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

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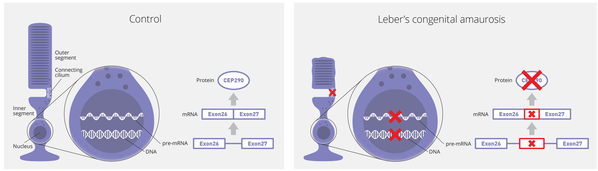
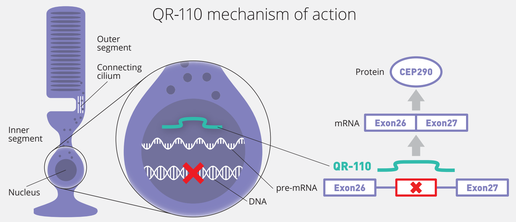
Introduction

- Leber's congenital amaurosis Type 10 (LCA10), caused by mutations in *CEP290*, is the most frequent form of LCA (~15%)[1] and generally results in the most severe LCA phenotype. p.Cys998X (also identified as c.2991+1655A>G) is the most frequently occurring *CEP290* mutation, especially in European countries and the US[2-4].
- This mutation creates a cryptic splice donor site in intron 26 of the pre-mRNA which results in the inclusion of an aberrant exon of 128 bases into the *CEP290* (mutant) 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 photoreceptors.



- QR-110 is an antisense, single stranded, fully phosphorothioate and 2'-O-methyl modified RNA oligonucleotide designed as a disease modifying therapy for patients with LCA10 due to the p.Cys998X mutation in *CEP290*.
- QR-110 targets the splicing mutation of the *CEP290* 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*.



Objectives

- **Assess if QR-110 restores CEP290 wild-type mRNA and protein levels in primary LCA10 patient fibroblasts carrying the p.Cys998X mutation in homozygosity and compound heterozygosity.**
- **Assess if QR-110 restores *CEP290* wild-type mRNA in LCA10 patient iPSC-derived optic cups carrying the p.Cys998X mutation in homozygosity.**

Materials & Methods

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

Generation of LCA10 patient **iPSC-derived three-dimensional optic cups** was described in Parfitt et al. 2016. Optic cups were generated from LCA10 patient cells carrying the p.Cys998X mutation in homozygosity.

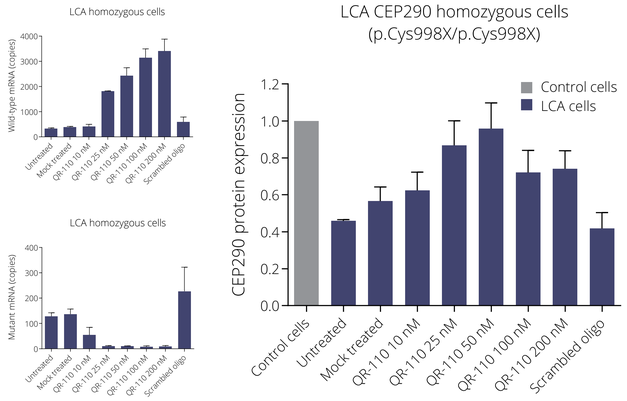
Fibroblast were **transfected** with QR-110 using polyethyleneimine. Optic cups were treated with QR-110 gymnotically (i.e. without a transfection reagent).

Quantification of *CEP290* wild-type and mutant mRNA was performed by isoform specific TaqMan droplet-digital PCR (dd-PCR) assays. Quantification of *CEP290* levels in optic cups was done by end-point PCR. Data was normalized to the housekeeping gene expression.

CEP290 protein levels were analyzed by Western blotting method. Data was normalized using protein load.

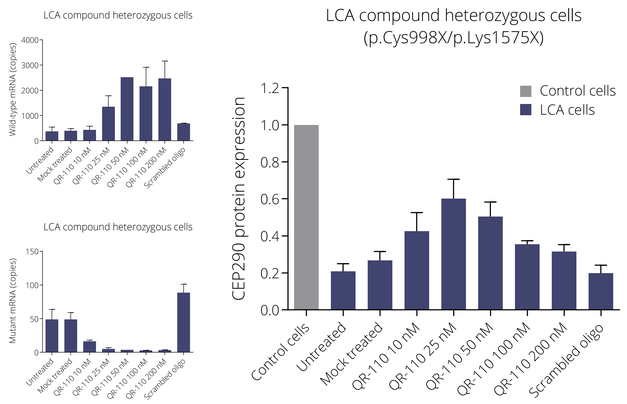
Results

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



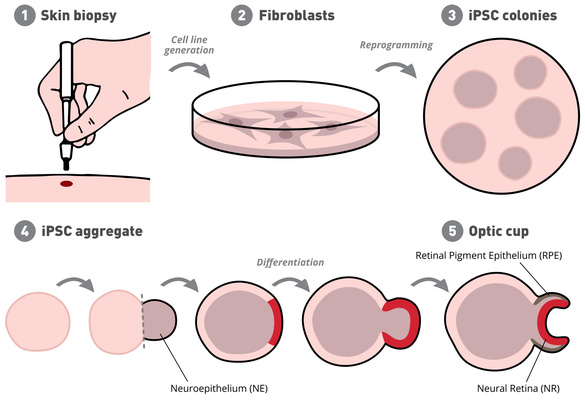
In LCA10 fibroblasts carrying the p.Cys998X mutation in homozygosity QR-110 treatment *in vitro* restored CEP290 protein levels to that of control cells. Error bars show mean with SEM calculated from three biological experiments. This effect was also evident at *CEP290* transcript level where QR-110 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.

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

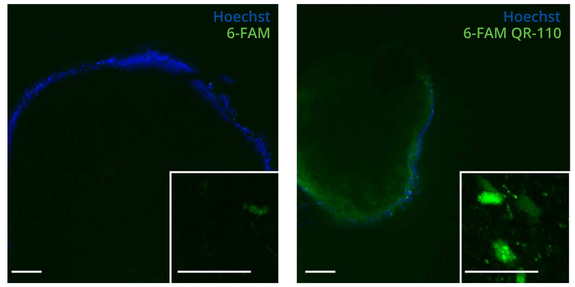


In LCA10 fibroblasts carrying the p.Cys998X mutation in compound heterozygosity QR-110 treatment *in vitro* restored CEP290 protein levels to ~50% of control cells. Error bars show mean with SEM calculated from three biological experiments. This effect is also evident at *CEP290* transcript level where 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

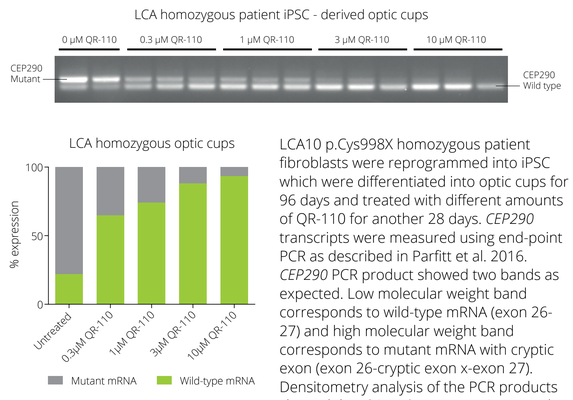


QR-110 reaches surrogate outer nuclear layer in control iPSC-derived optic cups



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

QR-110 increases wild-type *CEP290* mRNA levels in LCA10 patient iPSC-derived optic cups



LCA10 p.Cys998X homozygous patient fibroblasts were reprogrammed into iPSC which were differentiated into optic cups for 96 days and treated with different amounts of QR-110 for another 28 days. *CEP290* transcripts were measured using end-point PCR as described in Parfitt et al. 2016. *CEP290* PCR product showed two bands as expected. Low molecular weight band corresponds to wild-type mRNA (exon 26-27) and high molecular weight band corresponds to mutant mRNA with cryptic exon (exon 26-cryptic exon x-exon 27). Densitometry analysis of the PCR products showed that QR-110 treatment increased wild-type *CEP290* mRNA levels and decreased the mutant mRNA levels in a dose dependent manner.

Discussion

We have previously shown that QR-110 increases CEP290 mRNA and protein levels in primary LCA10 patient fibroblast cells carrying the p.Cys998X in homozygosity and reaches the outer nuclear layer, the target site for therapeutic benefit, following a single intravitreal injection in both mice and rabbits. We now show that QR-110 can restore CEP290 mRNA and protein levels in primary LCA10 compound heterozygous patient cells and homozygous optic cups in a dose dependent manner. In the absence of an animal model, iPSC-derived optic cups represent the closest analogue. Moreover, we showed that QR-110 enters the nuclei of the outer nuclear layer and corrects the splicing defect without requiring a transfection reagent.

Vast majority of LCA10 patients are compound heterozygous and unlike the p.Cys998X mutation other frameshift mutations are not expected to produce any wild-type *CEP290*. Therefore these patients represent the most severely affected population. Hence the ability to increase CEP290 levels in compound heterozygous patients broadens the target population of *CEP290* p.Cys998X splice correction therapies.

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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Acknowledgements

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