Therapeutic Correction of an LCA-Causing Splice Defect in the CEP290 Gene by CRISPR/Cas-Mediated Genome Editing

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Leber Congenital Amaurosis caused by mutations in the CEP290 gene
• Early onset retinal degeneration characterized by severe loss of vision in the first years of life.
• Approximately 20% of all LCA caused by mutations in the CEP290 gene.
• Most common mutation in CEP290 is the IVS26 c.2991+1655 A>G mutation in intron 26 which creates a strong splice donor. Boye et al., PLOS ONE 2014

 cép290

Targeted Genomic Deletion
Quantification of targeted genomic deletion in primary patient fibroblasts transfected with Cas9 and gRNAs by droplet digital PCR

Targeted Deletion Corrects Splicing
Increased expression of wildtype transcript and decreased expression of mutant transcript in primary patient fibroblasts transfected with Cas9 and gRNAs as measured by qRT-PCR

Increased CEP290 Expression
Increased expression of wildtype CEP290 protein as measured by Western blot in patient fibroblasts transfected with Cas9 and gRNAs

Gene Editing Strategy
DNA Exon 26 Exon 27
mRNA Exon 26 Exon 27
Targetable DNA Chr1:42755713 Cr1:97268500
mRNA Exon 26 Exon 27

Off-target editing rate (% indels)

Specificity Profiling of Candidate gRNAs reveals few off-target sites
Specificity analysis performed by GUIDE-Seq and targeted amplicon sequencing in U2OS cells and primary fibroblasts.

Targeted Genomic Deletion

Targeted Deletion Corrects Splicing

Increased CEP290 Expression

Conclusions
This work supports the development of a gene-editing approach for therapeutic treatment of CEP290-associated disease caused by the IVS26 c.2991+1655 A>G mutation. The use of the S. aureus CRISPR/Cas9 system enables efficient packaging of the Cas9 gene, as well as two gRNA genes, into a single AAV vector and provides a method for delivery into patient photoreceptors.