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 (CFEOM) 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 oculomotor and/or the trochlear nucleus with its related nucleus and nerve. According to the clinical difference in the phenotype, CFEOM is subdivided to seven types including CFEOM1 (OMIM 135700), CFEOM2 (OMIM 602078), CFEOM3A (OMIM 600638), CFEOM3B (OMIM 135700), CFEOM3C (OMIM 609384), Tukel syndrome (OMIM 609428), and CFEOM5 (OMIM 610047). 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), TUKLS (Tukel syndrome), KIF21A (Kinesin Family Member 21A), COL2A1 (Collagen Type XXV Alpha 1 Chain), and PHOX2A (Paired Like Homeobox 2a) 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. Inheritance pattern of CFEOM5, CFEOM2 and Tukel syndrome is autosomal dominant but CFEOM1 and CFEOM3 are autosomal recessive.

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. Funduscopic 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.
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 ºC, 35 cycles of 30 s at 95 ºC, 30 s at 57 ºC, and 1 min at 72 ºC. 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, uniport 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 15. 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.

KIF21A with 38 exons is located on 12q12 chromosome. It proceeds microtubule-stabilization through balancing polymerization and depolymerization 13. Mutational analysis of KIF21A in CFEOM1 confirmed 13 different mis-sense mutations (c.84C>G, c.1056C->G, c.2860C>T, etc.) (Figure 3).

Table 1. TUBB3 and KIF21A primer sequences for conventional PCR

<table>
<thead>
<tr>
<th>Gene (exon/s)</th>
<th>Forward sequence</th>
<th>Reverse sequence</th>
<th>Amplicon size</th>
</tr>
</thead>
<tbody>
<tr>
<td>tubulin beta-3(1)</td>
<td>CAGCTCCTCTGGGAGACA</td>
<td>CATCCCTTTGTTGAGGTGTT</td>
<td>485 bp</td>
</tr>
<tr>
<td>tubulin beta-3(2)</td>
<td>GAGGGCTAAAAAGGCTTCACA</td>
<td>GTTGCTGAGACCTGTGAGTT</td>
<td>272 bp</td>
</tr>
<tr>
<td>tubulin beta-3(3)</td>
<td>CGGGCACAGAATCTCAAGAA</td>
<td>TCATGTGAGAGCTAGACCAT</td>
<td>300 bp</td>
</tr>
<tr>
<td>tubulin beta-3(4)</td>
<td>TGCCCTTGGAGATGTTCAG</td>
<td>GGGATCCACTCCAGAAGTA</td>
<td>846 bp</td>
</tr>
<tr>
<td>tubulin beta-3(4)</td>
<td>GTTCGATGCCAAGAACATGA</td>
<td>AGCTCTTCTGCTGTCCCAG</td>
<td>862 bp</td>
</tr>
<tr>
<td>KIF21A(8)</td>
<td>TTTTGTACATTTTTAGGCTTTT</td>
<td>AAAAGTCCAGGCTTATGAGTG</td>
<td>306 bp</td>
</tr>
<tr>
<td>KIF21A(20-21)</td>
<td>TGTGTACTTAAATGAAAAATGGCTC</td>
<td>AGAGAATCTGAAAAAGCAAAGCAGG</td>
<td>794 bp</td>
</tr>
</tbody>
</table>
development of the oculomotor axons, neuromuscular through failure in transferring cargo essential to the
2.
1.
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disease prior to do Whole Exome/Genome Sequencing
to record the last update of known genes in the rare
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from Japan, Hung Kung, America, and Europe.
OM1A cases develop disease by the same way as cases
the present study, it was indicated that Iranian CFE-
served regions of the
14,15. Mutated
KIF21A
probably leads to CFEOM1
mutations. Scheme of the Kinesin protein structure and the relative most common variant locations of KIF21A.
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
14,15. Mutated KIF21A probably leads to CFEOM1
chain or extraocular muscles 8. Our data was in line
with the previous reports and existing in silico predic-
tors.
Chan et al genotyped a pedigree with CFEOM1
phenotype from Iran which they did not find any
known variant 16. In figure 4, 3 of most common path-
ologic variants are illustrated that disrupt the con-
erved regions of the KIF21A protein. Interestingly, in
the present study, it was indicated that Iranian CFE-
OM1A cases develop disease by the same way as cases
from Japan, Hung Kung, America, and Europe. KIF-
21A is a causative gene in more than 50% of CFEOM1
phenotype in the case reports that were not hitherto
pointed in Iranian families 14. Recurrence risk ratio of
affected offspring is approximately 50% in each gener-
ation in CFEOM1.

Conclusion
Therefore, results of this study demonstrated that
genetic clinicians should refer to the literature reviews
to record the last update of known genes in the rare
disease prior to do Whole Exome/Genome Sequencing
(WES/WGS) which most of families couldn’t afford it.
Sanger sequencing of known genes not only saves time
and needs less cost but also facilitates prognosis, genet-
ic counseling and Prenatal Diagnosis (PND).

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University of Medical Sciences.

Conflict of Interest
The authors declare no conflict of interest.

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