



# QRX-421

Antisense oligonucleotide targeting  
Exon 13 mutations in the USH2A gene  
for treatment of non-syndromic RP  
and RP in Usher syndrome type II

# RP associated with Usher Syndrome

*Genetic cause of combined deafness and blindness*

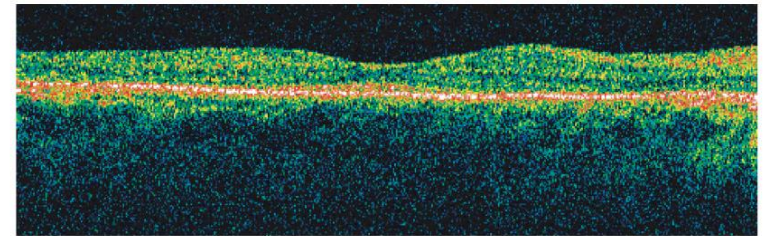
Symptoms: Pale optic nerve, thin vessels



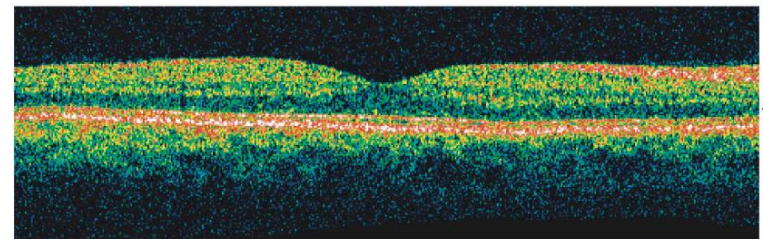
USH2A

Normal

Degeneration of Outer Nuclear Layer (ONL)



USH2A



Normal

←ONL

*From Sandberg et al. 2008*

# Targeting Strategy

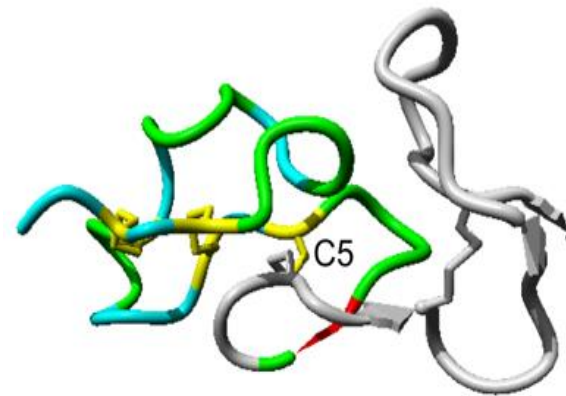
## *In frame removal of Ex13 (642nt)*

Many pathogenic mutations in exon 13, including the two most common variants

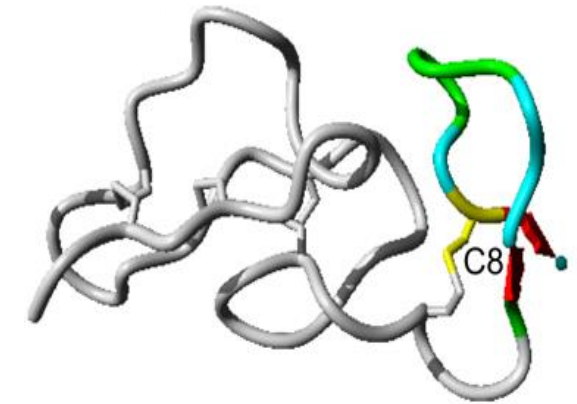
- G2299del (frameshift), causing Usher
- G2276T (Cys759Phe), causing RP

mRNA remains in frame (removal of 642 nt) Removes 4 laminin-EGF repeat domains (214 aa)

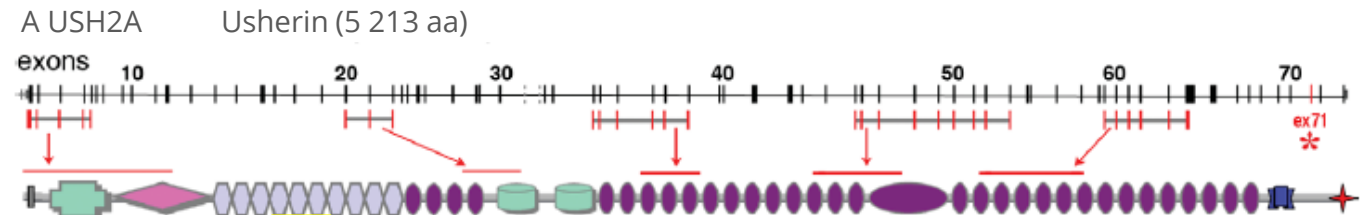
Strict requirement to show truncated (exon-13 deleted) mRNA leads to functional protein



Laminin-type EGF like domain 4

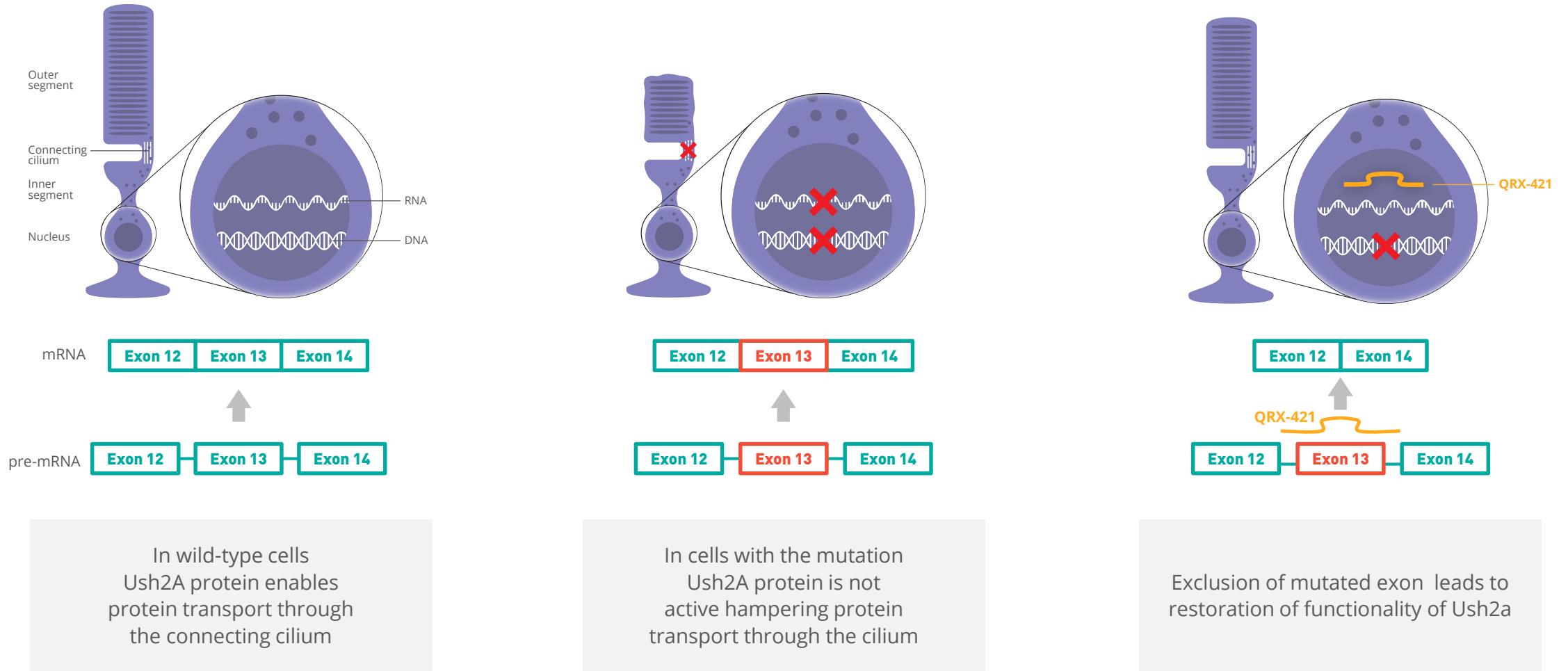


Laminin-type EGF like domain 8



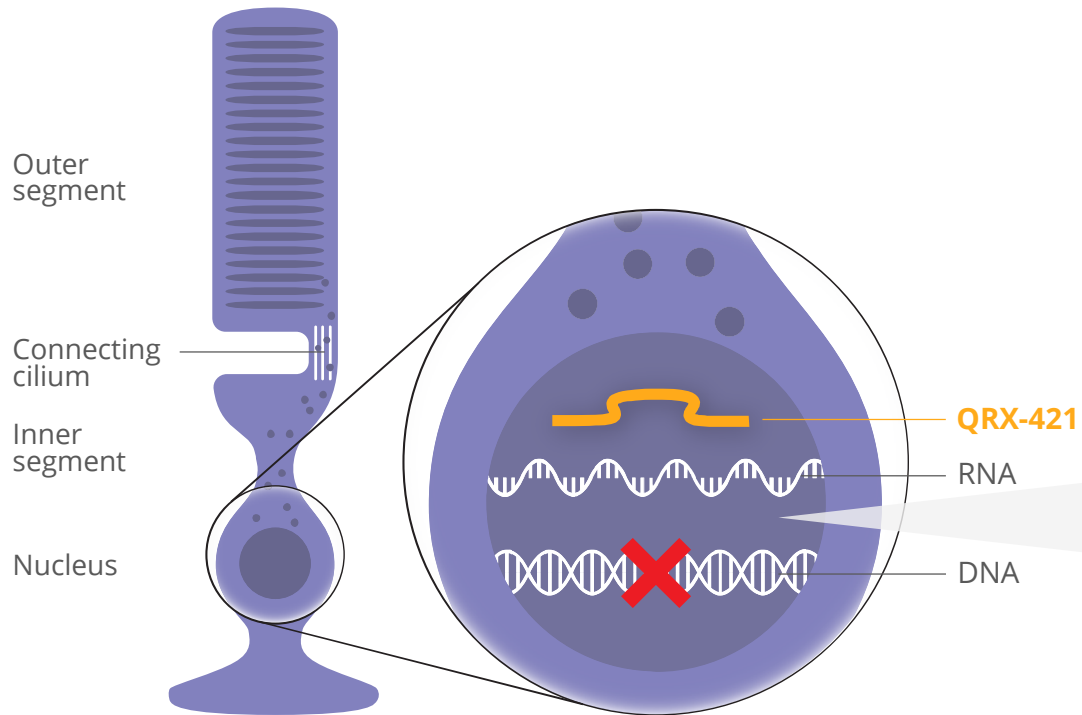
# QRX-421 for RP in Usher Syndrome

*USH2A* exon 13 splice correction



# QRX-421 for RP in Usher Syndrome

*USH2A* exon 13 splice correction



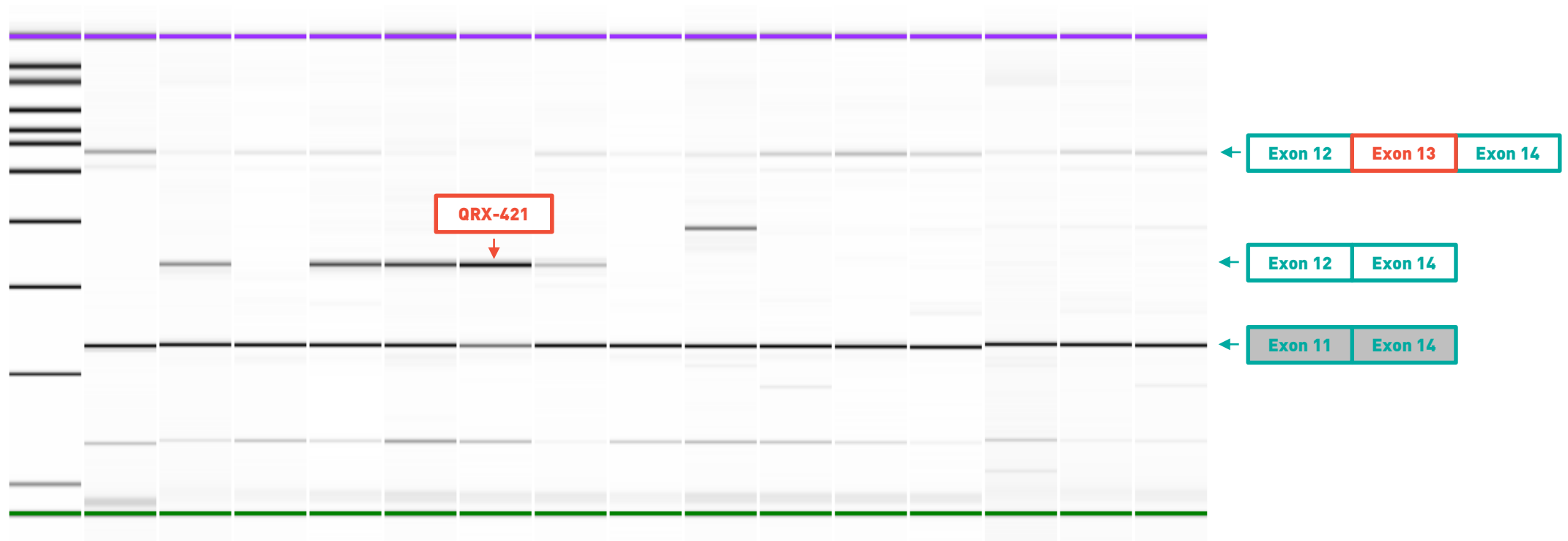
## QRX-421:

- Single stranded 21-mer RNA oligonucleotide
- P=S and 2'O-Me chemically modified for stability and uptake
- Designed to target *USH2A* exon 13 mutations

# QRX-421 mediates exon 13 skip in vitro

*Double exon-skipping in Ush2A is also present in wild-type cells*

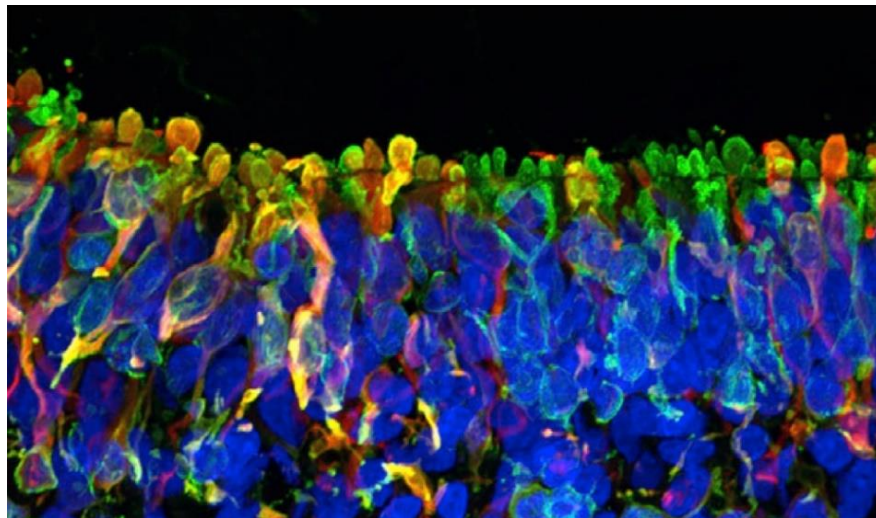
Wild type retinoblastoma cells treated with various AON's



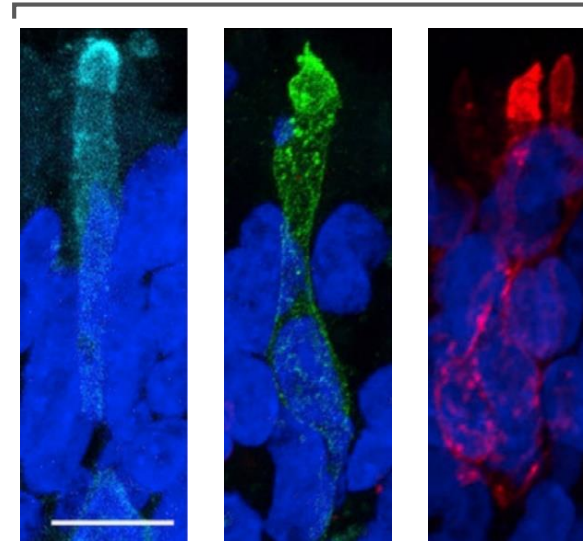
# Patient-derived iPSC optic cups

*Optic cup is an organoid model containing differentiated photoreceptor cells*

Recoverin cone-arrestin



Details

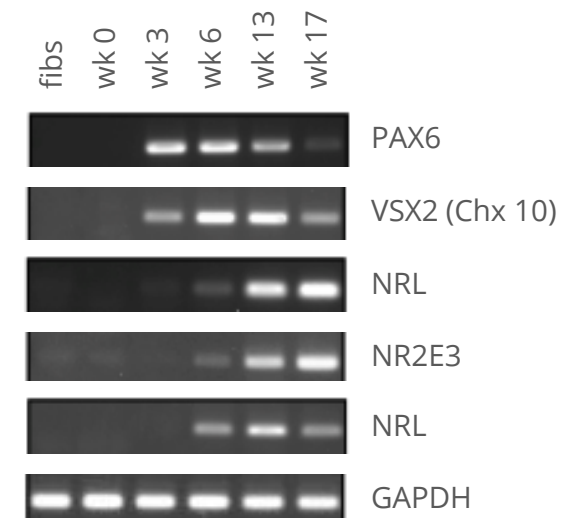


Rhodopsin

L/M-opsin

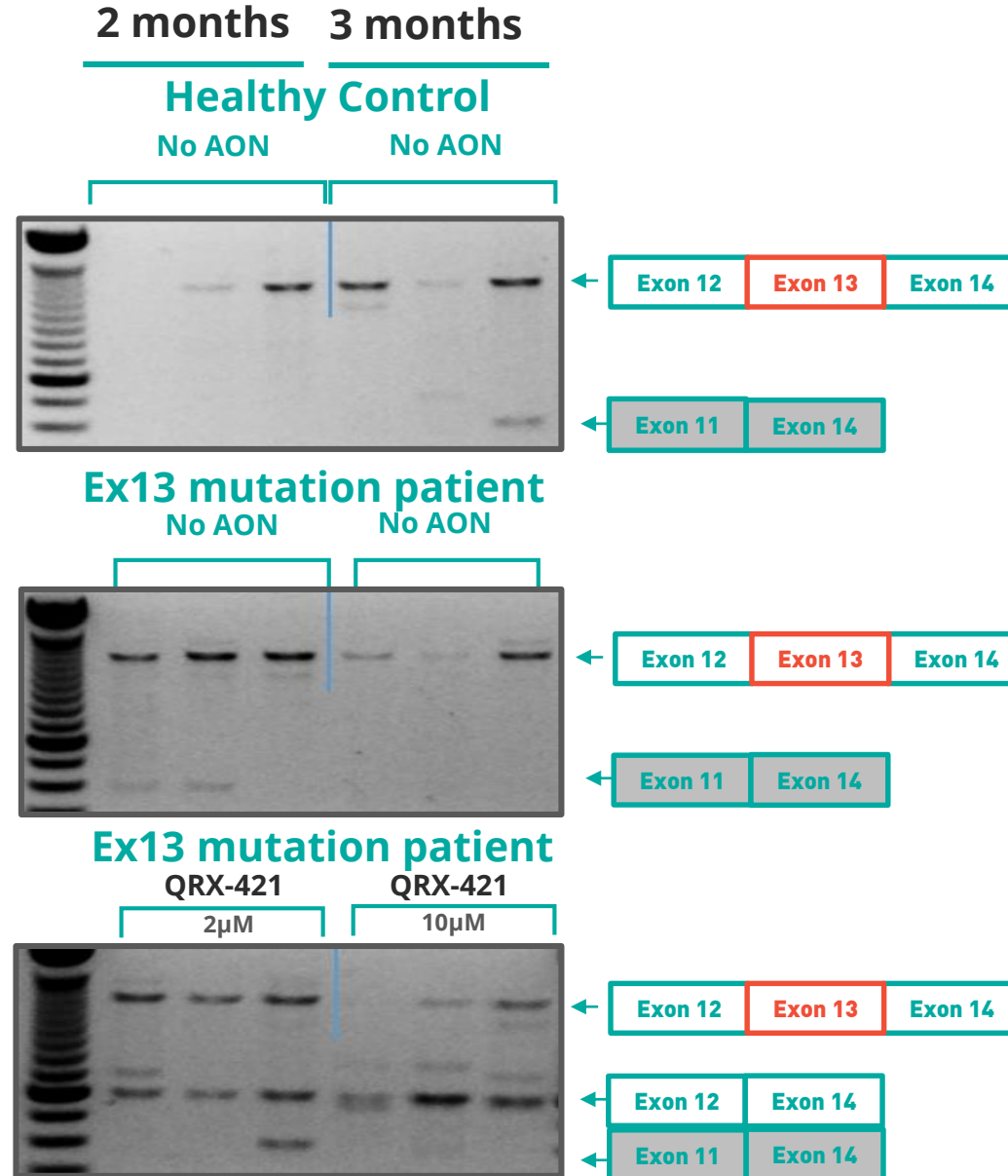
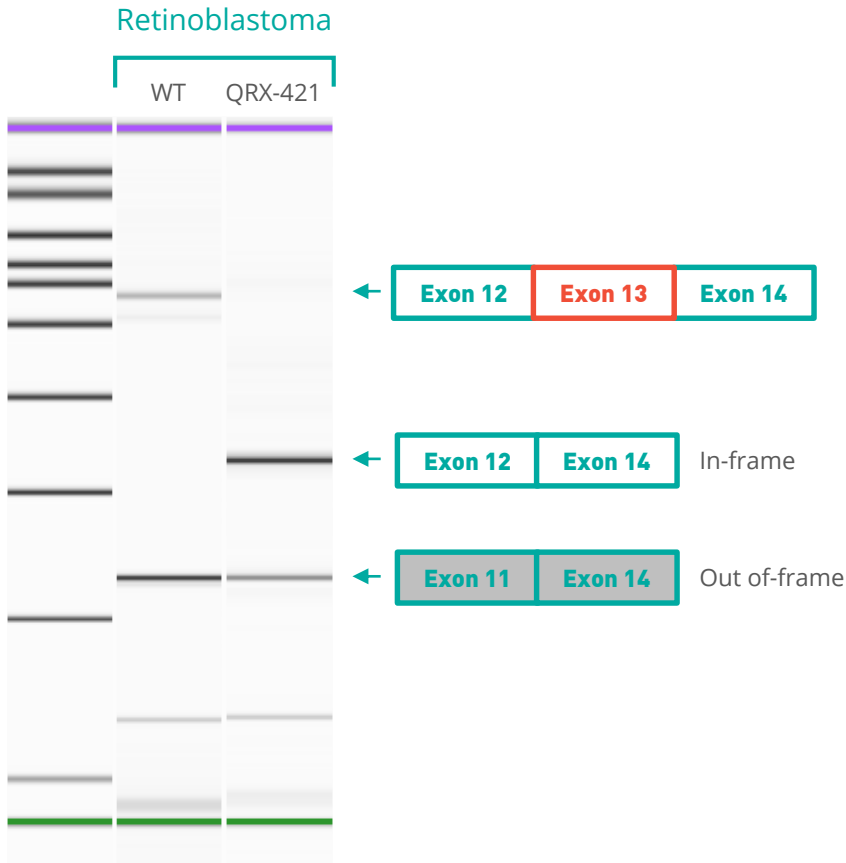
S-opsin

Optic cups



*Parfitt et al., 2016*

# QRX-421 mediated exon 13 skip in vitro and in optic-cups



Erwin van Wijk, Radboudumc, Nijmegen, the Netherlands





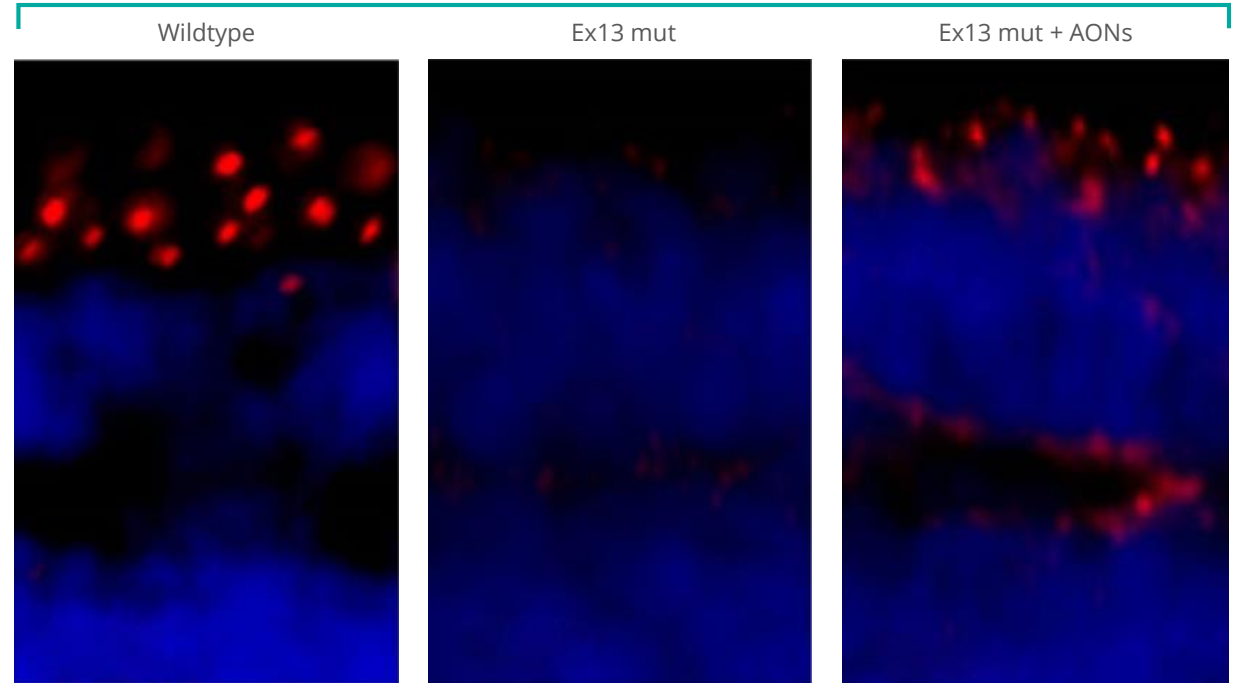
# AON targeting Ex13 skip modifies mRNA and restores protein localization in Zebrafish retina

*Restoration of Ush2a localization in zebrafish eyes*

RT-PCR: Ush2a Ex13m -/-



Ush2a antibody in fish retina showing localization at connecting cilia



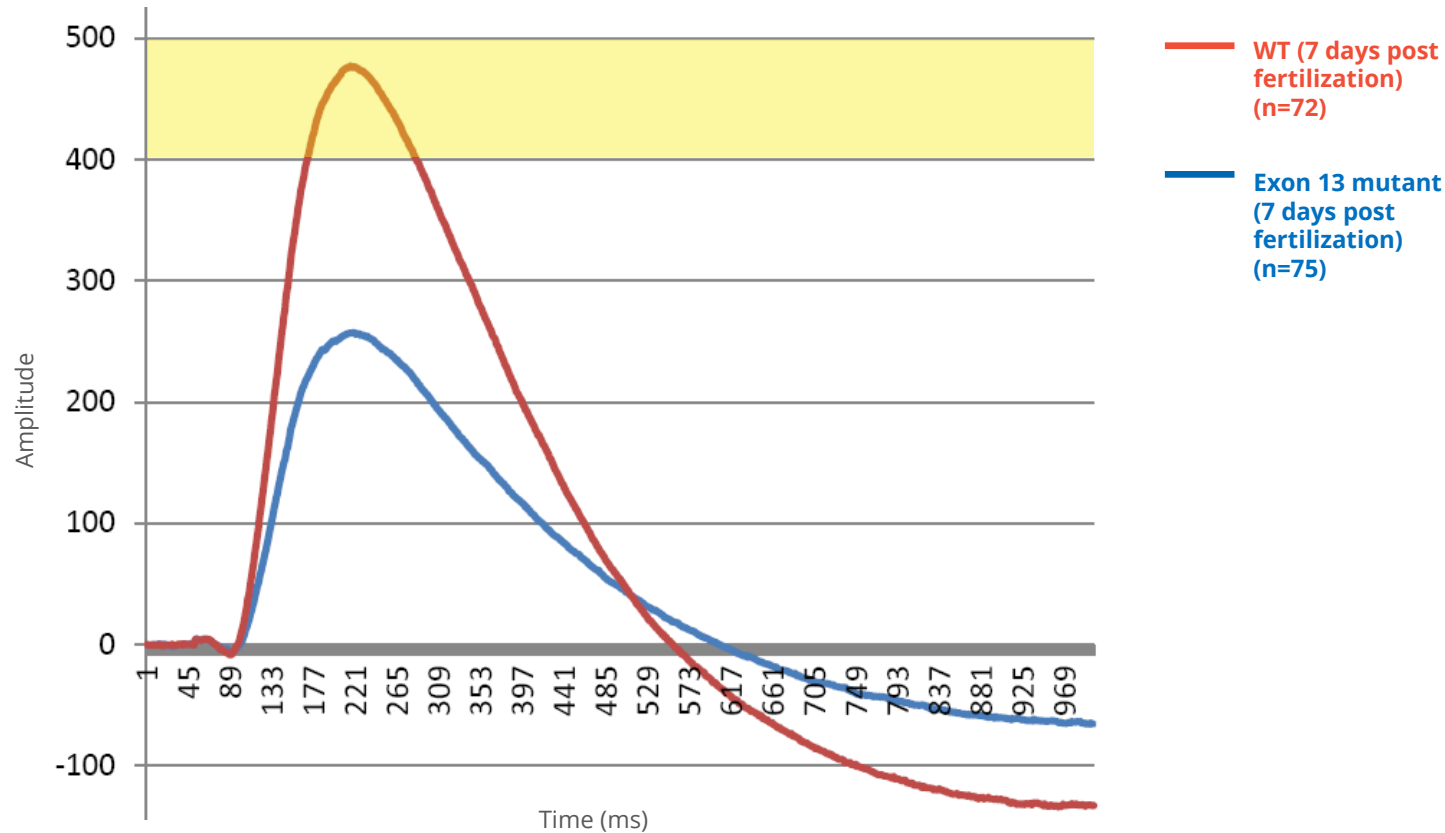
Co-staining with anti-centrin Ab showed Usherin localized at the connecting cilium

*Erwin van Wijk, Radboudumc, Nijmegen, the Netherlands*



# Loss of ERG in Exon 13 mutant Zebrafish

*Reduced b-wave ERG amplitude in Exon 13 mutant fish*



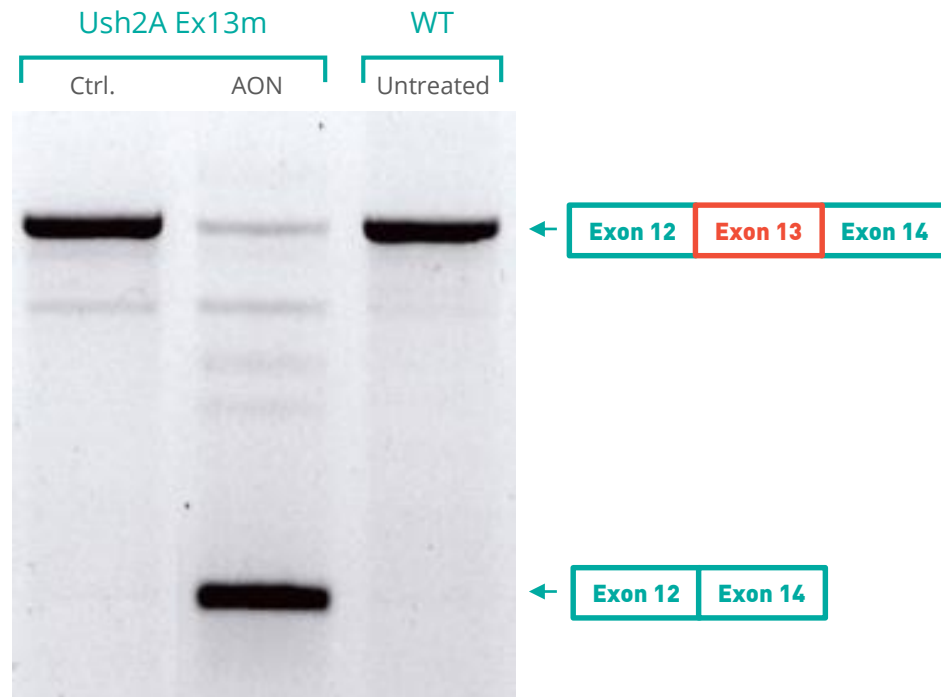
*Erwin van Wijk, Radboudumc, Nijmegen, the Netherlands*



# Restoration of b-wave ERG to wild-type level following Exon-13 deletion

*Exon 13 deleted mutant zebrafish*

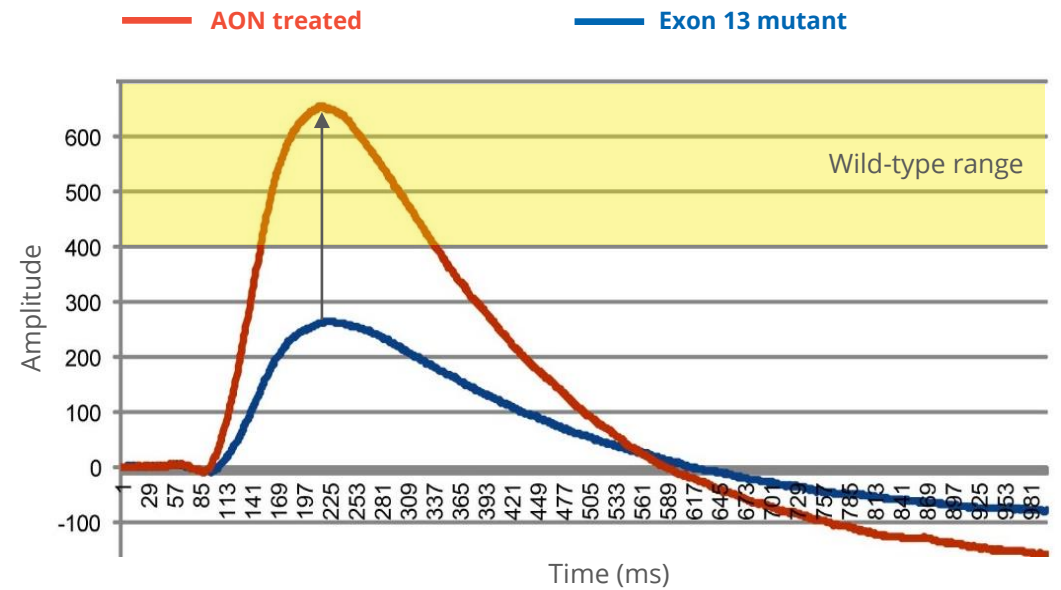
## Exon 13 skip in zebrafish model



Bands have been Sanger sequenced and confirmed to be Ex13-skipped

*Erwin van Wijk, Radboudumc, Nijmegen, the Netherlands*

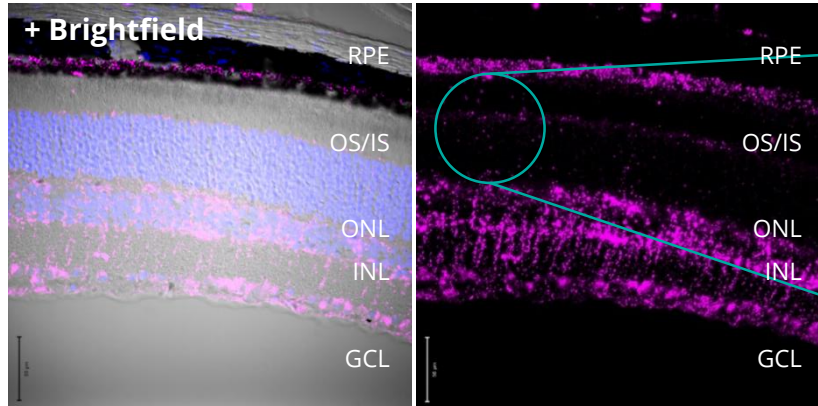
## AON treated zebrafish shows b-wave ERG amplitude restoration



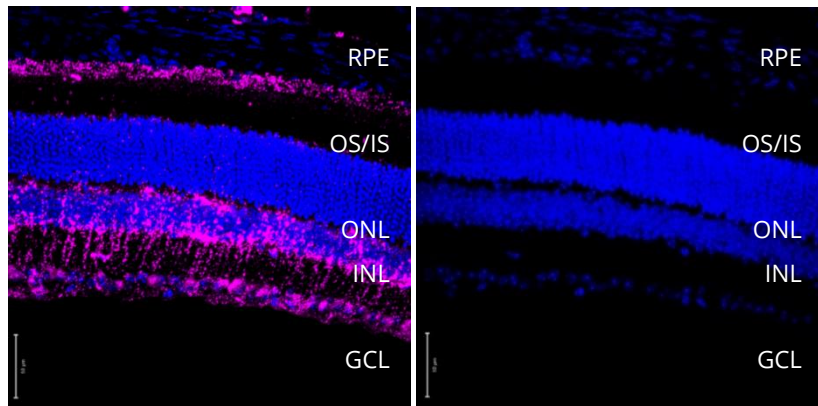


# Efficient delivery of QRX-421 all retinal layers

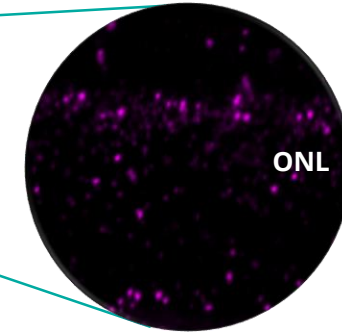
Efficient delivery of QRX-421 to outer nuclear layer (photoreceptor cells)



Immediately post IVT dose

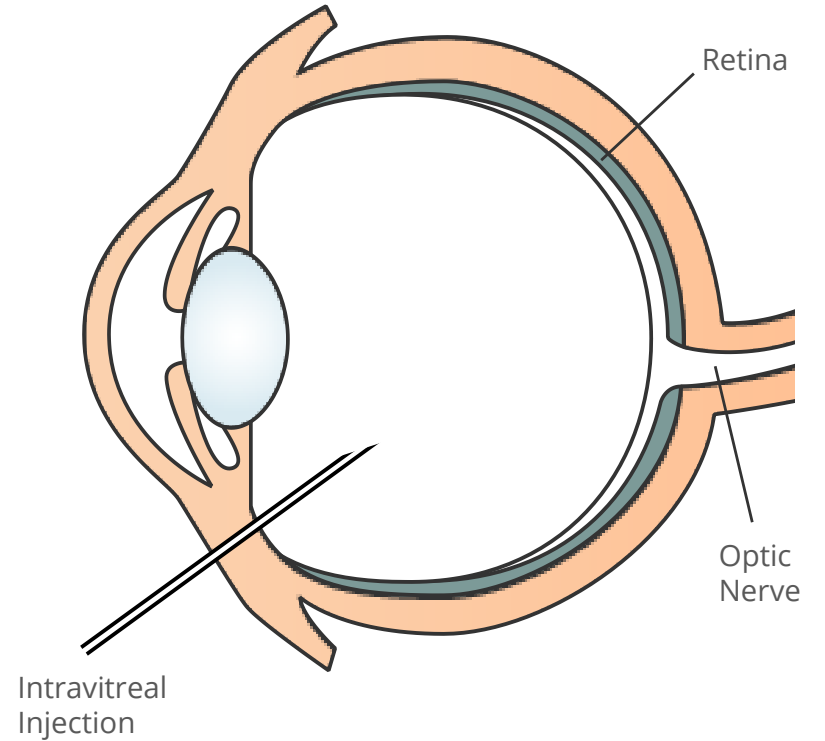


7 days POST IVT dose



**Dose**  
1  $\mu$ l 25  $\mu$ g/ $\mu$ l  
in C57/Bl6  
mice

 DAPI  
 Cy5-QRX-421



# Summary: QRX-421 for USH2A Exon 13

## mRNA profile restoration



mRNA profile with exon 13 skip

## Local (intravitreal) delivery to the eye



Eye well validated target for oligo's  
Efficient delivery to outer nuclear layer in the retina

## mRNA profile restoration in eye-cups



mRNA profile shows Ex13 Skip in patient -derived eye-cups

## Restoration ush2a protein levels



Significant increase in Ush2a protein levels

## Functional restoration in Fish model



protein and ERG restoration established

## Clinical candidate selected



QRX-421 selected as clinical candidate

# Acknowledgements



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Margot Dona  
Erik de Vrieze



**IT'S IN  
OUR RNA**