CTG REPEAT EXPANSION IN THE *TCF4* GENE IN THAI PATIENTS WITH FECD

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ABSTRACT

Fuchs Endothelial Corneal Dystrophy (FECD) is a hereditary non-inflammatory corneal disease affecting the corneal endothelial cells, resulting in cellular dysfunction, corneal edema, thickened Descemet's membrane, epithelial bullae, and vision loss. CTG repeat expansion in the *TCF4* gene is a well-established genetic risk factor for FECD. To determine the prevalence of CTG repeat expansion in Thai FECD patients, we recruited 100 FECD cases and 100 matched controls. Three-milliliter peripheral blood samples were collected from both cases and controls for DNA extraction. We performed the STR assay and triplet repeat primed (TP) PCR to identify CTG repeat expansion in *TCF4*. CTG repeat expansion with the repeat count exceeding 40 in the *TCF4* gene was identified in 0.5% (1 in 200 alleles) of both FECD cases and controls. No significant differences were observed between the two groups. These results indicate that CTG repeat expansion in the *TCF4* gene may not be the primary underlying factor responsible for FECD within this group of patients. It is possible that locus heterogeneity plays a role in the development of FECD, as other genes, which have not yet been identified, may be associated with this condition.

Keywords: Fuchs Endothelial Corneal Dystrophy (FECD), CTG repeat expansion, STR assay and TP-PCR

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INTRODUCTION

Fuchs Endothelial Corneal Dystrophy (FECD) is a hereditary non-inflammatory corneal disease affecting the corneal endothelial cells, resulting in cellular dysfunction, corneal edema, thickened Descemet's membrane, epithelial bullae, and vision loss. FECD can be caused by pathogenic variants in multiple genes and is classified into two types: early-onset FECD caused by defects in the *COL8A2* gene; and late-onset FECD which is a multifactorial disorder. The responsible genes include the *TCF4*, *TCF8*, *SLC4A11*, *LOXHD1*, and *AGBL1* genes. Genome-wide linkage analysis (GWAS) identified specific SNPs located within *TCF4* on chromosome 18 that exhibited a strong association with FECD. In addition, the most frequent variant associated with FECD is intronic CTG repeat expansion in the *TCF4* gene. (Fautsch et al., 2021) A previous study also revealed that the expansion of CTG repeats within the 18.1 allele significantly elevated the risk of developing FECD. (Mootha et al., 2014) CTG repeat expansion in the *TCF4* gene has demonstrated statistical significance when comparing FECD cases and controls in various populations, including those from Asia, Europe, and Africa. The prevalence of FECD varies across different populations. Globally, the estimated prevalence of FECD is approximately 7.33%, with North America reporting the highest prevalence of 21.62%. The number of individuals with FECD over the age of 30 is expected to increase from 300 million in 2020 to 415 million in 2050, primarily due to the aging population. (Aiello et al., 2022) FECD is more common in the Caucasian population compared to the Asian population and is found more frequently in females than males. (Kitagawa et al., 2002) For instance, the Icelandic Reykjavik Eye Study discovered corneal guttae in 11% of Caucasian females and 7% of Caucasian males. (Zoega et al., 2006) In contrast, a study in a rural southwestern island of Japan reported lower rates, with 5.8% of females and 2.4% of males showing corneal guttae. (Higa et al., 2011) A previous Thai cohort study reported 39% CTG repeat expansion in FECD cases, while no CTG repeat expansion was observed in the control group. (Okumura et al., 2020) This current case-control study aims to investigate CTG repeat expansion in the *TCF4* gene in a larger Thai population from Phanat Nikhom, a district in the northern part of Chonburi province, eastern Thailand. We hypothesize that CTG repeat expansion in the *TCF4* gene plays a significant role in the pathogenesis of FECD in this cohort.

LITERATURE REVIEWS

Fuchs Endothelial Corneal Dystrophy (FECD)

Fuchs Endothelial Corneal Dystrophy (FECD), a hereditary corneal disorder, is characterized by abnormally low numbers of endothelial cells, changes in the thickness of Descemet's membrane, and the formation of guttae. These guttae cause the cornea to thicken and result in impaired vision. In addition, FECD is a prevalent cause of corneal transplantation worldwide. Descemet's membrane consists of an anterior banded layer, a posterior nonbanded layer, and an endothelial cell layer with hexagonal corneal endothelial cells. In late-onset FECD, the anterior banded layer remains normal, but the posterior nonbanded layer is reduced or absent. Furthermore, there are additional abnormal layers, including a posterior banded layer with guttae, a border layer, a fibrillar layer, and an endothelial cell layer with pleomorphic corneal endothelial cells. (Matthaei et al., 2019)

FECD is influenced by several factors. Firstly, older age is associated with a higher risk of developing FECD. (Krachmer et al., 1978) A study by Higa et al. found that the prevalence of corneal guttae in individuals aged 40 and above in the Kumejima population was 4.1%.(Higa et al., 2011) Second, exposure to UVA light sources can lead to DNA damage and may contribute to the development of FECD. UVA has been linked to the activation of CYP1B1, which can convert estrogen to metabolites, potentially explaining why FECD is more common in females than males.(Liu et al., 2020) Third, smoking has been associated with advanced stages of FECD in grades 4-6, particularly in the Caucasian population. It is believed that

smoking may induce oxidative stress in corneal endothelial cells, leading to cell apoptosis and death. (Zhang et al., 2013)

The most common variant and molecular mechanism

Intronic CTG repeat expansion in the *TCF4* gene is the most common genetic variant associated with FECD, and its prevalence varies among different ethnic groups. The molecular mechanisms underlying CTG repeat expansion in corneal endothelial cells involve the generation of CUG repeats in sense mRNA and CAG repeats in antisense mRNA. These repeat expansions lead to the production of toxic peptides composed of poly-Cysteine, poly-Alanine, and poly-Cysteine from sense mRNA, as well as poly-Serine, poly-Alanine, and poly-Glutamine from antisense mRNA. These peptides are produced through non-AUG dependent (RAN) translation and tend to accumulate in corneal endothelial cells, ultimately contributing to the development of FECD. (Zhang et al., 2013)

Short tandem repeat (STR) assay and triplet repeat primed PCR (TP-PCR)

The STR assay utilizes polymerase chain reaction (PCR) to selectively amplify the target repeat expansion. Subsequently, capillary electrophoresis is employed to precisely determine the size of these repeats. This technique serves as a fundamental tool for evaluating repeat expansion.

TP-PCR, on the other hand, comes into play when the STR assay is limited in its ability to detect alleles. This method involves a modified approach, utilizing a forward primer with a fluorescence label and two reverse primers. The first reverse primer is designed to target the repetitive sequence specific to the repeats of interest. Meanwhile, the second reverse primer amplifies products generated in preceding PCR cycles, facilitating the identification of expanded repeats. Like the STR assay, TP-PCR culminates in capillary electrophoresis to ascertain the size of the repeat expansion. (Mootha et al., 2014; Warner et al., 1996)

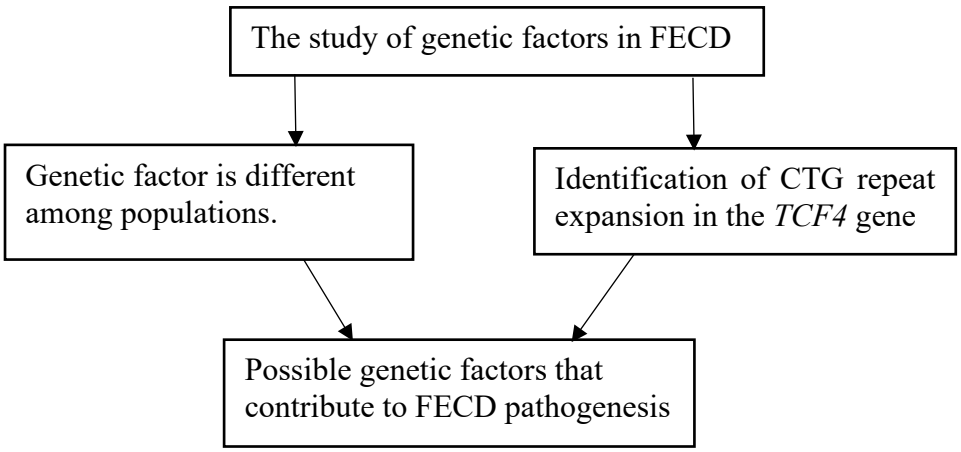

Figure 1 Conceptual Framework

RESEARCH METHODOLOGY

Collection of FECD patients and Eye examination

The study received approval from the Institutional Review Board (IRB) with reference number 0366/66, affiliated with the Faculty of Medicine, Chulalongkorn University. All affected FECD cases and controls were collected from Phanat Nikom, Chonburi, Thailand. Affected FECD cases were diagnosed with FECD by Snellen best-corrected visual acuity (BCVA), automated refraction by KR-8100 auto refractometer (Topcon®, Japan), anterior segment and fundus examinations by a slit-lamp biomicroscopy, intraocular pressure (IOP) measurement by the Goldmann applanation tonometry, and corneal endothelial cell count by Specular Microscopy (Topcon SP 3000-P®, Japan). The severity of FECD is assessed using a modified Krachmer grading system, ranging from grades I to VI. Grade I is characterized by the presence of scattered guttae numbering 12 or fewer, which are non-confluent. Grade II entails scattered

guttae exceeding 12 and remaining non-confluent. Grade III features confluent guttae with a width of 1-2 mm at the widest point. Grade IV involves confluent guttae with a width exceeding 5 mm, without stromal edema. Grade VI is designated when stromal edema is present. Furthermore, the selection of unaffected FECD controls was recruited according to criteria: individuals of both genders aged over 60 years who did not exhibit FECD and were collected from the same population.

Blood Collection and DNA extraction

Three milliliters of peripheral blood were collected from affected FECD patients and unaffected FECD controls in EDTA blood collection tubes for DNA extraction. DNA was extracted using Qiagen Blood Mini Kit (Qiagen, Germany).

Identification of CTG repeat expansion

STR assay was performed to detect the number of CTG repeats in the *TCF4* in all FECD cases and controls. The specific primers for the STR assay and TP-PCR are detailed in Table 1. The cycle of STR assay included initial denaturation at 95°C, 5 minutes, 35 cycles of 95°C, 30 seconds, 60°C, 30 seconds, 72°C, 30 seconds, and 72°C, 5 minutes. The TP-PCR cycle was performed as previously described.(Okumura et al., 2020) When the STR assay could only identify one allele or was unsuccessful in detecting any alleles, we utilized Triplet-Primed Polymerase Chain Reaction (TP-PCR) to ascertain the presence of repeat expansions. In this study, a repeat count exceeding 40 was categorized as an expansion, while counts below 40 were classified as non-expansion.

Table 1 STR assay and TP-PCR for detection of CTG repeat expansion in the *TCF4* gene (Mootha et al., 2014)

Primer	**Sequence**
STR assay	
P1 (Forward)	AATCCAAACCGCCTTCCAAGT
P2 (Reverse)	CAAAACTTCCGAAAGCCATTTCT
TP-PCR	
P1 (Forward)	AATCCAAACCGCCTTCCAAGT
P3 (1st Reverse)	TACGCATCCCAGTTTGAGACG
P4 (2nd Reverse)	TACGCATCCCAGTTTGAGACGCAGCAGCAGCAGCAG

Statistical analysis

Pearson's chi-square test was performed between cases and controls using SPSS 15.0 software. Statistical significance was determined with a threshold of $p<0.05$.

RESEARCH RESULTS

A total of 100 individuals diagnosed with FECD were included in the study, comprising 57 females and 43 males, with a mean age of 64.15 years. Additionally, 100 unaffected FECD individuals were recruited as controls, with 51 females and 49 males and a mean age of 68.57 years. STR assay and TP-PCR were used to identify CTG repeat expansion with a repeat count exceeding 40 being considered an expansion, while counts less than 40 were classified as non-expansions. Among the FECD cases, 99 individuals did not exhibit CTG repeat expansion in the *TCF4* gene, whereas only one individual with FECD had CTG repeat expansion in one allele (28, expansion). In the control group, 99 individuals did not exhibit CTG repeat expansion in the *TCF4*. Nonetheless, one individual among the controls was found to have CTG repeat expansion (11, expansion). CTG repeat expansion in the *TCF4* was identified 0.5% (1 in 200) in FECD cases and controls. In this study, CTG repeat expansion in the *TCF4* gene was identified only 0.5% of both FECD cases and controls. There was no statistically significant difference between two groups.

Table 2 Demographic data and CTG repeat expansion result

	FECD cases (n=100)	Control (n=100)
Gender (male/female)	43/57	49/51
Mean age (year)	64.15	68.57
Presence of CTG repeat expansion (the number of repeat)	1 (28, >40)	1 (11, >40)

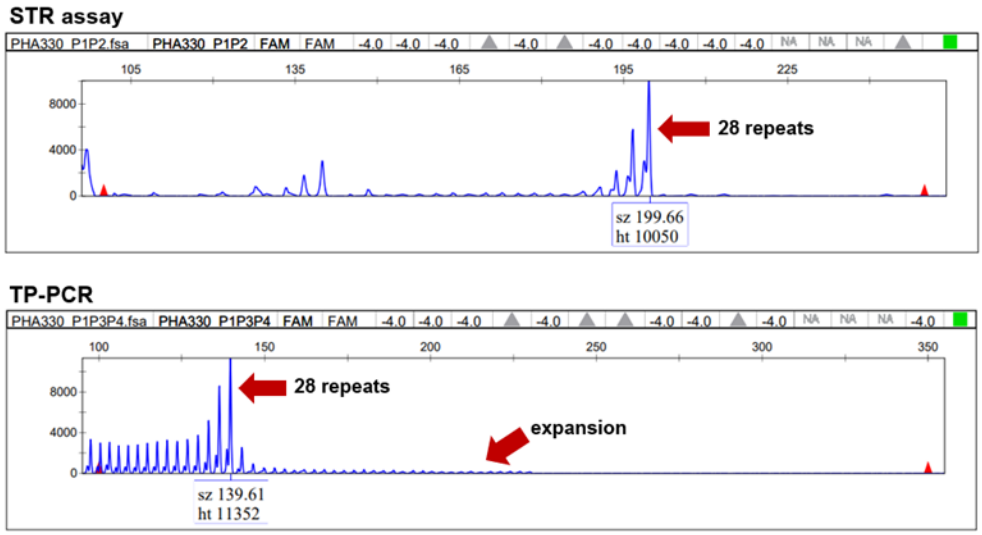

Figure 2 CTG repeat expansion in the *TCF4* gene in cases with 28 repeats and repeat expansion

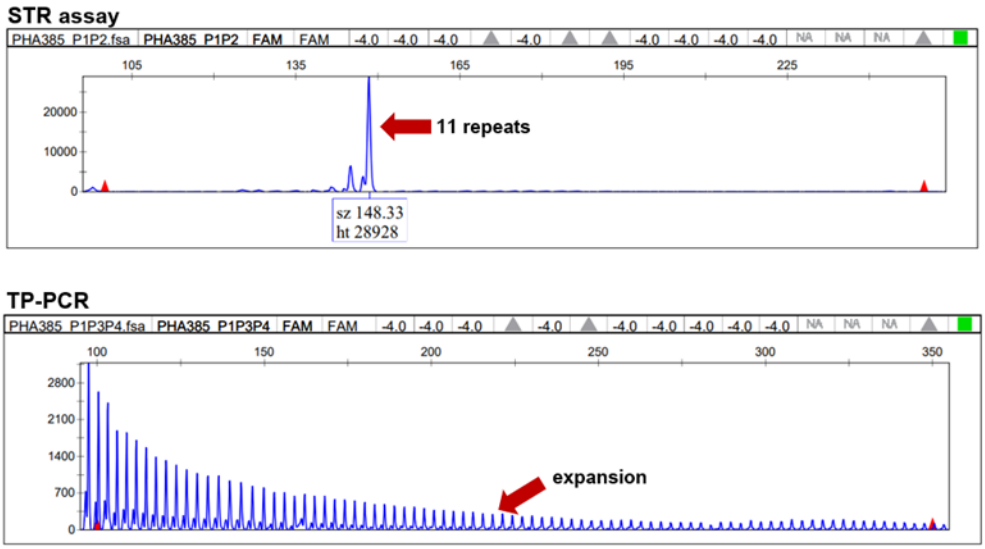

Figure 3 CTG repeat expansion in the *TCF4* gene in controls with 11 repeats and repeat expansion

DISCUSSION & CONCLUSION

This study investigated CTG repeat expansion in the *TCF4* gene in a larger Thai population from Phanat Nikhom, a district in the northern part of Chonburi province, eastern Thailand. The CTG repeat expansion in the *TCF4* was not different between cases and controls. These results did not support the findings of Okumura et al., (Okumura et al., 2020), revealing that *TCF4* repeat expansion in the Thai population was absent in controls but present in 39% of patients. The study of CTG repeat expansion in the *TCF4* has been performed in different populations. In the British population, 77.3% of FECD patients had CTG repeat expansion,

while only 4.2% of controls exhibited this expansion. (Zarouchlioti et al., 2018) Additionally, studies in the United States reported a range of 63-79% of FECD patients having CTG repeat expansion, while controls showed a range of 3-7% with this expansion. (Eghrari et al., 2017; Mootha et al., 2014; Wieben et al., 2012) In the Asian population, CTG repeat expansion (>50 CTG repeats) in the *TCF4* gene was detected in 26% of cases and was absent in the control group among the Japanese population. No clinical differences were observed between the cases and controls. (Nakano et al., 2015) It remains possible that CTG repeat expansion in the *TCF4* gene may not be the primary causative factor for FECD in the population of Phanat Nikhom, Chonburi, Thailand. Other genes could play a role in the development of FECD in this community. In summary, CTG repeat expansion in the *TCF4* gene is not associated with the development of FECD in the patients from Phanat Nikhom, Chonburi, Thailand. Further studies are required to determine other genes or factors associated with FECD.

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