QR-110 Restores CEP290 in p.Cys998X (c.2991+1655A>G) LCA 10 Models

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Introduction

• Leber’s Congenital Amaurosis 10 (LCA 10) is an ultra-rare orphan non-syndromic autosomal recessive disease of the retina due to mutations in the CEP290 protein in photoreceptor cells, leading to early and progressive loss of vision.

• p.Cys998X (also known as c.2991+1655A>G) is the most frequently occurring CEP290 mutation causing LCA 10, especially in European countries and the US, and generally results in the most severe phenotype1. Although prevalence rates vary in different geographic areas, p.Cys998X mutation is estimated to occur in approximately 2,000 patients in the world.

• LCA 10 is a 17-mer single stranded, fully phosphorothioated and 2′-O-methyl modified RNA oligonucleotide designed to target the p.Cys998X mutation. QR-110 masks the cryptic splice site created by the p.Cys998X mutation thus resulting in the production of wild-type CEP290.

• p.Cys998X mutation creates a cryptic splice donor site in intron 26 of the pre-mRNA which results in the inclusion of a 128-base sequence that includes a stop codon, and

• In wild-type cells, splicing results in correct transcripts producing a full-length mRNA and protein, whereas p.Cys998X LCA 10 models result in the inclusion of a cryptic splice site in intron 26 of the pre-mRNA which results in the inclusion of a 128-base sequence that includes a stop codon, and

• Expression levels relative to control cells are shown except for mutant wild-type transcripts which are represented relative to mock treated. Error bars show mean with SEM. Three biological experiments.

• In LCA 10 fibroblasts carrying the p.Cys998X mutation in homozygous QR-110 treatment in vitro restored CEP290 protein levels to that of control cells. This effect was specific for QR-110 as scrambled control oligonucleotide with the same chemistry and length did not alter the protein levels. CEP290 protein restoration was accompanied by increased wild-type mRNA levels and decreased mutant mRNA.

Results

Objectives


Materials & Methods

Primary fibroblast cell lines were generated from skin biopsies of LCA 10 patients carrying the p.Cys998X mutation in homozygosity or compound heterozygosity. Control cell lines were generated from healthy volunteers. LCA 10 patients carrying the p.Cys998X mutation in homozygosity or compound heterozygosity. Control cell lines were generated as described in Parfitt et al. 2016.

CEP290 mRNA and protein levels in untreated patient fibroblast cell lines at the baseline. Two cell lines for each genotype were used and seed age, value per plate of the CEP290 protein. Cell culture medium was supplemented with 10% fetal bovine serum and 1% penicillinn-streptomycin. CEP290 protein levels were measured by ELISA with mouse monoclonal anti-CEP290 antibody. The mean cilia length of 1.09 μm, which is a similar cilia incidence and length to control wild-type fibroblasts treated with different amounts of QR-110 at 24 h. In line with mRNA data, at 10 µM QR-110 concentration significant increase in not only p.Cys998X allele but also wild-type allele was observed.

Conclusion

QR-110, a promising investigational therapy for LCA 10, proved the capacity to rescue molecular and phenotypic defects associated with p.Cys998X as demonstrated by

• mRNA and protein profile in LCA 10 patient fibroblasts carrying the p.Cys998X mutation in homozygosity and compound heterozygosity,

• mRNA profile in LCA 10 patient derived optic cups, carrying the p.Cys998X mutation in homozygosity and compound heterozygosity,

• Functional protein restoration, as indicated by increased number of ciliated cells and length of cilia in optic cups.

References


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