QR-1011 CORRECTS SPLICING IN THE STARGARDT DISEASE TYPE 1-CAUSING VARIANT *ABCA4* c.5461-10T>C

Melita Kaltak[1,2], Petra de Bruijn[1], Sang-Eun Lee[3], Davide Piccolo[3], Kalyan Dulla[1], Rob W. J. Collin[2], Michael E. Cheetham[3], Gerard Platenburg[1], Jim Swildens[1]

[1]ProQR Therapeutics, Leiden, the Netherlands | [2]Radboud University Medical Center, Nijmegen, the Netherlands | [3]University College London, London, UK
Contact: mkaltak@proqr.com

Radboudumc
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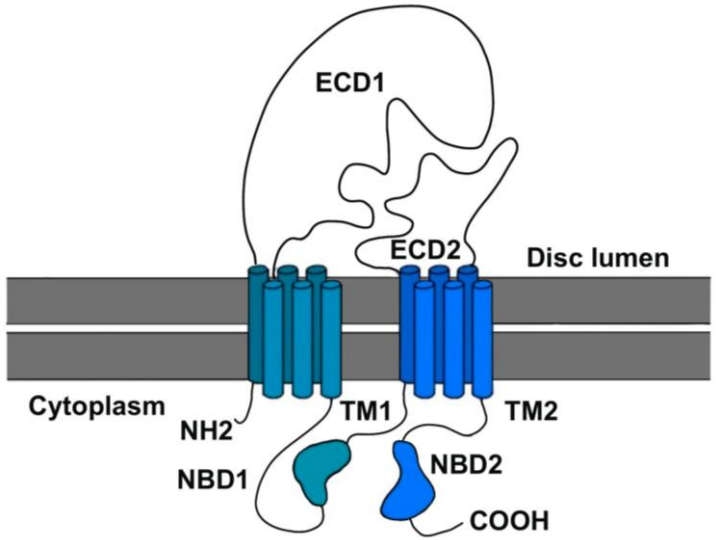
Autosomal recessive Stargardt disease (STGD1)

Clinical features caused by mutations in ABCA4

- The most commonly occurring maculopathy that leads to progressive degeneration of the retina
- More than **2000 disease causing-mutations** in the *ABCA4* gene*

ATP-Binding Cassette subfamily A member 4 protein (ABCA4)



TM: transmembrane domain

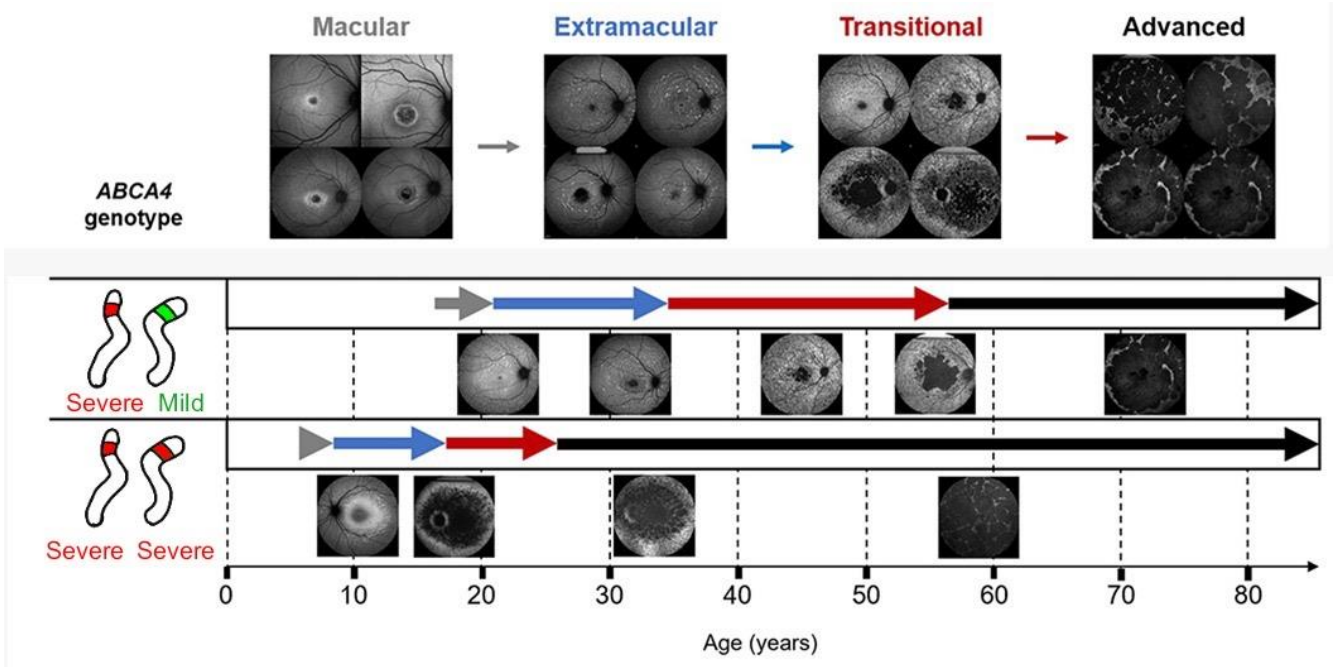
ECD: extracellular cytoplasmatic domain

NBD: nucleotide binding domain

*Cornelis et al, *AJHG*, 2022

STGD1-causing variant *ABCA4* c.5461-10T>C

- ***ABCA4* c.5461-10T>C is the most common severe STGD1-causing variant,** affecting ~7000 individuals in the Western World*
- Homozygous patients can reach the state of complete blindness by 2nd decade of life**



Classic STGD1

Childhood-onset STGD1

Adapted from: *Cremers et al.,Progress in retinal and eye research, 2020*

*Cornelis et al, *AJHG*, 2022 | **Sangermano et al, *Ophthalmology*, 2016

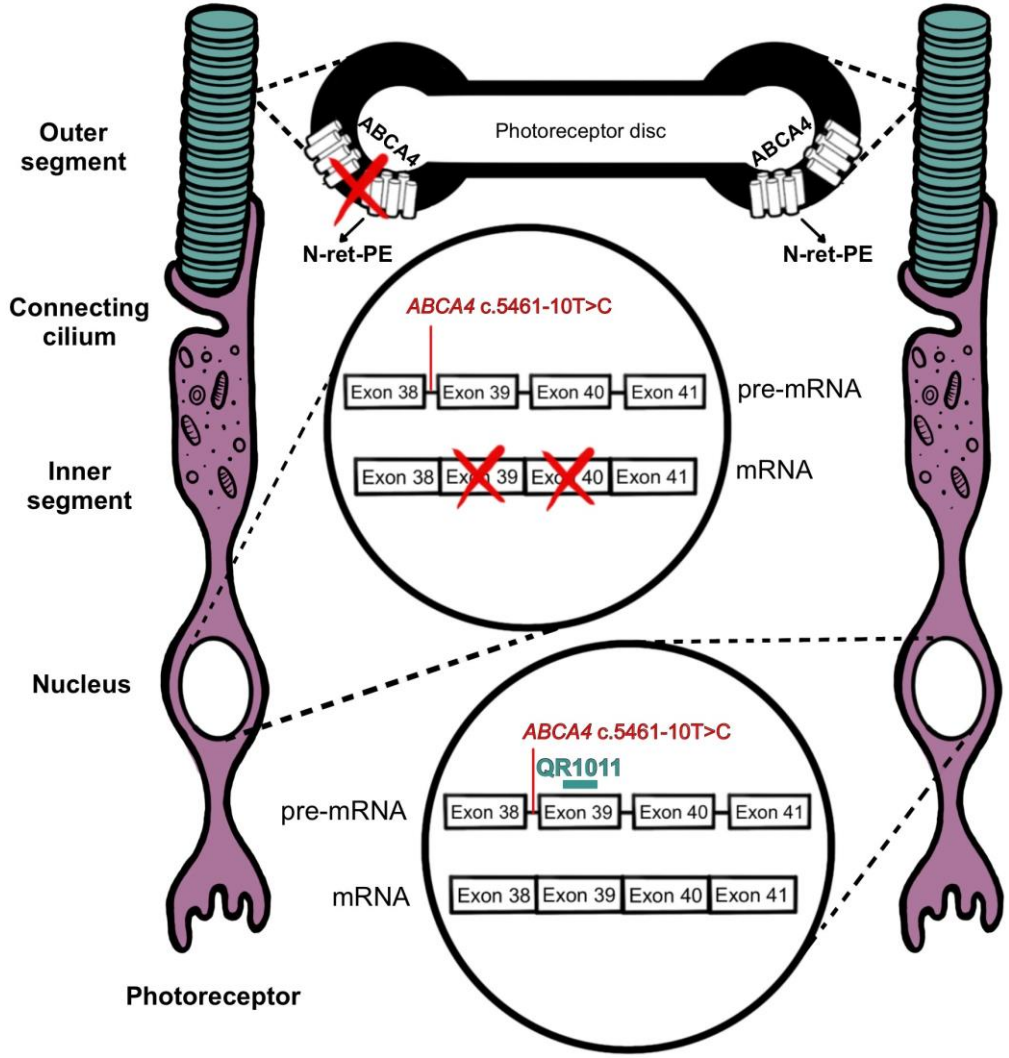
QR-1011: antisense oligonucleotide (AON) treatment for splicing correction

- ***ABCA4* c.5461-10T>C** leads to *ABCA4* isoforms lacking either exon 39 or exons 39 and 40

  ➡ shifts in open reading frame and premature stop codons

Objectives

- **Select splice-correcting AONs in *ABCA4* c.5461-10T>C midigene-transfected cells**
- **Assess transcript and protein restoration with best AON candidates in retinal organoids (ROs)**

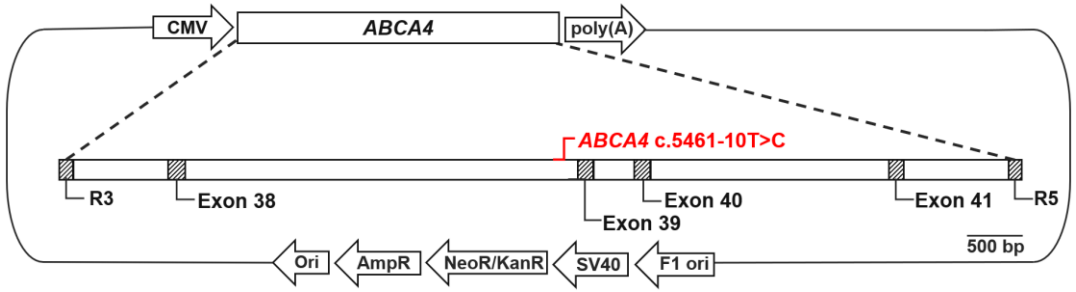


AON selection in midigene model

Splicing restoration in midigene-transfected HEK293 cells

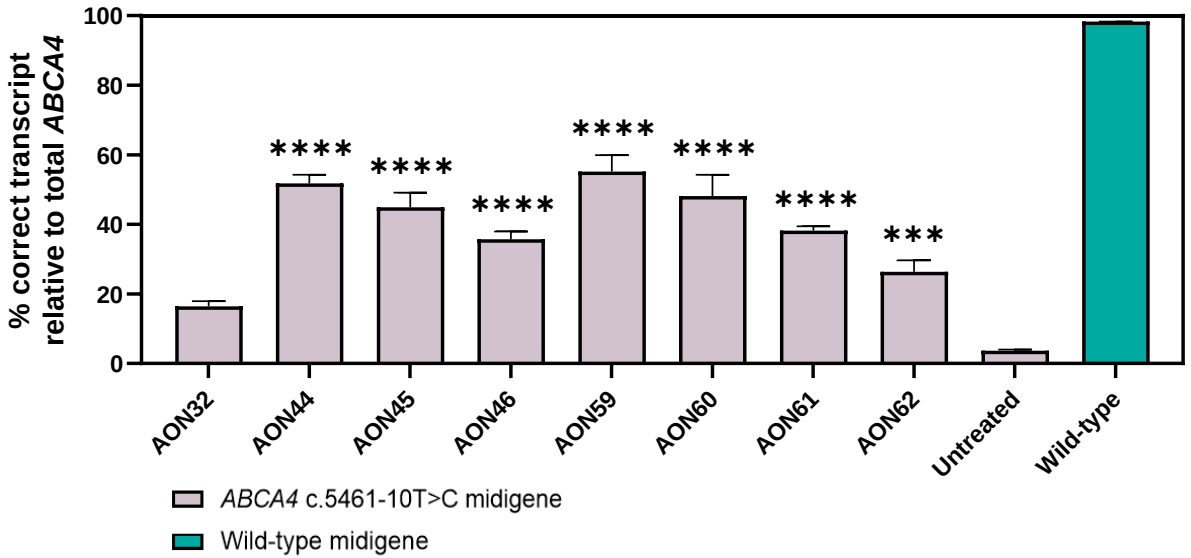
AON treatment of midigene-transfected HEK293 cells

→ isoform-specific analysis with quantitative PCR



Midigene model

- Clear splicing modulation activity with all AONs
- Restoration of **correct transcript achieved at >50%** after treatment
- Best splicing correctors were assessed in retinal organoids

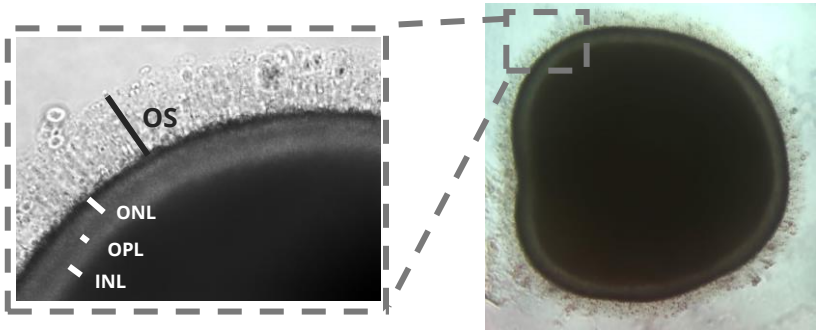


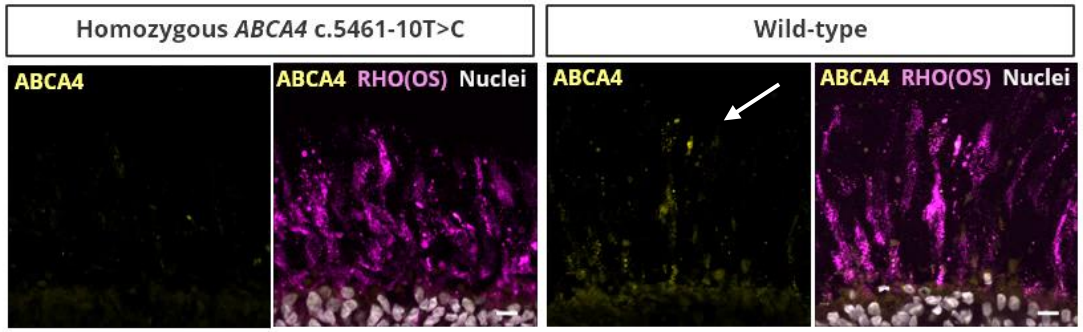
Data are shown as mean ± s.e.m. ***p<0.001, ****p<0.0001

Retinal organoids (ROs) as model for STGD1

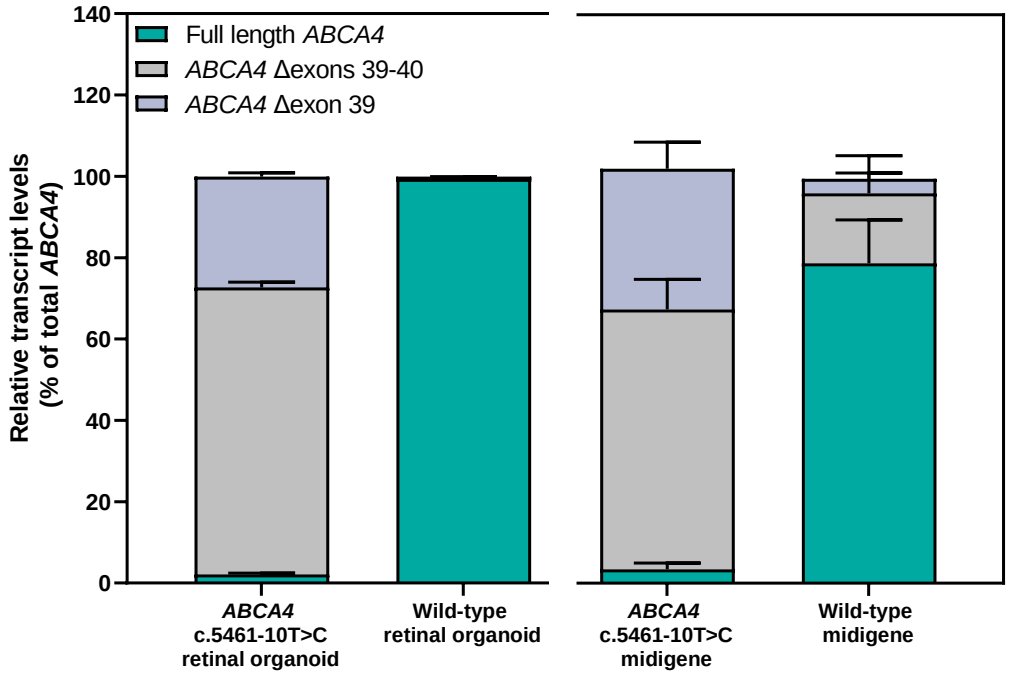
Identification of ABCA4 protein and transcripts in CRISPR-Cas9 homozygous c.5461-10T>C retinal organoids

- **Retinal organoids resemble the retina**





- **High similarities in RNA content between midigene and ROs**

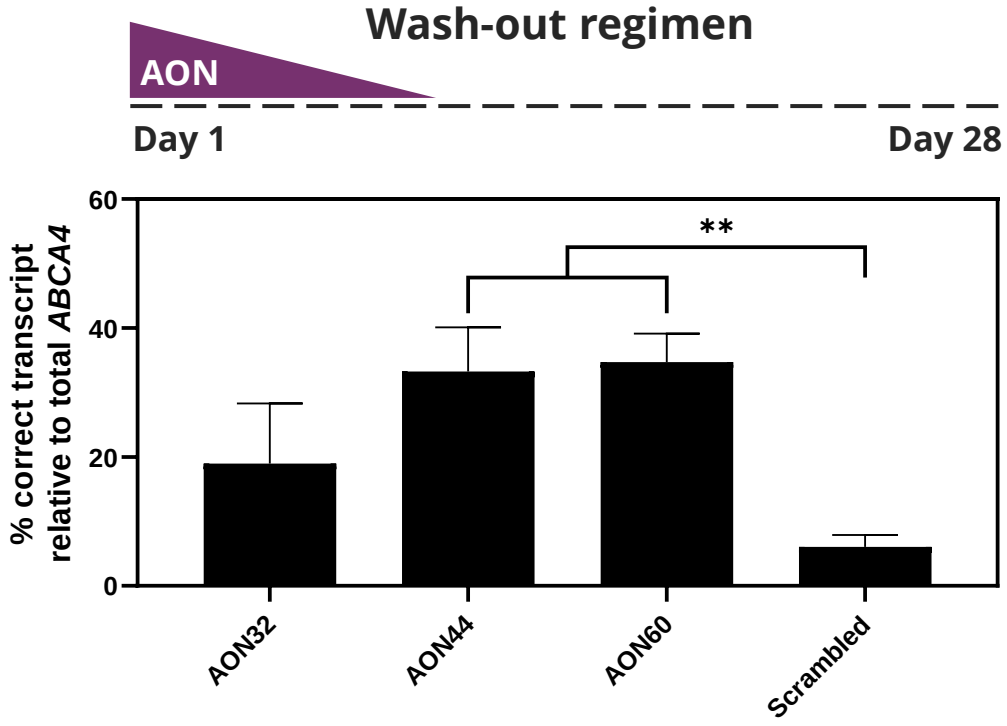


Data are shown as mean ± s.e.m.

Selection of lead AON in CRISPR-Cas9 edited ROs

Wash-out AON treatment with 3 different AON sequences

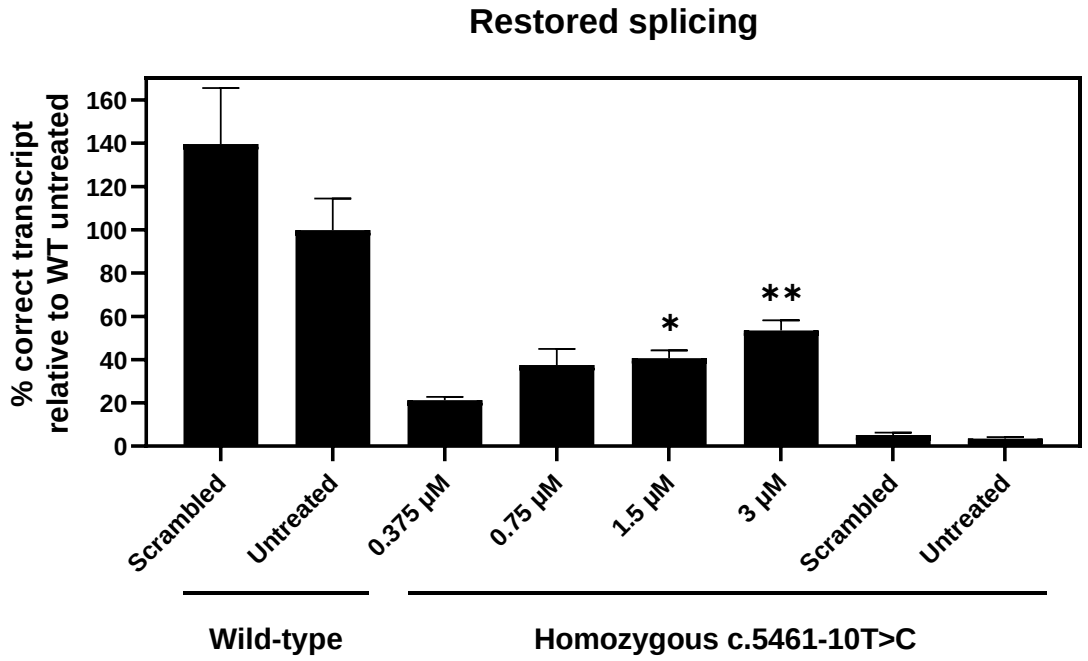
- Gene edited ROs homozygous for c.5461-10T>C treated with 3 AONs at **1.5 µM concentration**
- **Wash-out regimen 4 weeks long**
- RNA analysis detected **>30% of correct transcript**
- In the control oligo-treated group the full length *ABCA4* isoform was present at a low percentage

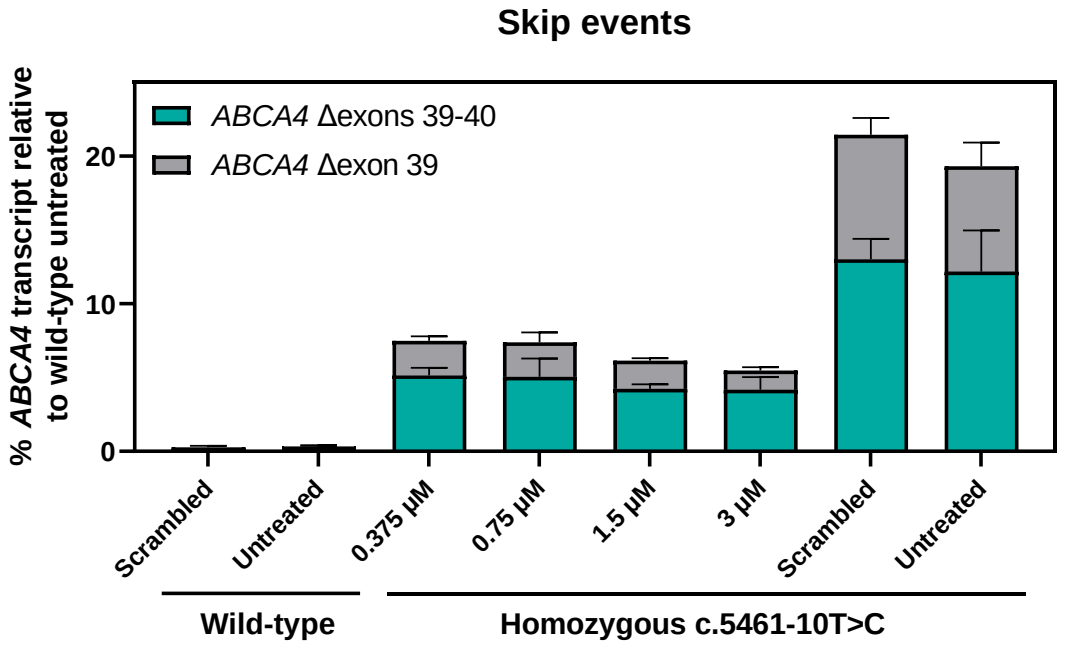


Data are shown as mean ± s.e.m., **p≤0.01

Characterization of QR-1011 in patient-derived ROs

- Organoids differentiated from patient-derived IPSCs homozygous for c.5461-10T>C
- QR-1011 applied at 4 different clinically relevant concentrations in an 8-week long wash-out treatment
- **53% of wild-type level *ABCA4* transcript measured after 3 µM treatment**
- All AON concentrations reduced the levels of aberrant isoforms



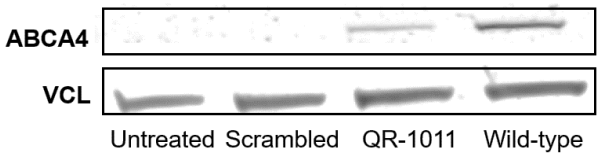


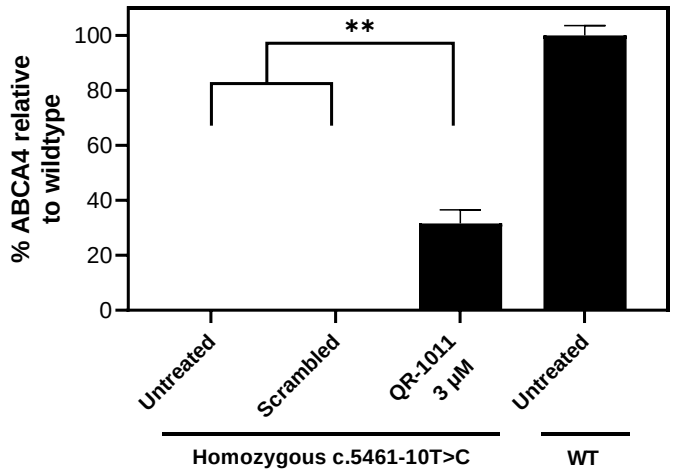
Data are shown as mean ± s.e.m., *p≤0.05, **p≤0.01

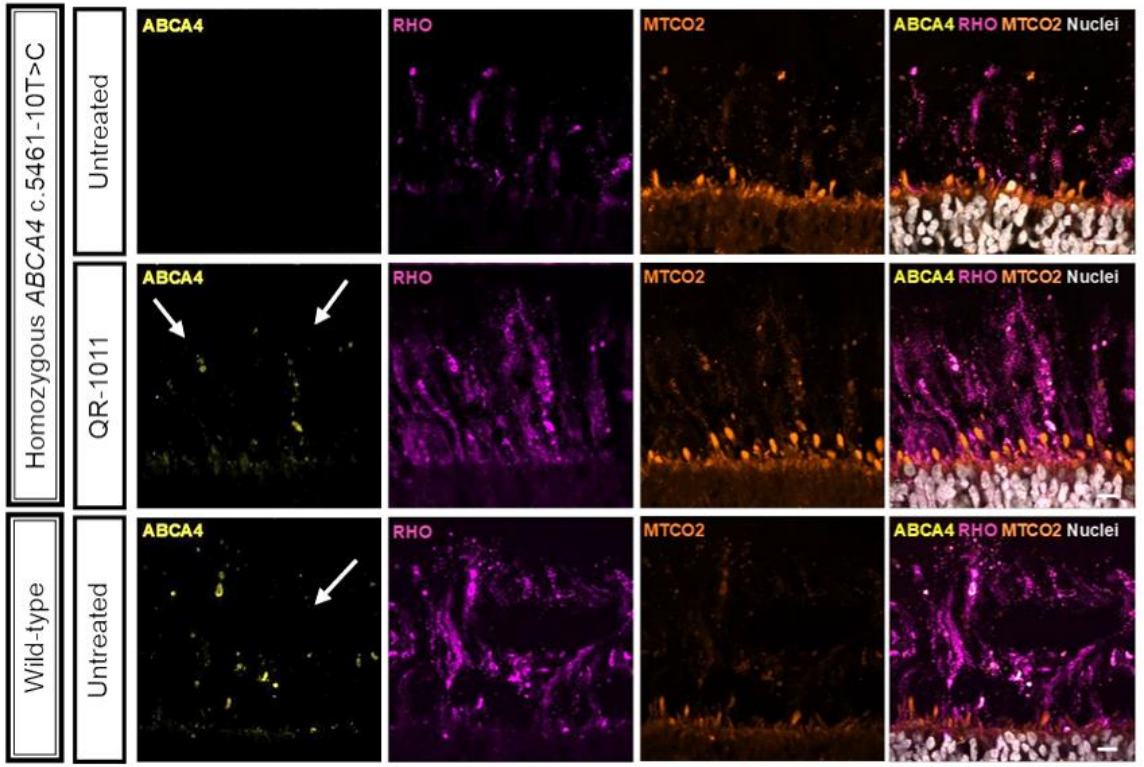
QR-1011 rescues ABCA4 protein expression

Quantification and localization of ABCA4 protein in patient-derived ROs

- **AON treatment generated >30% of wild-type protein** in c.5461-10T>C organoids
- No protein found in untreated and scrambled-AON groups
- Rescued ABCA4 co-localized with rhodopsin (RHO) in the outer segments (OS) of photoreceptor cells







Data are shown as mean ± s.e.m., **p≤0.01

Conclusions

- QR-1011 corrects mis-splicing due to *ABCA4* c.5461-10T>C
- Similar AON effect in midigene model and ROs
- Clinically relevant doses achieved significant restoration of wild-type transcripts and protein in patient-derived ROs

QR-1011 shows potential as therapy for STGD1 caused by the *ABCA4* c.5461-10T>C mutation.

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