**Case Report**

*KIF21A Gene c.2860C>T Mutation in CFEOM1A: The First Report from Iran*

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**Abstract**

Congenital Fibrosis of the Extra Ocular Muscles (CFEOM1) is an autosomal dominant condition, caused by mutation in the *KIF21A* and *TUBB3*. It is characterized by congenital non-progressive restrictive ophthalmoplegia and ptosis. Mutational analysis of the known genes in such rare diseases by Sanger sequencing not only prevents wasting the time and expenses but also speeds diagnosis process, genetic counseling, and the possibility of prenatal diagnosis. Here, for the first time, association of pathogenic variant c.2860C>T in *KIF21A* gene in an Iranian family with positive history of CFEOM1A was reported.

**Keywords**: Fibrosis of extra ocular muscles, Iran, Mutation, Prenatal diagnosis

**Introduction**

Congenital Fibrosis of the Extraocular Muscles (CFEOM) is characterized by congenital non-progressive ophthalmoplegia with or without ptosis affecting part or all of the ocular motor and/or the trochlear nucleus with its related nucleus and nerve 1. According to the clinical difference in the phenotype, CFEOM is subdivided to seven types including CFEOM1 (OMIM 135700) 1, CFEOM2 (OMIM 602078) 2, CFEOM3A (OMIM 600638) 3, CFEOM3B (OMIM 135700) 4, CFEOM3C (OMIM 609384) 5, Tukel syndrome (OMIM 609428) 6, and CFEOM5 (OMIM 610004) 7. Literature reviews revealed pathogenic variants in the *TUBB1* (Tubulin Beta 1 Class VI), *TUBB2* (Tubulin Beta 2 Class II), *TUBB3* (Tubulin Beta 3 Class III) 5, *TUKLS* (Tukel syndrome) 6, *KIF21A* (Kinesin Family Member 21A) 8, *COL2A1* (Collagen Type XXV Alpha 1 Chain) 7 and *PHOX2A* (Paired Like Homeobox 2a) 2 genes in different types of CFEOM.

Classic CFEOM shows bilateral ophthalmoplegia with the eyes fixed in an infraducted position about 20 to 30 degrees below the horizontal midline. But CFEOM3 phenotype has more variable clinical features as unilateral eye involvement and may be able to raise the eyes above midline 9. Inheritance pattern of CFEOM5, CFEOM2 and Tukel syndrome is autosomal dominant but CFEOM1 and CFEOM3 are autosomal recessive 10.

The first time, Yamada et al reported mutations in the *KIF21A* in 45 patients with CFEOM1 phenotype 8. This study for the first time reported association of c.2860C>T *KIF21A* in the CFEOM1A phenotype in an Iranian family.

**Case Presentation**

Proband was a 31-year-old man (III2) referred to Ophthalmology Department, Vasei Hospital on Dec. 2016 with severe bilateral restricted eye movements and ptosis since birth (Figure 1). His intellectual and social ability were satisfying and there were no other clinical symptoms as growth parameters abnormality, abdominal, respiratory and cardiovascular problems. Eye examination showed significant limitation of abduction, limitation of adduction and limitation of depression bilaterally. To compensate ptosis, 20 degree chin-up head position was noted. Fundoscopic observation detected no pigmented retinopathy and optic atrophy. Pupillary function and anterior segment examinations were within normal limits. Due to the positive family history with similar ocular abnormalities across three generations (Figure 2), proband and his family received clinical genetic service.

**Keywords**: Fibrosis of extra ocular muscles, Iran, Mutation, Prenatal diagnosis

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**Figure 1. External photograph of II:7, III:2, III:9.**
Patient II:7 is a 54 year old man who was born with bilateral ophthalmoplegia and ptosis. Levator function was absent in both eyes. Primary vertical position of each eye was infraducted. Patient III:9 was a 14 year old boy who was born with typical signs of ptosis and complete restriction in eye movements. Ptosis was slightly improved after surgery at the age of 6 in the right eye.

All 3 patients had a normal cornea, iris, lens, and fundus appearance. Phenotype of the referring family has been suspected to be similar to the CFEOM 1. For time and cost saving, instead of doing Whole Exome Sequencing (WES) or performing Sanger sequencing on the known genes, according to the literature reviews, only KIF21A and TUBB3 were sequenced which are involved in the most common form of CFEOM.

Sanger sequencing
Ethical committee of Sabzevar University of Medical Sciences confirmed the study. Consent form was collected from all the members of the family that participated in the study. For performing molecular experiments, 5 ml peripheral blood was collected from each sample and was kept in EDTA tubes. According to the extraction kit (C.N. DN 8115C Sina colon, Iran), genomic DNA was extracted from peripheral blood. Considering the mutation reports of KIF21A and TUBB3 in the literatures, exons 8, 20, 21 of the KIF21A gene and exons 1, 2, 3, 4 of TUBB3 gene were amplified using sequence specific primers (Table 1).

Optimal temperature conditions were as following: 5 min at 95°C, 35 cycles of 30 s at 95°C, 30 s at 57°C, and 1 min at 72°C. Then, Sanger sequencing was performed on purified amplicons (high throughput Applied Biosystems 3730XL sequencers). To analyze the results, the sequences were monitored using Finch TV software version 1.4.0.

Results
Data showed a heterozygote mutation c.2860C>T in the exon 21 of the KIF21A. c.2860C>T mutation changed the 954th amino acid of KIF21A from Arginine to Tryptophan (p.Arg954Trp). For validating the pathogenic variant, segregation was extended on the rest of family members (wild type and patient individuals). Segregation results confirmed c.2860C>T variant in the patients (Figure 3).

Discussion
In this paper, for the first time, the association of pathogenic variant c.2860C>T in KIF21A gene in an Iranian family with positive history of CFEOM1A was reported. NM_001173464.1 (KIF21A): c.2860C>T is known in ClinVar, uniprot and dbSNP databases as a pathogenic variant and predictor tool such as phyloP, Grantham, SIFT and Mutation Taster if this change is deleterious and disease causing [Alamut Visual version 2.9 (Interactive Biosoftware, Rouen, France)].

CFEOM1 is subdivided to CFEOM1A and CFEOM1B with mutation in KIF21A and TUBB3, respectively 11. CFEOM1A is the most common form of CFEOM1 with autosomal dominant inheritance pattern that is characterized by congenital non-progressive restrictive ophthalmoplegia and ptosis 12.

c.1067T>C, c.2830G>C, c.2839A>G, c.2840T>G, c.2840T>C, c.2841G>A, c.2860C>T, c.2861G>A, c.2861G>T, c.3022G>C, c.3029T>C) and a deletion c.3000_3002delTGA (p.Asp1001del) at codon 1001


nese family with congenital fibrosis of the extraocular muscles type 1. Medicine (Baltimore) 2017;96(38):e8068.
