificity of IRBP or GRK1 promoters in transducing and driving gene expression in non-human primate photoreceptors is lacking. In addition, obtaining a stable full-length RPGR-ORF15 cDNA expressing the intact and native RPGR protein is a challenge due to the complexity of the purine-rich region in the RPGR ORF15 exon.

In the present study, we evaluated two AAV capsids, AAV5 and an AAV2 capsid variant with 3 tyrosine to phenylalanine mutations (AAV2tYF), and two promoters, IRBP and GRK1, in non-human primates for their efficiency and specificity in driving GFP reporter gene expression in photoreceptors. The GRK1 promoter was significantly more efficient than the IRBP promoter at directing expression in primate photoreceptors and the AAV2tYF capsid was more effective in delivering the vector to primate photoreceptors. We also designed and tested a stable, full-length RPGR-ORF15 cDNA expressing the intact, native RPGR protein.

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.

The ability of IRBP and GRK1 promotors, and AAV2tYF and AAV5 capsids to target photoreceptors was evaluated in non-human primates by subretinal injection of AAV vectors containing a GFP expression cassette. Cynomolgus macaques received bilateral subretinal injections of 0.1 mL of AAV vector at a concentration of 5×10^{11} or 2×10^{12} vg/mL, and were followed for up to 12 weeks. GFP expression was evaluated by observation of fluorescence in-life and by qRT-PCR and immunohistochemistry post-mortem. The study design is summarized in **Table 1**.

A human RPGR cDNA was rationally designed based on the reference hRPGR cDNA sequence published in GenBank (variant C; NM_001034853). The redesigned hRPGR cDNA encodes a full length RPGR protein whose amino acid sequence is 100% identical to the published sequence. Synthesized hRPGR cDNA was first cloned in a conventional cloning vector and then into an AAV vector plasmid used for AAV production. Stability of the hRPGR cDNA sequence was confirmed by DNA sequencing at each step of cloning and also in multiple AAV preparations.

Table 1 NHP Study Design

Group	# of animals	Vector		Dose level		
		Left Eye	Right Eye	vg/mL	vg per eye	Volume
1	1	AAV2tYF-hGRK1-GFP	AAV5-hGRK1-GFP	5×10^{11}	1×10^{11}	2 × 0.1 mL
2	1	AAV5-hGRK1-GFP	AAV5-IRBP-GFP	5×10^{11}	1×10^{11}	2 × 0.1 mL
3	1	AAV5-IRBP-GFP	AAV5-hGRK1-GFP	5×10^{11}	1×10^{11}	2 × 0.1 mL
4	1	AAV5-hGRK1-GFP	AAV2tYF-hGRK1-GFP	5×10^{11}	1×10^{11}	2 × 0.1 mL
5	1	AAV2tYF-hGRK1-GFP	AAV5-IRBP-GFP	5×10^{11}	1×10^{11}	2 × 0.1 mL
6	1	AAV5-IRBP-GFP	AAV2tYF-hGRK1-GFP	5×10^{11}	1×10^{11}	2 × 0.1 mL
7	4	AAV2tYF-hGRK1-GFP	AAV5-hGRK1-GFP	5×10^{11}	5×10^{10}	1 × 0.1 mL
8	4	AAV2tYF-hGRK1-GFP	AAV5-hGRK1-GFP	2×10^{12}	2×10^{11}	1 × 0.1 mL

RESULTS

In non-human primates, AAV-GFP vectors packaged in either AAV2tYF orAAV5 capsids transduced photoreceptors efficiently, with the AAV2tYF capsid slightly more efficient. The GRK1 promoter directed robust and specific expression of GFP in photoreceptors, while GFP expression was barely detected in eyes injected with the AAV5-GFP vector driven by the IRBP promoter (**Figure 1** and **Figure 2**). The observation of minimal or no GFP expression in non-human primate eyes after subretinal injection of the AAV5-IRBP-GFP vector contrasts with the efficient GFP expression reported after subretinal injection of the AAV5-IRBP-GFP vector in dogs.

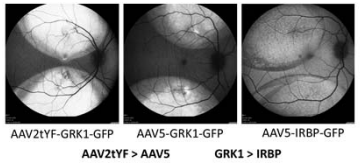

Figure 1 Fundus autofluorescence (FAF) images of primate retinas at 4 weeks post-treatment. AAV2tYF appears to be a preferred capsid and GRK1 promoter drives strong GFP expression. Fluorescence with the IRBP promoter is barely detectable even in overexposed images.

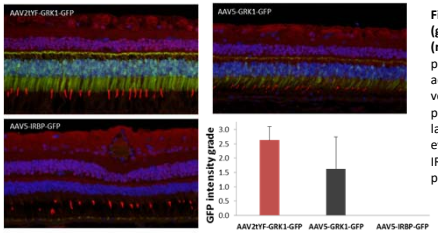

Figure 2 IHC staining for GFP (green) and red/green opsins (red). Specific transduction of primate photoreceptors was achieved only with AAV-GFP vectors driven by a GRK1 promoter (top panels). No GFP labeling was present in any eyes treated with an AAV5-IRBP-GFP vector (bottom panels).

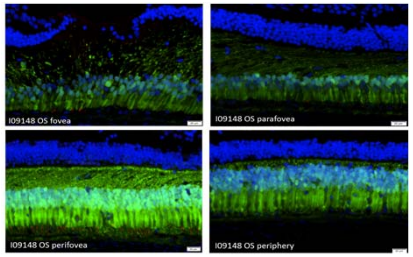

Figure 3 IHC staining for GFP (green) and red/green opsins (red) in eyes receiving high dose of AAV2tYF-GRK1-GFP vector. Robust and specific transduction of primate photoreceptors was achieved with an AAV2tYF-GFP vector driven by a GRK1 promoter in different locations of the retina (fovea, parafovea, perifovea, and periphery)

Robust and specific transduction of primate photoreceptors was achieved with an AAV2tYF-GFP vector driven by a GRK1 promoter in different locations of the treated area of retina, including the fovea that contains only cone photoreceptors, cone photoreceptor-rich parafoveal areas and rod photoreceptor-rich perifoveal and periphery areas. (**Figure 3**).

RESULTS (CONTINUED)

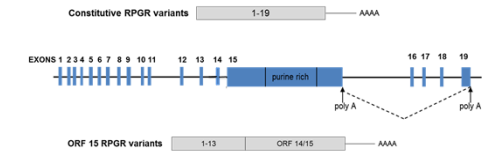

Figure 4 Open reading frames (ORFs) and mRNA isoforms. The mRNA for constitutive RPGR contains all 19 exons of the gene, while the mRNA for isoform RPGR^{ORF15} contains exons 1 to 15 and a large part of intron 15. Intron 15 is a purine-rich region that contains highly repetitive sequences coding for glutamate and glycine repeats.

Figure 5 Alignment of DNA sequences of the purine-rich region of RPGR. DNA sequences were obtained from plasmids and AAV vectors from multiple production runs with both plasmid transfection and HSV-based methods.

The redesigned full-length RPGR cDNA was readily cloned into a conventional cloning plasmid and a plasmid used for the production of AAV vectors, and was stable during large scale plasmid production.

DNA sequencing data obtained from multiple AAV vector preparations manufactured by both a plasmid transfection method and the AGTC proprietary herpes simplex virus based manufacturing system demonstrated no mutation in any of the large scale AAV preparations, including the material that will be used in a toxicology study (**Figure 5**).

CONCLUSIONS

The GRK1 promoter was much more efficient than the IRBP promoter at directing transgene expression in primate photoreceptors and the AAV2tYF capsid was more efficient than the AAV5 capsid in transducing primate photoreceptors.

A stable RPGR cDNA that encodes full-length, native RPGR protein was obtained. These results have informed the design of an AAV vector for treatment of patients with XLRP caused by RPGR mutations.

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For efficacy data of AAV-GRK1-RPGRco vector, Please visit Dr. Beltran's poster Board Number: 785 - C0074

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