Instability of TCF4 Triplet Repeat Expansion With Parent–Child Transmission in Fuchs’ Endothelial Corneal Dystrophy

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PURPOSE. Fuchs’ endothelial corneal dystrophy (FECD) caused by the CTG triplet repeat expansion in the TCF4 gene (CTG18.1 locus) is the most common repeat expansion disorder. Intergenerational instability of expanded repeats and clinical anticipation are hallmarks of other repeat expansion disorders. In this study, we examine stability of triplet repeat allele length and FECD disease severity in parent–child transmission of the expanded CTG18.1 allele.

METHODS. We studied 44 parent–child transmissions of the mutant expanded CTG18.1 allele from 26 FECD families. The CTG18.1 polymorphism was genotyped using short tandem repeat analysis, triplet repeat primed PCR assay, and Southern blot analysis. FECD severity was assessed using modified Krachmer grading (KG) system. Triplet repeat length of mutant allele and KG severity were compared between generations.

RESULTS. Instability of the expanded allele was seen in 14 of 44 (31.8%) parent–child transmissions, and the likelihood of an unstable event increased with the size of the parental allele (P = 5.9 × 10−5). A tendency for contraction was seen in transmission of large alleles (repeat length > 120), whereas intermediate alleles (repeat length between 77 and 120) had predilection for further expansion (P = 1.5 × 10−3). Although we noted increased KG severity in the offspring in three pairs, none of these transmissions were associated with allele instability.

CONCLUSIONS. We observed instability of the TCF4 triplet repeat expansion in nearly a third of parent–child transmissions. Large mutant CTG18.1 alleles are prone to contraction, whereas intermediate mutant alleles tend to expand when unstably transmitted. Intergenerational instability of TCF4 repeat expansion has implications on FECD disease inheritance.

Keywords: Fuchs’ endothelial corneal dystrophy, triplet repeat expansion, TCF4, genetics
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METHODS

Study Participants

The study was conducted with the approval of the institutional review board of the University of Texas Southwestern Medical Center (UTSW) and was in compliance with the tenets of the Declaration of Helsinki. All study subjects were recruited from the cornea referral practice at UTSW after informed consent.

The UTSW FECD cohort of 640 individuals belonging to 309 families was reviewed. We identified all parent–child pairs in the cornea referral practice at UTSW after informed consent. The study was conducted with the approval of the institutional review board of the University of Texas Southwestern Medical Center (UTSW) and was in compliance with the tenets of the Declaration of Helsinki. All study subjects were recruited from the cornea referral practice at UTSW after informed consent.

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Parent Generation,

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Parent Generation, N = 35</th>
<th>Offspring Generation, N = 44</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, male, no. (%)</td>
<td>12 (34.3)</td>
<td>13 (29.5)</td>
<td>8.1 × 10^{-1}</td>
</tr>
<tr>
<td>FECD affected, no. (%)</td>
<td>34 (97.1)</td>
<td>31 (70.5)</td>
<td>2.2 × 10^{-3}</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>76.5 (11.5)</td>
<td>49.1 (9.3)</td>
<td>3.4 × 10^{-7}</td>
</tr>
<tr>
<td>Expanded CTG18.1 allele, median (IQR)</td>
<td>87.0 (59.0)</td>
<td>87.0 (59.0)</td>
<td>7.9 × 10^{-1}</td>
</tr>
<tr>
<td>KG, mean (SD)</td>
<td>5.3 (1.3)</td>
<td>3.1 (2.2)</td>
<td>3.5 × 10^{-7}</td>
</tr>
</tbody>
</table>

Three individuals appeared in both generations. IQR, interquartile range.

* Fisher’s exact test was performed when comparing sex and FECD affection distribution; two-sample unpaired t-test was performed when comparing age; Wilcoxon-Mann-Whitney test was performed when comparing CTG18.1 expansion; and two-sample paired t-test was performed when comparing KG.

RESULTS

Demographics of Study Subjects

There were a total of 44 parent–child pairs consisting of 76 individuals from 26 families meeting the inclusion criteria. All families were white except for one black family. The demographic information is summarized in Table 1 and Supplementary Table S1. There were 52 individuals that appeared only in the parent generation, 41 only in the child generation, and 3 in both generations. There were more affected individuals in the parent generation (P = 2.2 × 10^{-5}). There were more females in both generations (P = 9.0 × 10^{-2} and 9.6 × 10^{-3} by binomial exact test), without significant difference between the two generations (P = 8.4 × 10^{-1}). There were 3, 11, 10, and 20 male-to-male, male-to-female, female-to-male, and female-to-female pairs, respectively, which indicates no sex-to-sex bias in transmission (P = 5.0 × 10^{-1} by Fisher’s exact test). The expanded CTG18.1 allele appeared in all parents (in this cohort, there was no occurrence of an allele that expanded from <40 repeats to >40 repeats when transmitted). The KG was significantly greater in the parent generation by the paired comparison; however, the difference diminished to insignificance after adjusting for age effect (P = 1.2 × 10^{-1}; Supplementary Fig. S1).

CTG18.1 Polymorphism Genotyping

Genomic DNA of subjects was extracted from leukocytes of peripheral blood samples with the NucleonBlood Extraction Kit (Amersham, Biosciences, Buckinghamshire, UK). The CTG18.1 trinucleotide repeat polymorphism was genotyped using a combination of short tandem repeat (STR) assay, triplet repeat primed PCR (TP-PCR) assay, and Southern blot analysis as our group has previously described.

Statistical Analysis

In transmission investigation, parent–offspring pairs were partitioned into three groups with similar sample sizes based on the parental CTG18.1 repeat length of largest allele: small, \( \leq 77 \) (n = 15); intermediate, 78 to 120 (n = 15); large, >120 (n = 12) repeats. The KG of the more severely affected eye for each subject was reported and used for statistical analysis. Age adjustment of the KG was performed according to the linear regression model: \( KG = age + age^2 + \text{residual} \), based on all individuals. Comparisons of the demographic features were performed by exact tests, paired and unpaired t-tests, and the Wilcoxon-Mann-Whitney test, wherever it is proper, as clarified at each table. Associations between the parental CTG18.1 triplet repeat length and the intergenerational stability and length change of the repeats, as well as KG severity change, were examined by the trend test. Software R (version 3.3.3) was used for statistical analysis.
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Mutant CTG18.1 Allele Length in Parent–Child Transmission

Instability of the expanded mutant CTG18.1 allele was noted in 14 of 44 (31.8%) parent–child pair transmissions (Table 2; Fig. 1; Supplementary Table S1). There were five pairs with further expansion of the mutant allele, of which four were mother–offspring pairs ($P = 3.8 \times 10^{-3}$ by binomial exact test). One mother was present in three of these five pairs. There were seven pairs with contraction of the mutant allele, of which four were father–offspring pairs ($P = 5.2 \times 10^{-1}$ by Fisher’s exact test compared with the noncontraction groups). One father was present in two of these seven pairs. There were three pairs with a difference of $<10$ CTG repeats between generations.

There were two instances of somatic mosaicism or variation in the repeat allele length of parent: one pair wherein the mother carried one allele with mosaic repeat length of 100 to 500 and the daughter inherited an allele with repeat length of 130; another pair wherein the mother carried one allele with mosaic repeat length of 100 to 800 and the daughter inherited an allele with repeat length of 160. Comparisons between the groups are summarized in Table 2.

Next, we examined the association between parental CTG18.1 repeat length and its transmission stability (Table 3). The two pairs with mosaic alleles in mother were excluded. The rate of instability increased with the length of parental repeat length ($P = 6.7 \times 10^{-3}$).

Table 2. Comparison Between Groups With Unstable Transmission of the Mutant CTG18.1 Allele

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Mosaic Group</th>
<th>Expansion Group*</th>
<th>Contraction Group*</th>
<th>$P$ Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parent generation</td>
<td>$N = 2$</td>
<td>$N = 3$</td>
<td>$N = 6$</td>
<td></td>
</tr>
<tr>
<td>Sex, male, no. (%)</td>
<td>0 (0)</td>
<td>1 (33.3)</td>
<td>3 (50.0)</td>
<td>1.0</td>
</tr>
<tr>
<td>FECD affected, no. (%)</td>
<td>2 (100)</td>
<td>3 (100)</td>
<td>6 (100)</td>
<td>1.0</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>79.5 (0.7)</td>
<td>78.0 (9.5)</td>
<td>72.7 (11.8)</td>
<td>$5.0 \times 10^{-1}$</td>
</tr>
<tr>
<td>Expanded CTG18.1 allele, median (IQR)</td>
<td>-</td>
<td>81.0 (23.0)</td>
<td>130.0 (0.0)</td>
<td>$1.1 \times 10^{-2}$</td>
</tr>
<tr>
<td>KG, mean (SD)</td>
<td>5.5 (0.7)</td>
<td>5.3 (1.2)</td>
<td>6.0 (0.0)</td>
<td>$1.6 \times 10^{-1}$</td>
</tr>
<tr>
<td>Offspring generation</td>
<td>$N = 2$</td>
<td>$N = 5$</td>
<td>$N = 7$</td>
<td></td>
</tr>
<tr>
<td>Sex, male, no. (%)</td>
<td>0 (0)</td>
<td>2 (40.0)</td>
<td>1 (14.3)</td>
<td>$5.2 \times 10^{-1}$</td>
</tr>
<tr>
<td>FECD affected, no. (%)</td>
<td>1 (50.0)</td>
<td>3 (60.0)</td>
<td>6 (85.7)</td>
<td>$5.2 \times 10^{-1}$</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>50.5 (4.9)</td>
<td>47.6 (6.1)</td>
<td>47.7 (9.6)</td>
<td>$9.8 \times 10^{-1}$</td>
</tr>
<tr>
<td>Expanded CTG18.1 allele, median (IQR)</td>
<td>145.0 (15.0)</td>
<td>130.0 (20.0)</td>
<td>87.0 (7.0)</td>
<td>$1.1 \times 10^{-2}$</td>
</tr>
<tr>
<td>KG, mean (SD)</td>
<td>1.5 (2.1)</td>
<td>3.0 (2.8)</td>
<td>4.1 (2.2)</td>
<td>$4.3 \times 10^{-1}$</td>
</tr>
<tr>
<td>Trans-generation</td>
<td>-</td>
<td>$N = 5$</td>
<td>$N = 7$</td>
<td></td>
</tr>
<tr>
<td>CTG18.1 allele length difference, median (IQR)</td>
<td>-</td>
<td>53.0 (20.0)</td>
<td>-49.0 (22.0)</td>
<td>-</td>
</tr>
</tbody>
</table>

* There are five pairs in the expansion group with one mother appearing in three pairs, and there are seven pairs in the contraction group with one father appearing in two pairs.
† The statistical comparison is only between the expansion and contraction groups. Fisher’s exact test was performed when comparing sex and FECD affected distribution; two-sample unpaired t-test was performed when comparing age, KG; Wilcoxon-Mann-Whitney test was performed when comparing CTG18.1 expansions.

Figure 1. Correlation of CTG18.1 allele length difference between generations and the parental allele length. Parent–offspring pairs were partitioned into three groups, small, intermediate, and large, with similar sample sizes based on the parental CTG18.1 repeat length of largest allele: $<77$ ($n = 15$), 78 to 120 ($n = 15$), and $>120$ ($n = 12$) triplet repeats. Association was examined by a trend test ($P = 6.7 \times 10^{-3}$).
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TABLE 3. Stability of Mutant CTG18.1 Allele in Parent-Offspring Transmission

<table>
<thead>
<tr>
<th>Stability</th>
<th>Small *(N = 15)†</th>
<th>Intermediate *(N = 15)†</th>
<th>Large *(N = 12)</th>
<th>P Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable</td>
<td>12</td>
<td>10</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Unstable</td>
<td>1</td>
<td>4</td>
<td>7</td>
<td>5.9 × 10⁻³</td>
</tr>
<tr>
<td>Expansion</td>
<td>1</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Contraction*</td>
<td>0</td>
<td>0</td>
<td>7</td>
<td>1.3 × 10⁻³</td>
</tr>
</tbody>
</table>

* Parent–offspring pairs were partitioned into three groups, small, intermediate, and large, based on the parental CTG18.1 repeat length of largest allele: <77, 78 to 120, and >120 triplet repeats. The two pairs with mosaic alleles in mother were not included in this analysis.
† There are three pairs with the measured repeat length difference <10 and were not classified as either stable or unstable.
‡ The trend test was performed to compare the proportions of unstable mutant CTG18.1 allele transmission with the parental CTG18.1 repeat length categories; Fisher’s exact test was performed to compare expansion/contraction ratio between the large and nonlarge groups.

whereas all five unstable transmissions in the nonlarge group were expansions (P = 1.3 × 10⁻³). There is no significant difference of CTG18.1 expansion length between the two generations by a crude comparison (Table 1); however, there is a clear trend of contraction when parental CTG18.1 triplet repeat length is >120 (Fig. 1; P = 6.7 × 10⁻³).

FECD Severity in Parent–Child Transmission of the Mutant CTG18.1 Allele

There are three pairs in which the KG was greater in the child compared with the parent. However, the mutant repeat allele length was stable in all of them. We found no association between KG disease severity difference of a parent–offspring pair and parental repeats length after age adjustment (P = 9.8 × 10⁻²; Fig. 2).

**DISCUSSION**

In this study, we noted instability of the expanded TCF4 CTG18.1 allele in nearly a third of the parent–child transmissions in families with FECD. Additionally, the likelihood of an unstable event increased with the size of the mutant parental allele. We found intermediate-sized mutant CTG18.1 alleles have a tendency for further expansion in contrast to large mutant alleles that have a tendency for contraction in unstable transmissions from parent to child. These patterns of intergenerational instability of the TCF4 triplet repeat expansion may directly impact FECD inheritance because disease severity is positively correlated to repeat length of the mutant allele.5

Because we are at an early stage of understanding FECD molecular pathogenesis and disease inheritance mediated by the TCF4 triplet repeat expansion, it is important for us to learn lessons from other repeat disorders. Parent–child transmission of noncoding repeat expansions is best characterized for myotonic dystrophy type 1 (DM1) caused by a CTG expansion in the 3’ untranslated region of the DMPK gene. There are important parallels between CTG triplet repeat expansions in the TCF4 and DMPK genes; we recently reported an increased risk for FECD in subjects with DM1 via a similar accumulation of toxic CUG repeat RNA nuclear foci in corneal endothelium.6,8

DNA repair and replication mechanisms have been implicated in trinucleotide repeat instability. Trinucleotide repeat expansion occurs in various stages of human germ cell development and is sensitive to the sex of the transmitting parent.13 Six of seven expansions in our cohort were seen with maternal transmission of the mutant TCF4 allele. Although not statistically significant, our observation in FECD parallels that of DM1, as we also found a trend toward loss of expansion and gain of contraction in stable transmissions in the maternal transmission group (Table 1).

**FIGURE 2.** Correlation of age-adjusted KG difference between generations and the parental allele length. Age adjustment of the KG was performed according to the linear regression model: KG = Age + Age² + residual. Parent–offspring pairs were partitioned into three groups, small, intermediate, and large, with similar sample sizes based on the parental CTG18.1 repeat length of largest allele: <77 (n = 15), 78 to 120 (n = 15), and >120 (n = 12) triplet repeats. Association was examined by a trend test (P = 9.8 × 10⁻²).
of other noncoding CTG trinucleotide repeat disorders including DM1, Huntington's disease-like type 2 type, and spinocerebellar ataxia 8, in which expansions occur almost exclusively through maternal transmission.13 In DM1, expansion has been noted at the two-cell stage in preimplantation embryos, and the length of expansion is related to maternal age; both of these observations support the notion that expansion occurs in quiescent oocytes involving DNA repair mechanisms rather than DNA replication mechanisms.13

Five of eight contractions in our FECD cohort occurred with paternal transmission of the mutant TCF4 repeat allele. In the sperm of DM1 males, large trinucleotide repeat expansions contract, and the frequency of the contraction in male transmission is positively correlated to repeat length.15

Our study was adequately powered to detect the instability of the CTG18.1 repeat locus in parent-child transmissions with increasing sizes of parental allele. However, a limitation of this study was that the small sample size precluded a rigorous evaluation of role of sex. Studies on larger FECD cohorts are certainly warranted to assess the role of sex in the transmission of expanded repeat. We speculate that a shared pattern of repeat mutation may emerge in patients with FECD and DM1 with noncoding CTG repeat expansions.

We noted two examples of unstable transmissions in the setting of somatic mosaicism of the parental allele. Both somatic and germline instability of repeat DNA are thought to contribute to unstable intergenerational transmission and disease.15

Anticipation and instability studies of Huntington disease, DM1, and other repeat expansion disorders have used genomic DNA extracted from peripheral blood leukocytes.17-19 Studies on DM1 leukocytes over a 5-year period suggest that the repeat length in patient’s blood cells may continue to expand with time due to somatic mutation.20 Instability of repeats in leukocytes during the lifetime of an individual may contribute to larger alleles in the parental generation. We did not examine the repeat number in endothelial tissue in this study and such experiments are warranted to understand how somatic instability in endothelial tissue impacts disease.

Clinical anticipation is a hallmark of the genetics of DM1 and other repeat disorders characterized by increasing severity of disease and/or earlier onset of disease in subsequent generations. This phenomenon in DM1 has been attributed to the instability of the CTG repeat expansion in the DMPK gene and its tendency to expand in successive generations. However, anticipation was a rare event in our cohort of FECD subjects with the CTG repeat expansion in the TCF4 gene. We found three examples in which the KG of FECD disease inheritance has implications on FECD disease inheritance.

We noted instability of the expanded TCF4 triplet repeat expansions in nearly a third of parent-child transmissions in FECD families. Large mutant CTG18.1 alleles are prone to contraction, whereas intermediate mutant alleles tend to expand when unstably transmitted from parent to child. Intergenerational instability of the TCF4 triplet repeat expansion has implications on FECD disease inheritance.

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References


