Longitudinal analysis of somatic instability of CTG repeats in a Fuchs endothelial corneal dystrophy patient cohort

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What is Fuchs endothelial corneal dystrophy (FECD)?

- Fuchs endothelial corneal dystrophy (FECD) is a genetic condition usually diagnosed later in life and is the leading reason for transplants of the cornea, in the eye, with an incidence of 4–5% among individuals over 40 years old (1).
- Caused by a mutation, meaning a change in an individual's genetic code (DNA). Our DNA is made up from a four lettered-code, consisting of A, T, C, G.
- In this disease, a DNA segment in the *TCF4* gene, consisting of a repeating CTG sequence is changed (or mutated), making this repeat longer.

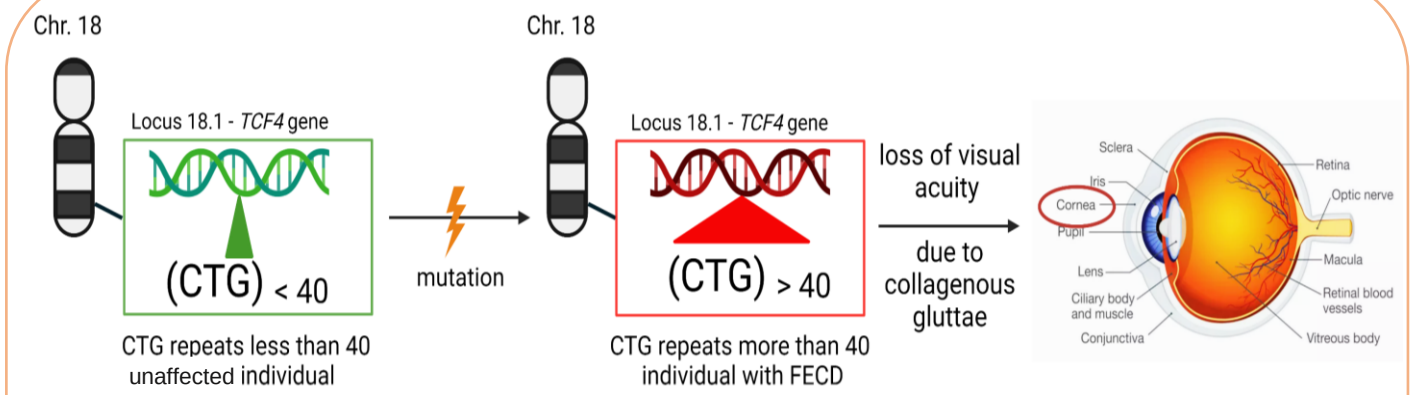

Figure 1: Health implications of Fuchs endothelial corneal dystrophy (FECD) mutation. Left: normal *TCF4* gene, with few CTG repeats. Middle: repeat expansion mutation, causing repeats to increase to more than 40. Right: anatomical representation of the human eye, with the cornea - defective in FECD - highlighted in red circle. Author's own figure, information drawn from (3) and (4).

But, why is all this important?

- This repeat expansion mutation has been proposed to be somatically unstable, meaning increasing in length throughout an individual's life-time, similarly to other repeat expansion mutations in disorders like Huntington disease and myotonic dystrophy.
- Longer repeats typically associate with increased disease severity and earlier age of onset (2).

Objectives and hypotheses

Establish whether the repeat is indeed somatically unstable in FECD patients Also, is there a link between inherited repeat length (ePAL) and somatic instability? What about ePAL and age at symptom onset? This is already the case for other repeat expansion diseases (5).

The main **hypotheses** are:

1. The trinucleotide CTG repeat in the *TCF4* gene is somatically unstable
2. More somatic instability correlates with larger inherited repeats

Methods

•Cohort of 21 patients, for each: two samples. One at t=0 (time at diagnosis/disease onset) and a second time t, after 1.5 to 11 years after diagnosis. One patient had samples from three time points.

•Analysis of both samples for each patient through small-pool polymerase chain reaction (SP-PCR) (6) – a modified version of the common PCR method used for COVID-19 - and visualization through X-ray imaging (autoradiography). Summary in ***Figure 2.***

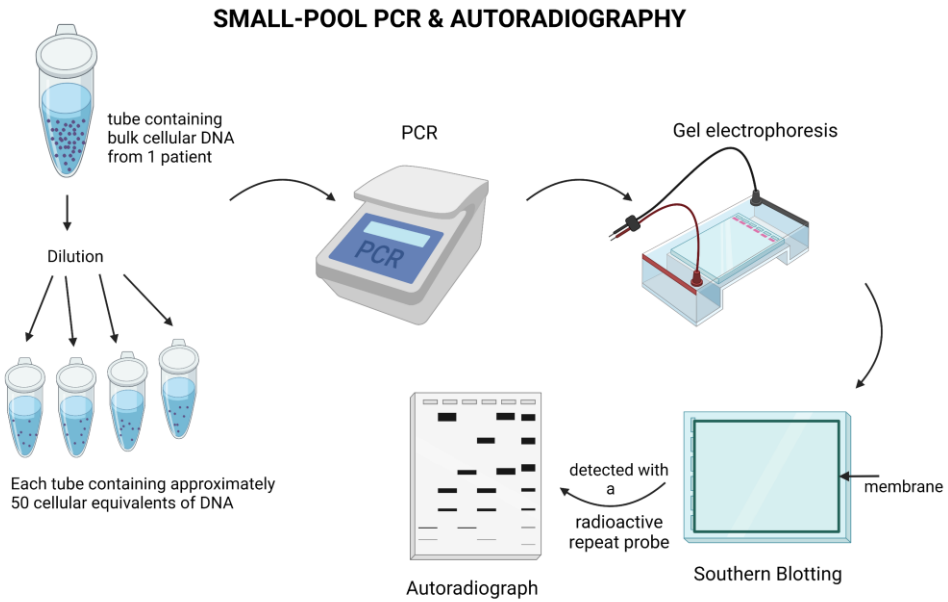

Figure 2: Methods employed for sample analysis. First, bulk DNA was diluted to aliquots containing 0.3 ng of DNA (or 50 cell equivalents) and underwent PCR, followed by overnight gel electrophoresis, Southern blotting, and subsequent membrane hybridization with a radioactive repeat probe. Results visualized through X-ray autoradiography.

Results - SOMATIC INSTABILITY OF CTG REPEAT IN FECD PATIENTS

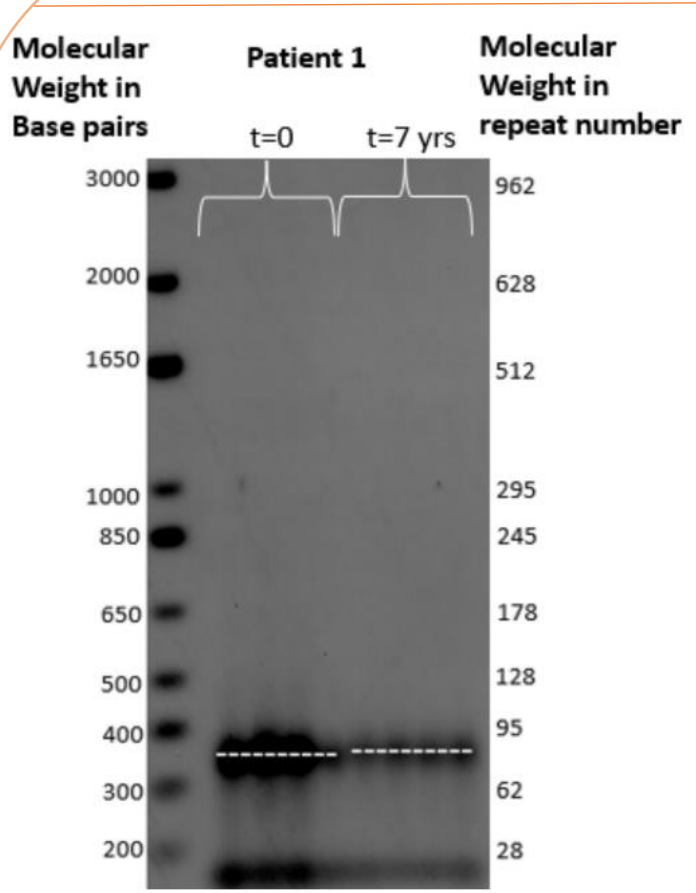

Figure 3: Patient 1, showing **no clear somatic instability**. This was the case for approximately 30% of the patients analyzed. Left: patient data from time at diagnosis (t=0) and right: after 7 years (t=7). Dashed white lines denote the mode (most common repeat length), which here coincides with the progenitor allele (ePAL), at around 75 repeats. Below, non-disease associated allele, at around 18 repeats. The mode between t=0 and t=7 is unchanged, so the CTG repeat appears stable.

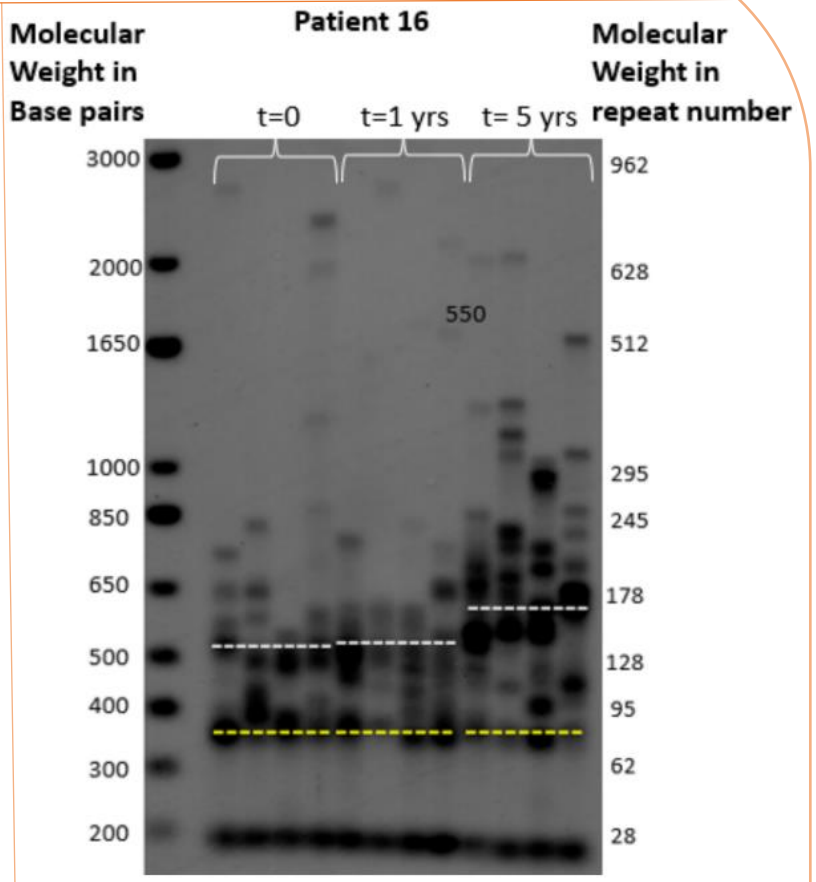

Figure 4: Patient 16, showing **somatic instability.** This was the case for approximately 70% of the patients analyzed. From left to right, patient data from time at diagnosis (t=0), after one year (t=1) and after 5 years (t=5). Dashed white lines represent the mode (most common repeat length). Dashed yellow lines denote the inherited number of repeats (ePAL), at around 84 repeats. Below, the non-disease associated allele at around 26 repeats. The mode between t=1 and t=5 has shifted slightly upwards, indicating that more cells have now more repeats. Thus, the repeat is likely unstable.

INHERITED REPEATS (ePAL) CORRELATION WITH SOMATIC INSTABILITY AND AGE OF DISEASE ONSET

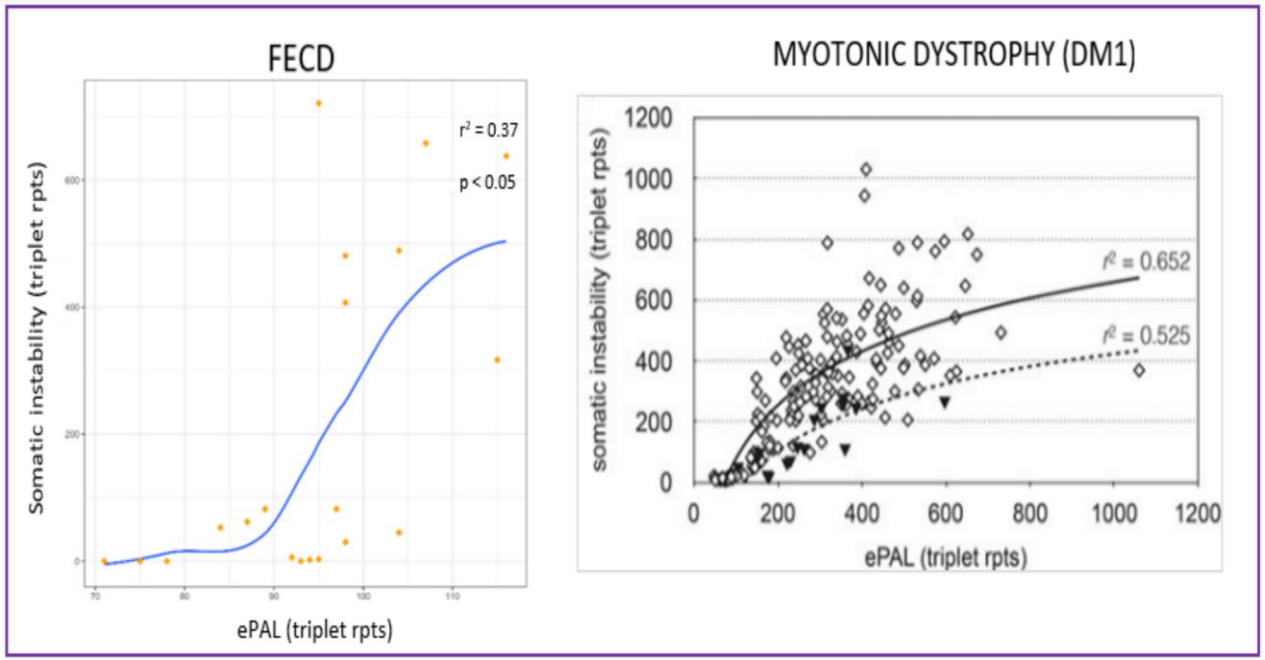

Figure 5: Relationship between inherited repeats and somatic instability. Somatic instability: Mode minus ePAL. Left: data yielded for Fuchs endothelial corneal dystrophy (FECD), right: published data for myotonic dystrophy, another repeat expansion disease. Figure reproduced with author's permission (5)

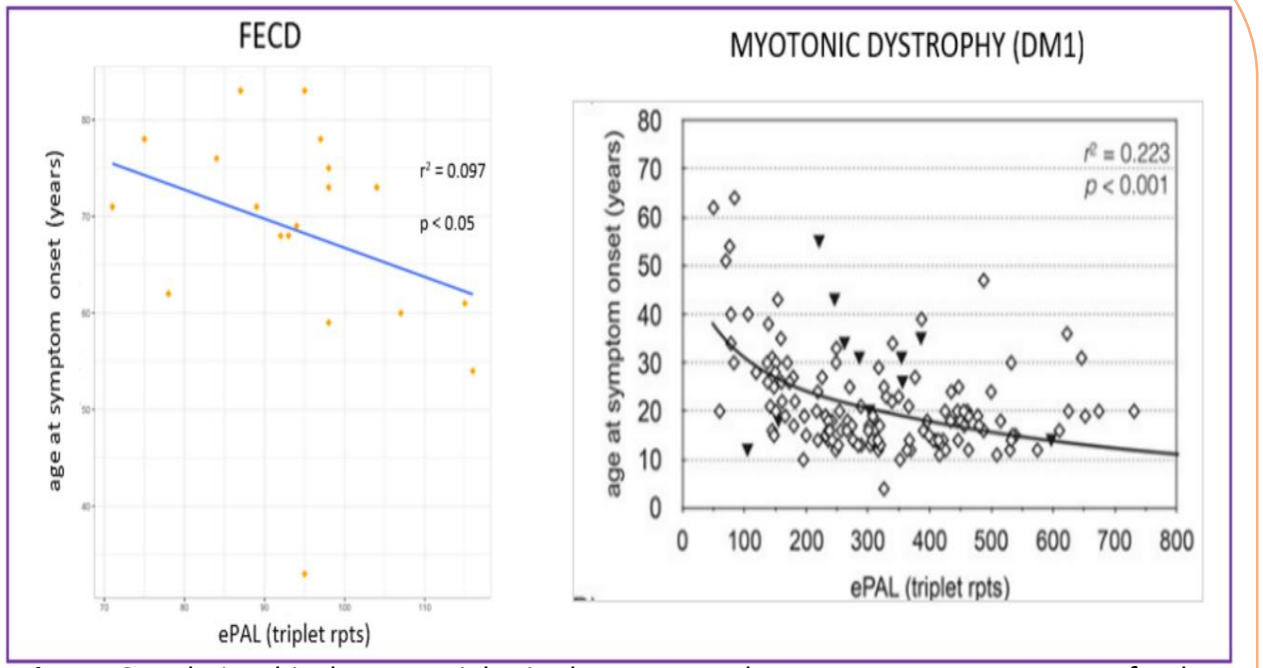

Figure 6: Relationship between inherited repeats and age at symptom onset. Left: data yielded for Fuchs endothelial corneal dystrophy (FECD), right: published data for myotonic dystrophy, another repeat expansion disease. Figure reproduced with author's permission (5).

Conclusions

Based on results yielded, the main conclusions to answer the research questions are:

Somatic instability present in FECD in approximately 70% of the patient samples analyzed

•The fact that 30% of patients display no somatic instability sheds light to additional mechanisms of pathogenesis. This supports existing literature (7).

Somatic instability is modified by inherited repeats, in a non-linear relationship

•Larger inherited repeats are inversely correlated with age at symptom onset. This is already established for other repeat expansion diseases, like myotonic dystrophy (DM1) (5) (8).

What does the future hold?

•Why do some patients have somatic instability, while others don't? Is this modifier genes or something else?

•To what extent do FECD and other repeat expansion diseases share other similarities/differences? Could maybe FECD be used as a model for therapeutical interventions for other diseases with similar causes?

•Present findings shed light to somatic instability, but what about intergenerational instability?

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