QR-110 Treatment for Leber’s Congenital Amaurosis Type 10: Restoration of CEP290 Levels in Optic Cup and Fibroblast Models

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Introduction

• Leber’s congenital amaurosis Type 10 (LCA10), caused by mutations in CEP290, is the most frequent form of LCA (~5% and generally results in the most severe LCA phenotype. p.Cys998X (also identified as c.2991G>A/V997X) is the most frequently occurring CEP290 mutation, especially in European countries and the US4.

• This mutation creates a cryptic splice donor site in exon 26 of the pre-mRNA which results in the inclusion of an aberrant exon of 128 bases into the CEP290 transcript mRNA. This cryptic exon introduces a premature stop codon leading to truncated CEP290 protein. However, a fraction of CEP290 pre-mRNA with the p.cys998x mutation is also spliced into wild type mRNA which translates into a wild type protein. The CEP290 protein is involved in the formation and stability of the connecting cilium in photoreceptors which facilitates the transport of proteins to the outer segment. When CEP290 is absent, protein trafficking is perturbed leading to degeneration of the photoreceptor cells.

• QR-110 is an anti-sense, single stranded, fully phosphorothioate and 2'-O-methyl modified 21mer oligonucleotide designed as a dosing modifying therapy for patients with LCA due to the p.Cys998X mutation in CEP290.

• QR-110 targets the splicing mutation in CEP290 (c.2991G>A) through a mechanism of splice correction by which it skips the inclusion of the cryptic exon and thus restoring the open reading frame of CEP290.

Results

QR-110 increases wild-type CEP290 mRNA and protein levels in LCA10 homogenous fibroblasts

In LCA10 fibroblasts carrying the p.Cys998X mutation in homozygosity QR-110 treatment in vitro restored CEP290 protein levels to that of control cells. Error bars show mean with SEM evaluated from three biological experiments. This result was also evident in CEP290 transcript level where QR-110 increased wild type mRNA levels and decreased mutant mRNA. Moreover, the effect was specific for QR-110 as scrambled control oligonucleotide with the same chemistry did not alter the mRNA or protein levels.

QR-110 increases wild-type CEP290 mRNA and protein levels in LCA10 compound heterozygous fibroblasts

In LCA10 fibroblasts carrying the p.Cys998X mutation in compound heterozygosity QR-110 treatment in vitro restored CEP290 protein levels to 50% of control cells. Error bars show mean with SEM evaluated from three biological experiments. This effect is also evident in CEP290 transcript level where QR-110 increased wild type mRNA levels and decreased mutant mRNA. Moreover, this effect was specific for QR-110 as scrambled control oligonucleotide with the same chemistry did not alter the mRNA or protein levels.

Generation of LCA patient iPSC-derived optic cups

To investigate the localization of QR-110, optic cups were treated with 10 μM 6-FAM-QR-110 for 48 hours and then imaged and stained with mAb to identify the surrogate outer nuclear layer. Live (unfixed) optic cups were imaged using fluorescence two-photon microscopy. Inset is higher magnification of 6-FAM-QR-110 (green) channel showing nuclear and perinuclear localization of QR-110.

QR-110 reaches surrogate outer nuclear layer in control iPSC-derived optic cups

In LCA10 fibroblasts carrying the p.Cys998X mutation in homozygosity and compound heterozygosity, the p.Cys998X population of CEP290 homogenous and compound heterozygous fibroblasts was increased in wild type CEP290 mRNA levels and decreased the mutant mRNA levels in a dose dependent manner.

Materials & Methods

Primary fibroblast cell lines were generated from skin biopsies of LCA10 patients carrying the p.Cys998X mutation in homozygosity or compound heterozygosity.

Conclusion

• QR-110 restored CEP290 mRNA and protein levels in primary LCA10 fibroblasts carrying the p.Cys998X mutation in homozygosity to approximately 100% and in compound heterozygosity to approximately 50% as is expected when there is only one p.Cys998X allele.

• QR-110 readily enters the LCA10 patient iPSC-derived optic cups without requiring a transfection reagent and restores CEP290 wild-type mRNA in a dose dependent manner.

References


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