# RNA toxicity induced by *TCF4* CTG expansions is ameliorated by antisense therapeutics in a patient-derived cell model of Fuchs corneal endothelial dystrophy

**Dr Alice Davidson**

**UCL Institute of Ophthalmology**

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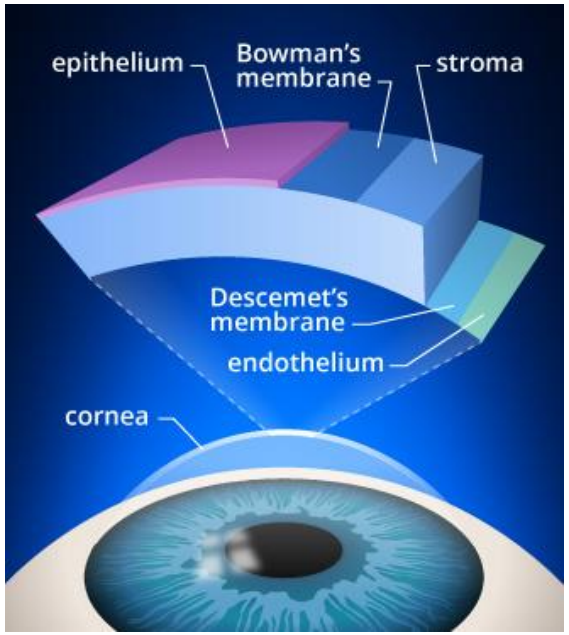
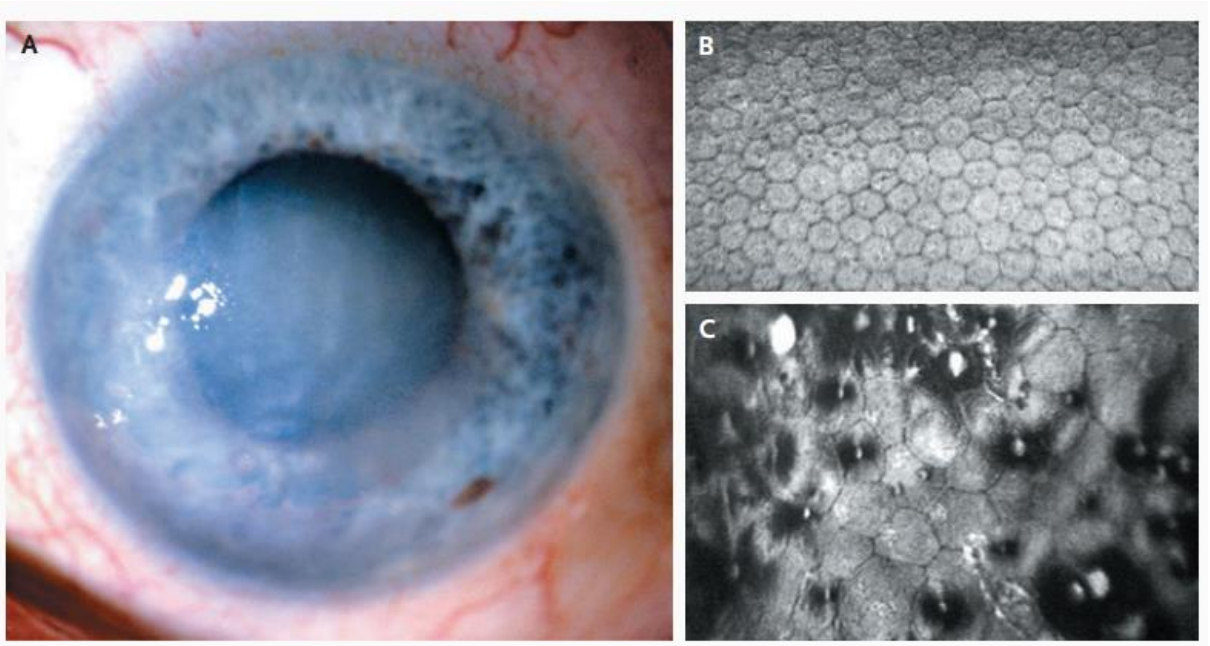
# Fuchs Endothelial Corneal Dystrophy (FECD)



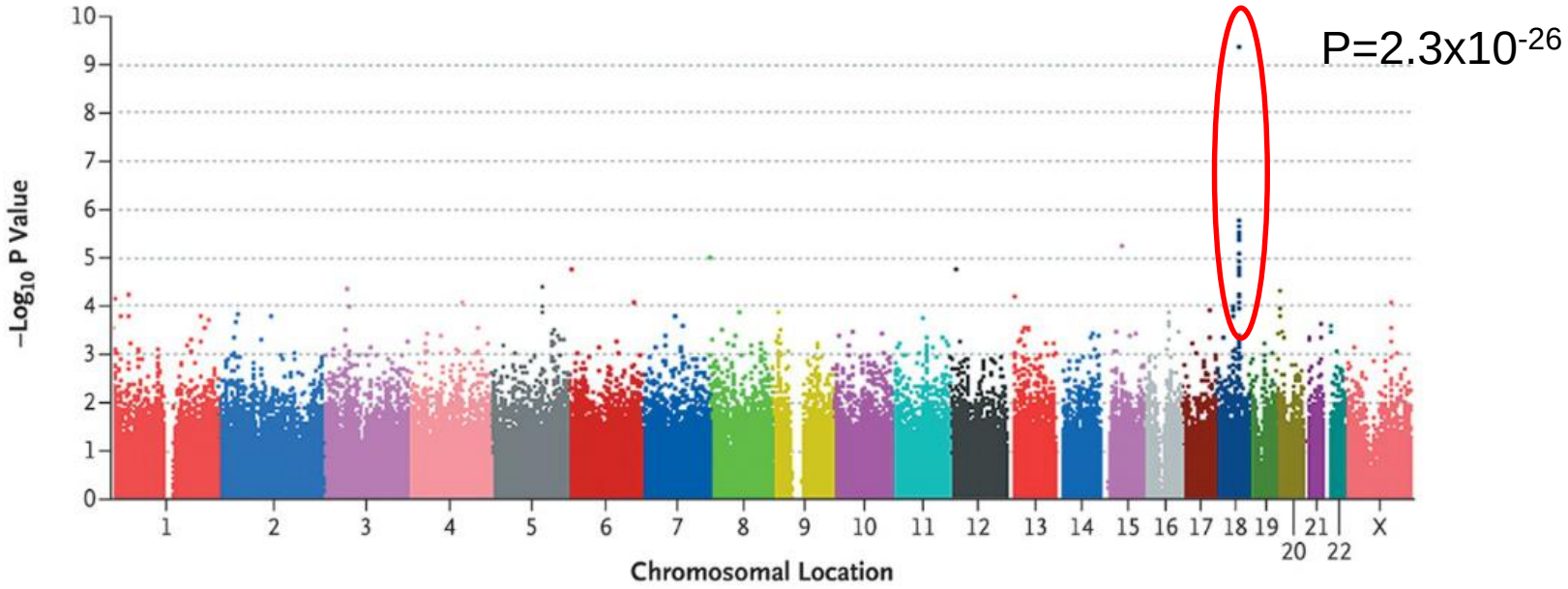




Baratz, *et al.* 2010 (NEJM )

Common, degenerative and age-related condition

# Investigating the genetic architecture of FECD

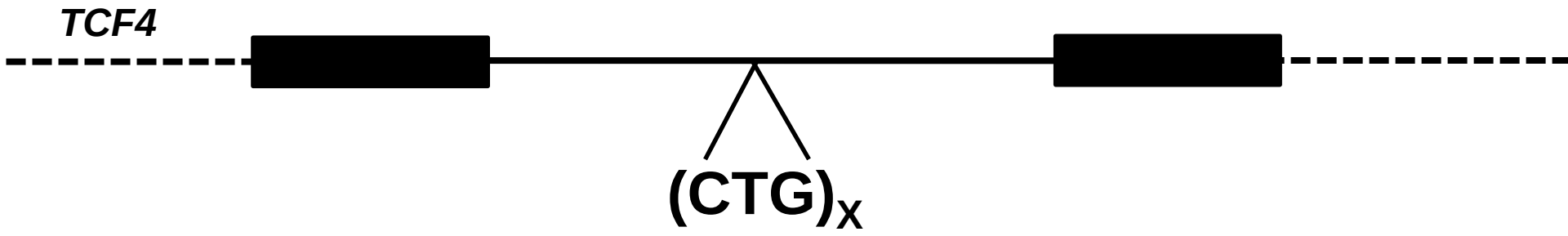
- GWAS identified common *TCF4* SNPs significantly associated with FECD




**Odds ratio (OR) = 5.5 (1 risk allele); 30 (2 risk alleles)**

**Baratz KH, *et al.* 2010 (NEJM)**

# FECD is predominantly a trinucleotide repeat disorder




- CTG18.1 expansion proposed as a functional variant for FECD

79% cases

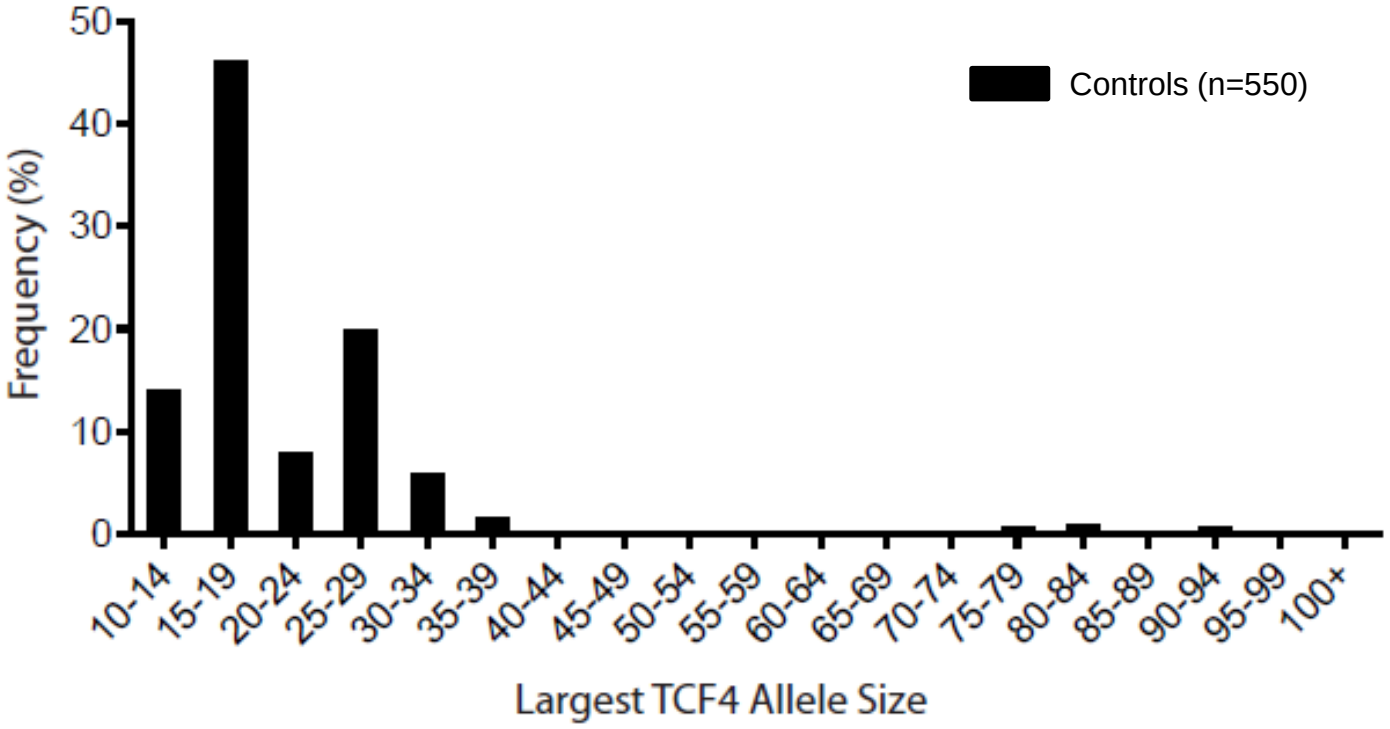
3% controls

→

≥50 copies of the repeat expansion

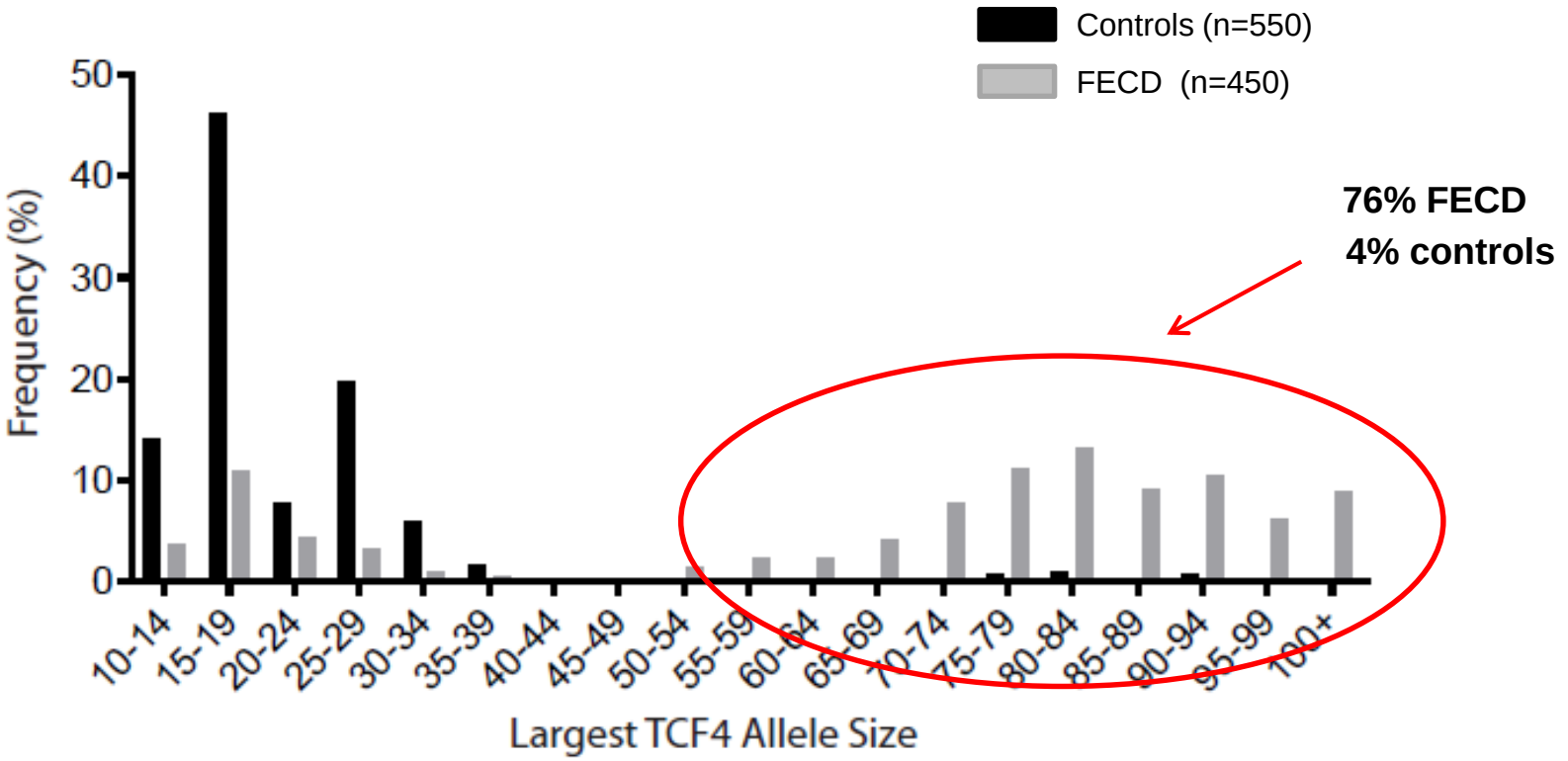
**Wieben *et al.* 2012 (PLoS One)**

# Screening the *TCF4* repeat expansion within a control population




Zarouchlioti *et al.* 2018 (AJHG)

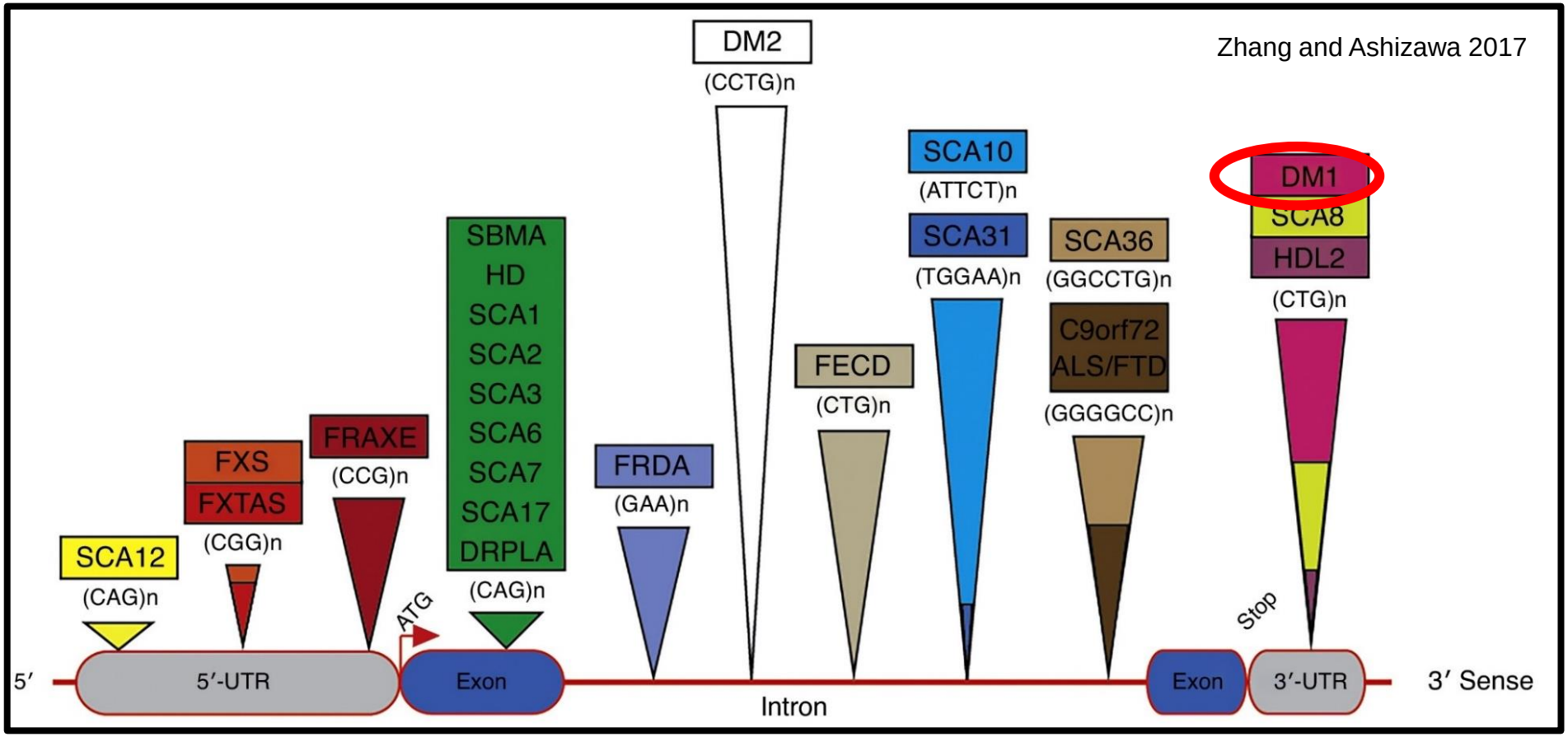
# Screening the *TCF4* repeat expansion within a British and Czech FECD cohort




**Repeat length ≥50 is significantly associated with FECD in the British and Czech FECD cohort** (OR=76.47; 95% CI: 47.45-123.2; $p=5.69 \times 10^{-74}$)

Zarouchlioti *et al.* 2018 (AJHG)

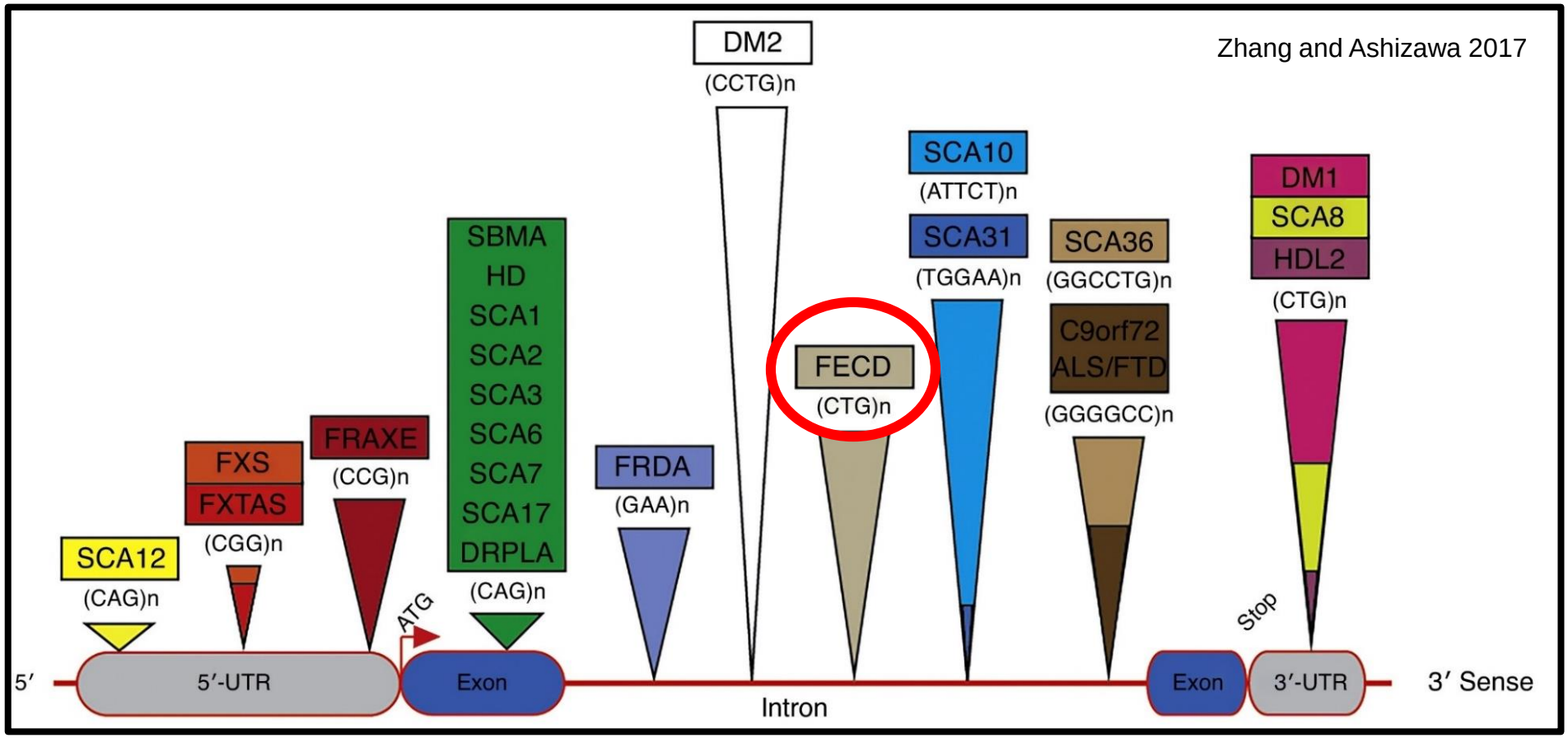
# Repeat expansion-mediated disease




**Myotonic dystrophy type 1 (DM1): non-coding CTG expansions induced RNA toxicity**

- RNA aggregates (foci) accumulate in patient tissue
- Induce toxic gain-of-function effects
  - Sequester RNA binding proteins
  - Global disruption in pre-mRNA splicing

# Repeat expansion-mediated disease



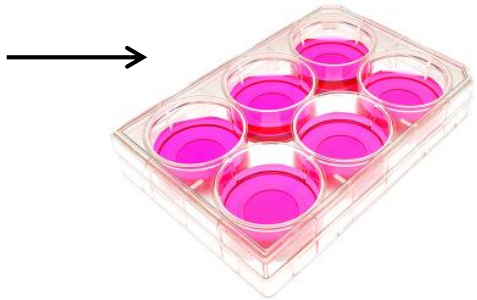

**FECD: non-coding CTG expansions induced RNA toxicity - Du *et al.* 2015**

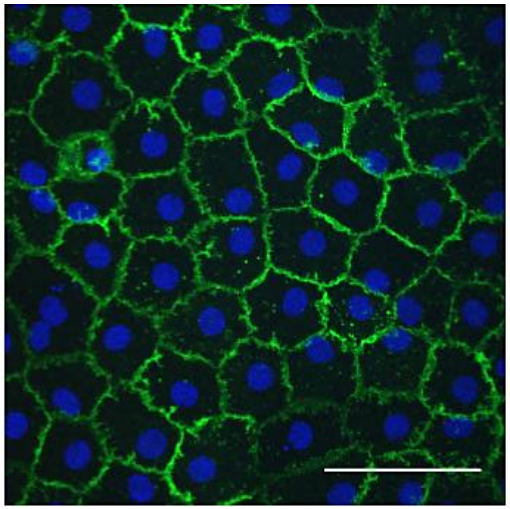
- RNA aggregates (foci) accumulate in patient tissue
- RNA toxicity model to explain FECD pathophysiology

# *In vitro* disease model of FECD

- ✓ **Probe disease mechanism**
- ✓ **Test therapies**

Isolate and expand primary, patient-derived, corneal endothelial cells (CECs)

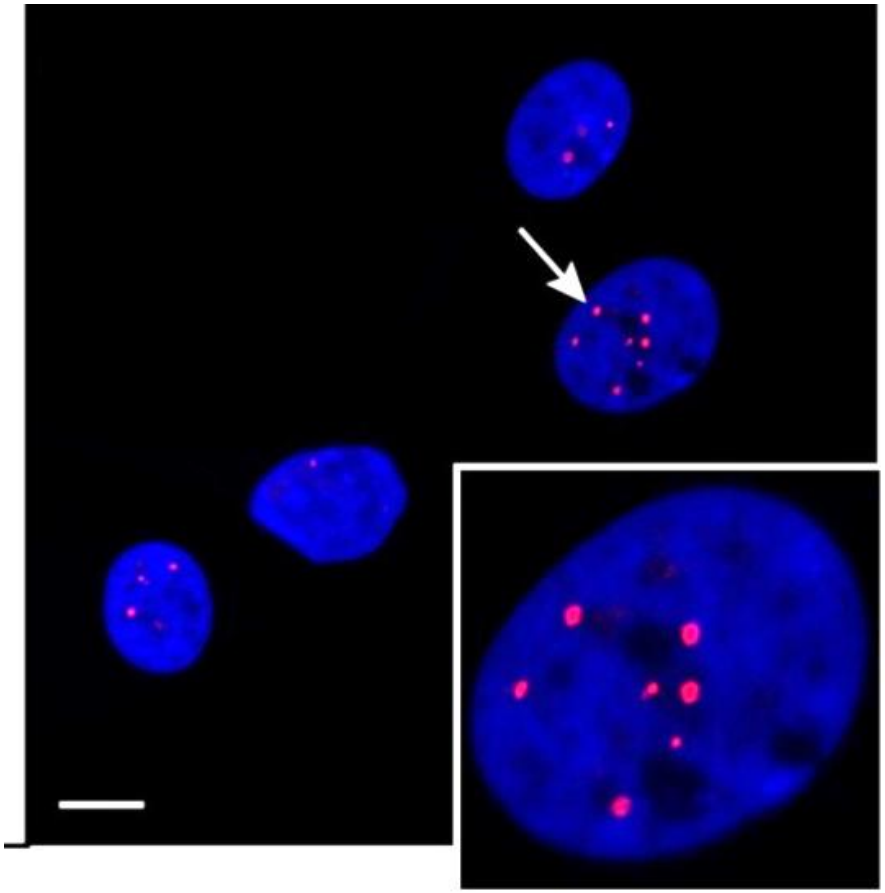




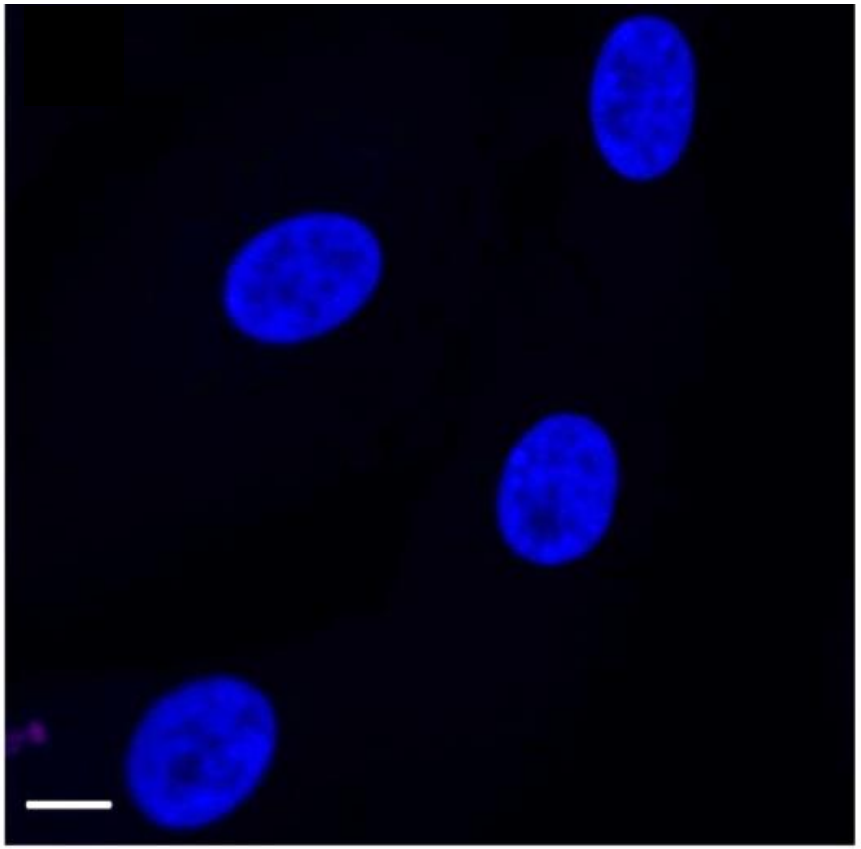
**Methods:** Propagation of CECs using a dual media approach
Peh *et al.* 2015 (Cell Transplantation)

# *In vitro* disease model of FECD

**Fluorescence *in situ* hybridisation (FISH):** Cy3-$(CAG)_7$ FISH probe



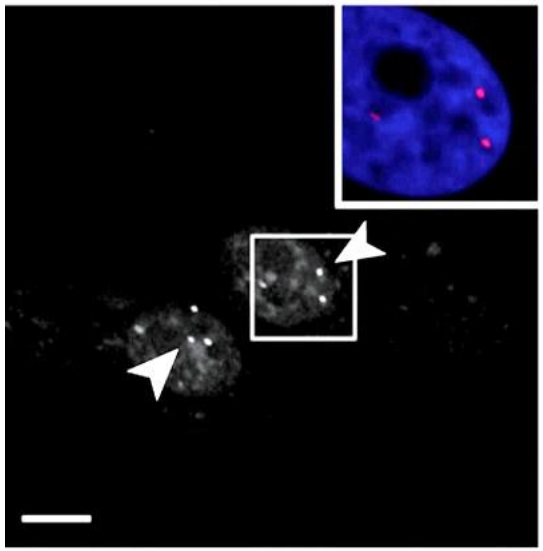
**Expansion positive**



**Expansion negative**

# *In vitro* disease model of FECD

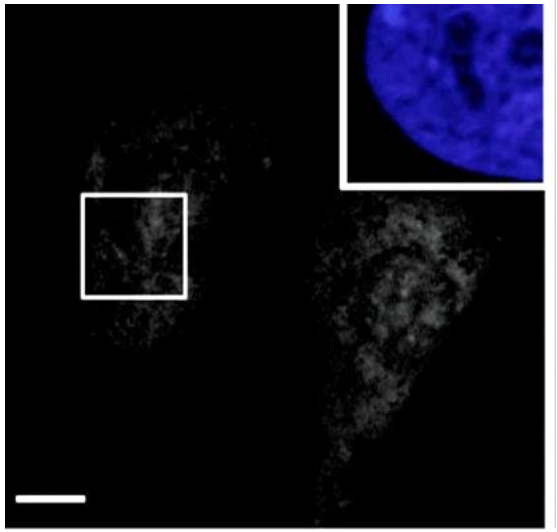
**CECs**



✓ **Foci positive**

**Foci occurrence is cell-type dependent**

**Fibroblasts**



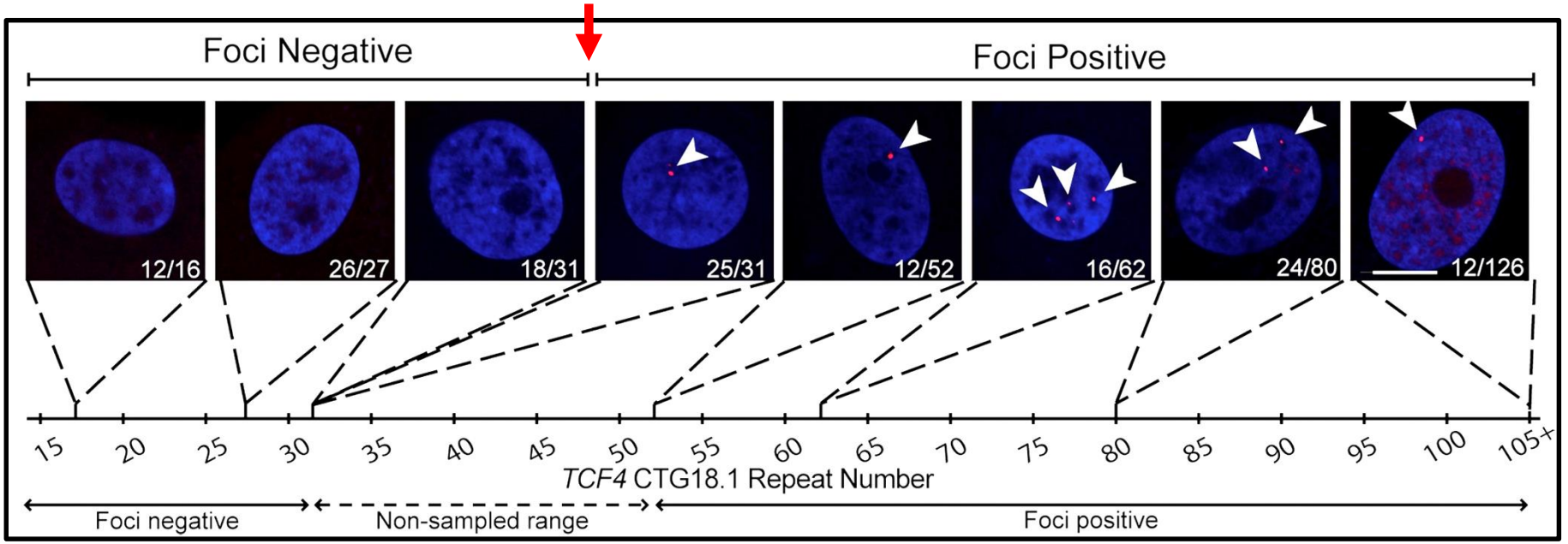
*x* **Foci negative**

n=6, repeat range sampled = 53-108

Zarouchlioti *et al.* 2018 (AJHG)

# *In vitro* disease model of FECD

## Identifying a repeat length dependant threshold for foci

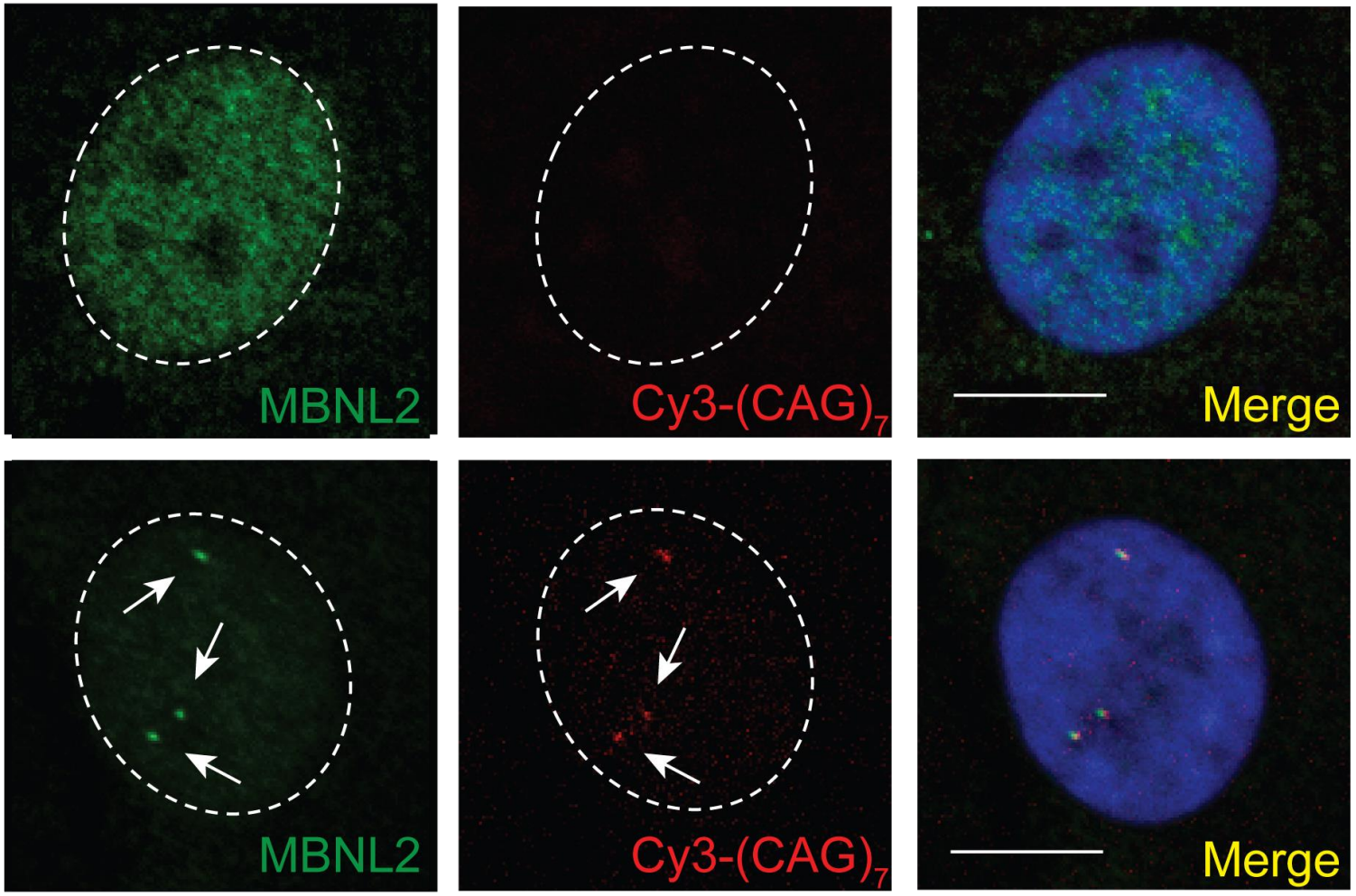



**n=36**

Zarouchlioti *et al.* 2018 (AJHG)

# *In vitro* disease model of FECD

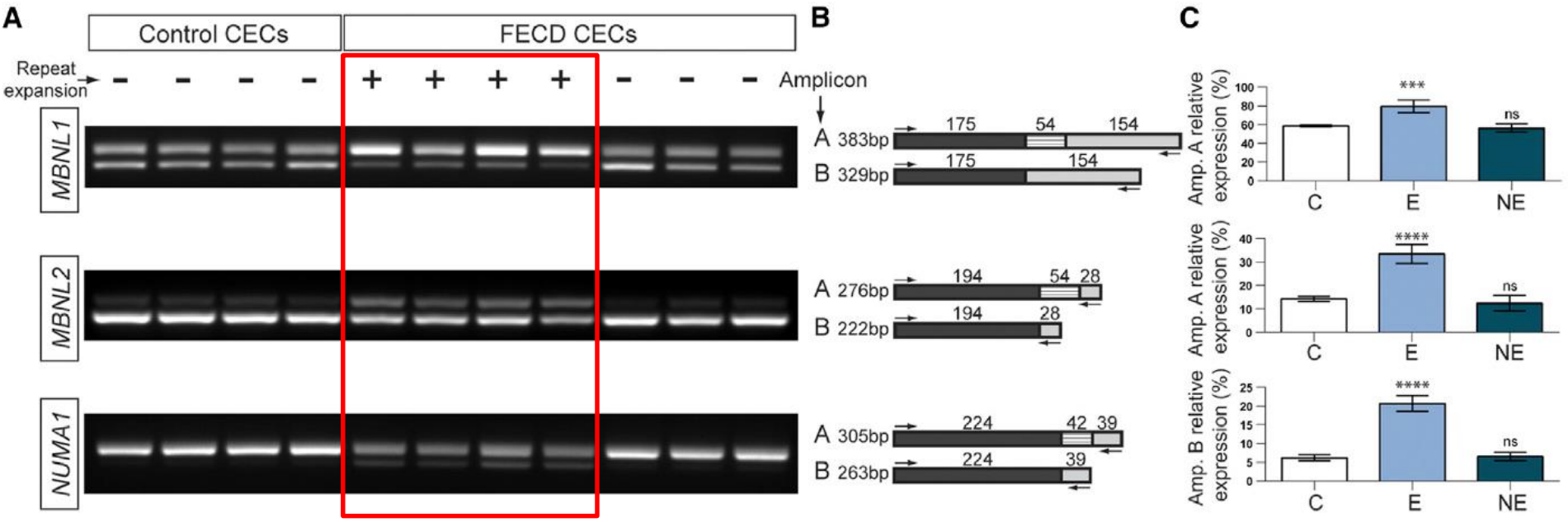
## Nuclear distribution of RNA splicing factors




## MBNL1 and 2 are recruited to RNA foci

# *In vitro* disease model of FECD

## RT-PCR assays: signatures of dysregulated splicing



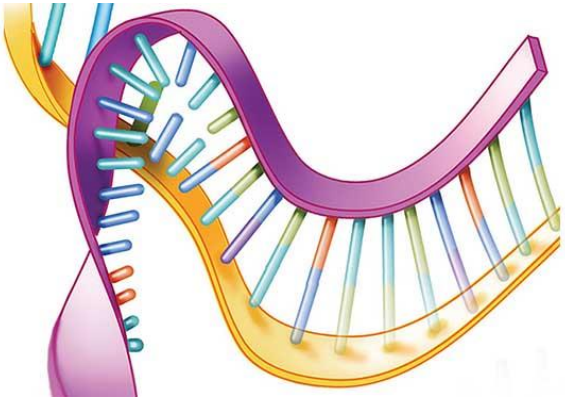

C = Control
E = Expanded
NE = Non-Expanded

**Repeat expansion pathology is associated with dysregulated splicing patterns in CECs**

Zarouchlioti *et al.* 2018 (AJHG)

# Antisense oligonucleotide (ASO) therapy

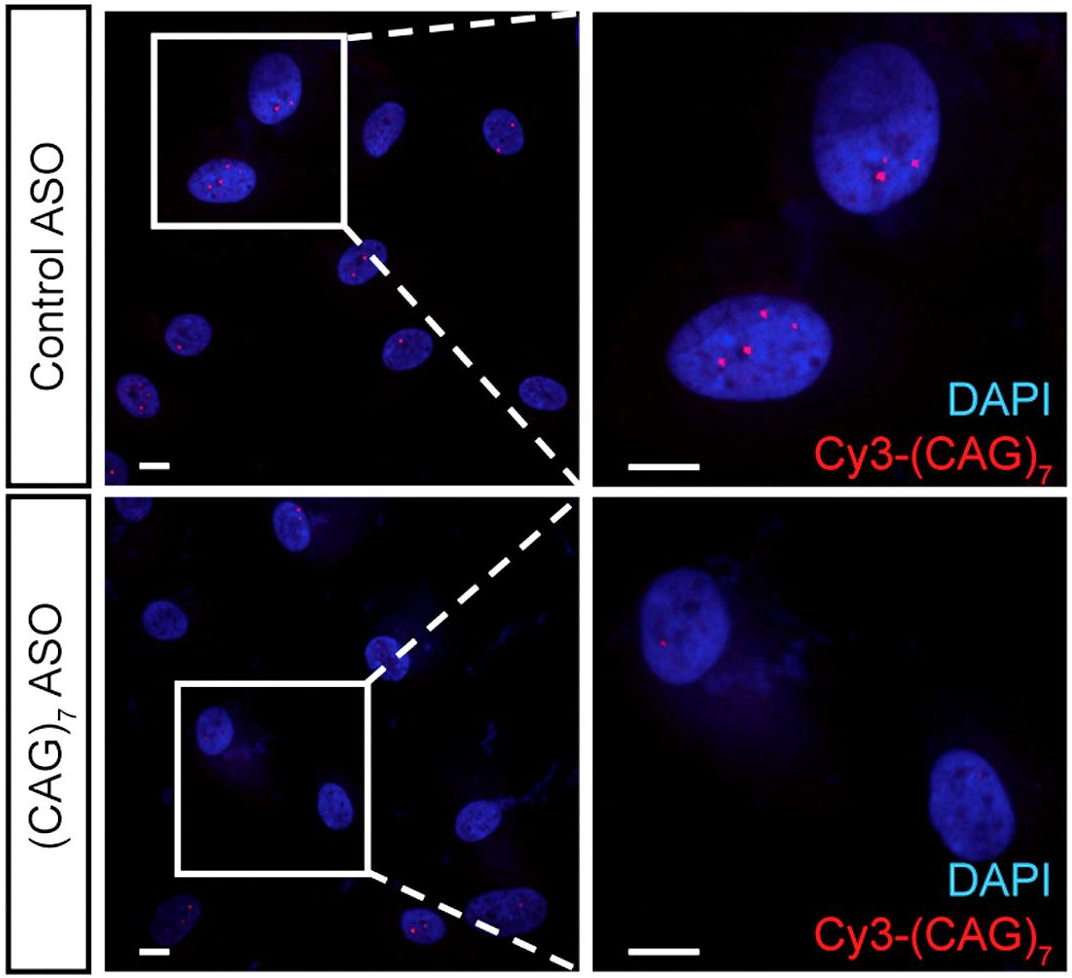
**Aim:** Test the efficacy of ASO treatment for FECD using primary, patient-derived, CECs

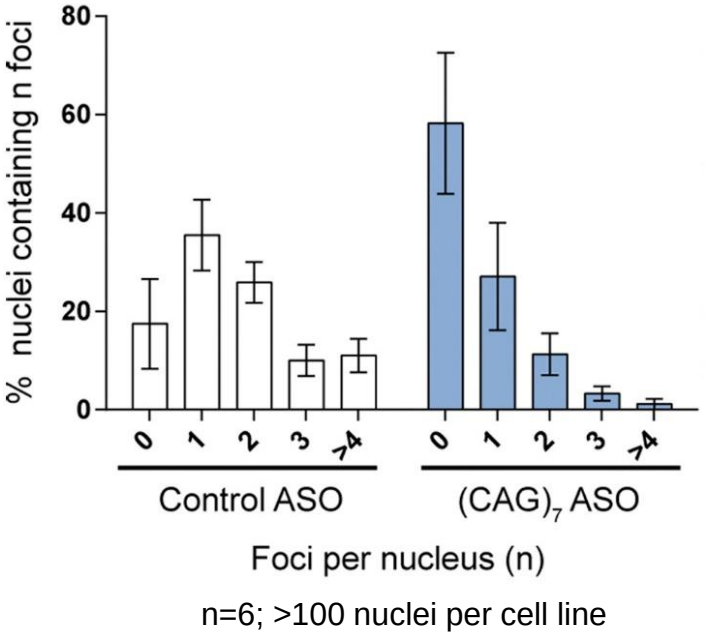


ProQR®

# ASO Treatment

**Treated:** 200 nM 2'-O-methyl-phosohorothioate modified $(CAG)_7$ ASO for 24 hours



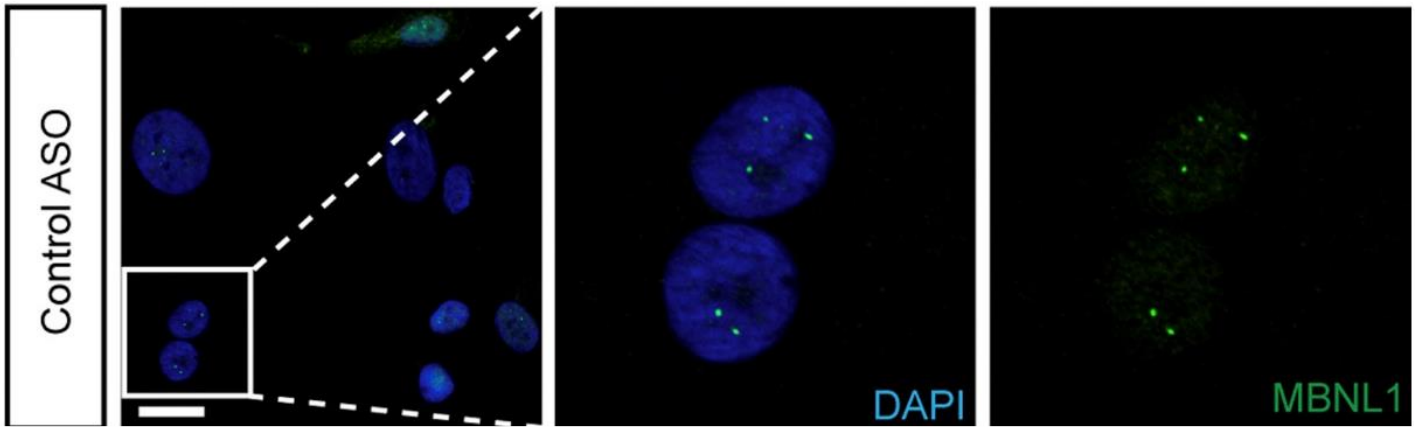



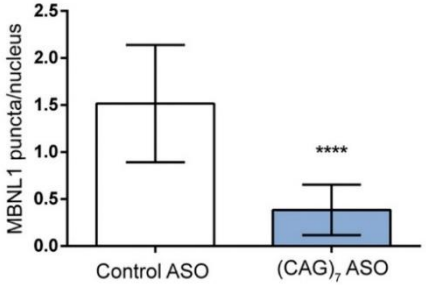

n=6; >100 nuclei per cell line

**ASO treatment significantly reduces the incidence of nuclear RNA foci**

Zarouchlioti *et al.* 2018 (AJHG)

# ASO Treatment: MBNL1 nuclear localisation



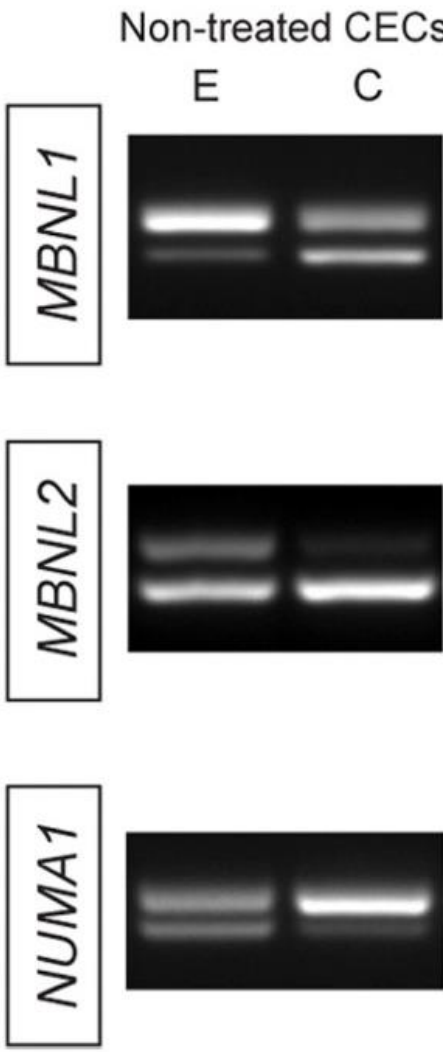




n=4; >100 nuclei per cell line

## ASO treatment rescues MBNL1 nuclear localization

Zarouchlioti *et al.* 2018 (AJHG)

# ASO Treatment: Splicing Dysregulation




**ASO treatment reduces aberrant mRNA processing**

Zarouchlioti *et al.* 2018 (AJHG)

# Summary

- Expanded copies of the *TCF4* repeat are significantly ($p=5.69 \times 10^{-74}$) associated with FECD in the British and Czech patient populations

- Primary patient-derived CECs display hallmarks of RNA toxicity
  - RNA foci
  - Sequester splicing factors
  - Dysregulated signatures of pre-mRNA splicing

- $CAG_7$ ASO treatment ameliorates features of RNA toxicity
  - Reduces foci numbers
  - Redistributing RNA-splicing factors
  - Reduces levels of differential splicing events

# Conclusions

- Patient derived primary CECs provide an excellent system to test therapies and probe disease mechanism
  - Investigating the repeat within it's genomic and cellular context

- Targeted genetic therapies for FECD is now a realistic goal
  - CTG18.1 expansion represent an ideal target for gene directed therapy
  - ASO offer a promising therapeutic avenue for *TCF4*-mediated FECD given that prevalence of *TCF4* repeat-mediated FECD and the accessibility of the cornea

***UCL Institute of Ophthalmology***
**Christina Zarouchlioti**
**Beatriz Sanchez-Pintado**
**Nathan Hafford Tear**
**Alison Hardcastle**
Mike Cheetham
Anthony Vugler
Ma'ayan Semo

***Moorfields Eye Hospital***
**Stephen Tuft**
Kirithika Muthusamy

***ProQR Therapeutics***
**Peter Adamson**
Pontus Klein
Kalyan Dulla

***King's College London***
Pirro Hysi

***Charles University, Prague***
Petra Liskova
Lubica Dudakova
Pavlina Skalicka

***University of Liverpool***
Hannah Levis



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