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

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Fuchs Endothelial Corneal Dystrophy (FECD)

Common, degenerative and age-related condition

Baratz, et al. 2010 (NEJM)
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

TCF4

$(CTG)_x$

- 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 $\geq 50$ 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)
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
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

✗ Foci negative

n=6, repeat range sampled = 53-108

Zarouchlioti et al. 2018 (AJHG)
Identifying a repeat length dependant threshold for foci

In vitro disease model of FECD

Zarouchlioti et al. 2018 (AJHG)
In vitro disease model of FECD

Nuclear distribution of RNA splicing factors

Expansion negative

Expansion positive

MBNL1 and 2 are recruited to RNA foci
Repeat expansion pathology is associated with dysregulated splicing patterns in CECs

Zarouchlioti et al. 2018 (AJHG)
Aim: Test the efficacy of ASO treatment for FECD using primary, patient-derived, CECs
Treated: 200 nM 2'-O-methyl-phosphorothioate modified (CAG)$_7$ ASO for 24 hours

ASO treatment significantly reduces the incidence of nuclear RNA foci

Zarouchlioti et al. 2018 (AJHG)
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 x10^{-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