

# KIF21A novel deletion and recurrent mutation in patients with congenital fibrosis of the extraocular muscles-1

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**Abstract.** Kinesin family member 21A (KIF21A) mutation is the most common cause for congenital fibrosis of the extraocular muscles type 1 (CFEOM1) in populations worldwide. However, only 12 missense mutations have been reported to date. In this study, KIF21A screening was performed in two Chinese families with CFEOM1. Ophthalmological examinations were performed. The coding exons and adjacent intronic regions of KIF21A were analyzed with cycle sequencing. The novel mutation identified was further evaluated in 150 normal control individuals and available family members. Two heterozygous mutations in KIF21A, c.3000\_3002delTGA (p.Asp1001del) and c.2861G>A (p.Arg954Gln), were detected in two families. The novel deletion involves a conserved residue in the coiled-coil domain of KIF21A and is co-segregated with the disease in the examined family, yet was absent in the 300 control chromosomes. In addition, apart from typical phenotypes for CFEOM1, optic disc hypoplasia was also observed in two patients. Deletion mutation in KIF21A has not been previously reported. Our study expands the KIF21 mutation spectrum. This study adds to the current state of knowledge about KIF21A mutations and CFEOM1, which may improve future clinical practice.

## Introduction

Congenital fibrosis of extraocular muscles (CFEOM), a rare hereditary eye-movement disorder with prevalence of 1/230,000 (1), is characterized by non-progressive restrictive external ophthalmoplegia and ptosis caused by hypotrophia of cranial neurons that are related to ocular motility and its corresponding nerves (2,3). CFEOM sometimes accompanies progressive cerebellar ataxia, epilepsy caused by central nervous system malformation, psychomotor retardation, and muscle hypotonia. By relying on different types of inheritances and phenotypes,

CFEOM has been classified into three types: CFEOM1 (autosomal dominant: MIM 135700), CFEOM2 (autosomal recessive: MIM 602078), and CFEOM3 (genetically and clinically heterogeneous: CFEOM3A, MIM 600638; CFEOM3B, MIM 135700; and CFEOM3C, MIM 609384). Mutations in kinesin family member 21A (KIF21A, MIM 608283) are responsible for CFEOM1 (4) and CFEOM3B (5) while mutations in ARIX (MIM 602753) affect CFEOM2 (6).

CFEOM1, the most common form of CFEOM, is characterized by bilateral congenital anchoring of the eyeballs in a downward gaze, ptosis, and a backward tilt of the head. The disease is transmitted as an autosomal dominant trait that is fully penetrable. All patients have the same typical phenotypes, including ptosis and eyes fixed in an infraducted position about 20 to 30 degrees below the horizontal midline (3). Neurological research revealed hypoplasia of oculomotor and trochlear neurons (7). The majority of CFEOM1 patients have symptoms caused by mutation in KIF21A that is located in 12q12 (4). To date, only 12 heterozygous missense mutations of KIF21A have been reported (4,5,8-10).

In this study, we identified a novel deletion mutation c.3000\_3002delTGA (p.Asp1001del) in one Chinese family and a recurrent missense mutation c.2861G>A (p.Arg954Gln) of KIF21A in another.

## Materials and methods

**Subjects.** This study was approved by the Institutional Review Board of the Zhongshan Ophthalmic Center. We obtained written informed consent from the families to collect clinical data and genomic samples. We adhered to the tenets of the Declaration of Helsinki and followed the Guidance of Sample Collection of Human Genetic Diseases (National 863-Plan) by the Chinese Ministry of Public Health of China. Ophthalmological examinations included a visual acuity test, slit lamp examination, fundus observation, and measurement of eye movement. The diagnosis of CFEOM1 was based on previous literature (4,11). Genomic DNA from two unrelated families with CFEOM1 was retrieved from our Genomic DNA repository for hereditary eye diseases.

**Mutation screening.** The individual exon and the adjacent intronic regions of KIF21A (NCBI human genome build 37.2, NC\_000012.11, NM\_001173464.1, NP\_001166935.1) were amplified by polymerase chain reaction (PCR) using

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**Key words:** kinesin family member 21A, congenital fibrosis of the extraocular muscles type 1, novel deletion, optic disc hypoplasia

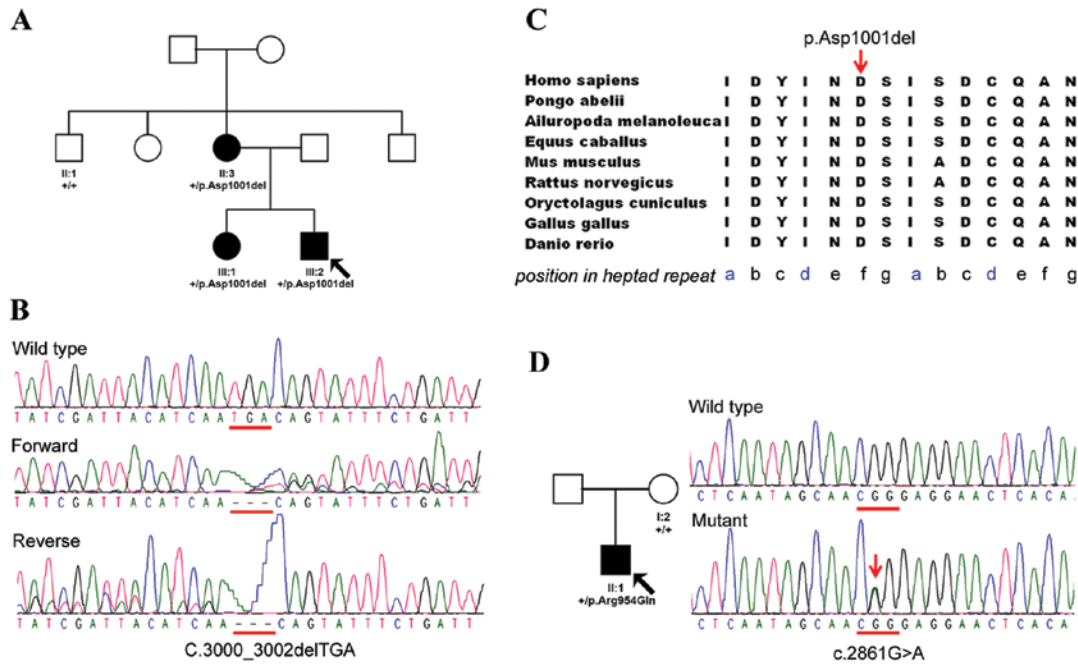


Figure 1. KIF21A mutations in patients with CFEOM1 (congenital fibrosis of the extraocular muscles 1). (A) The dominant pedigree QT742 with CFEOM1. (B) Sequencing chromatograms showing the novel KIF21A mutation in the QT742 family (indicated by the red underlining). (C) The mutation c.3000\_3002delTGA caused the deletion of a highly conserved residue 1001 in KIF21A (p.Asp1001del, indicated by the red arrow). Positions a-g of the heptad repeat sequence are denoted. (D) The recurrent mutant c.2861 G>A was detected in a sporadic case in the QT198 family (indicated by the red arrow).

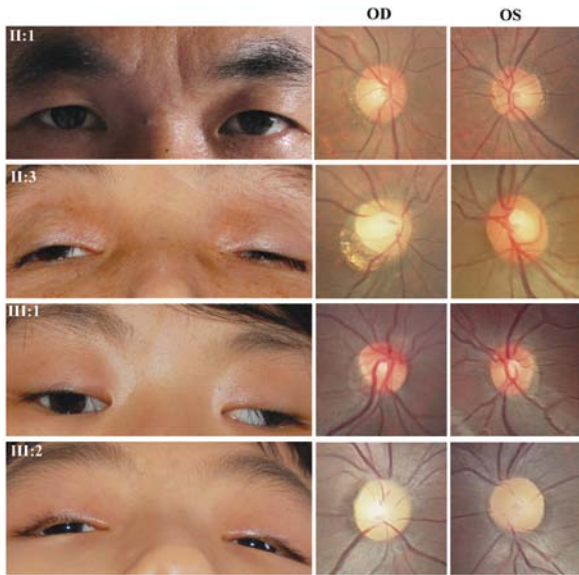


Figure 2. The typical phenotypes of CFEOM1 in family QT742. When compared with the control individual II:1, typical CFEOM1 phenotypes including narrow palpebral fissure, ptosis, and raised arched eyebrows could be observed in II:3, III:1 and III:2 in the QT742 family (left column). Fundus photographs of QT742 family members are shown in the middle (OD) and right (OS) columns. Optic nerve hypoplasia was observed in patient II:3 and patient III:1.

previously reported primers (4). Amplicons were analyzed by cycle sequencing using the ABI BigDye Terminator cycle sequencing kit v3.1 and the ABI 3100 Genetic Analyzer (ABI Applied Biosystems, Foster City, CA, USA). Sequencing results were compared with consensus sequences of KIF21A using the SeqMan II program of the Lasergene package (DNASTar, Inc., Madison, WI, USA). Mutation descriptions followed the

nomenclature recommended by the Human Genomic Variation Society (<http://www.hgvs.org/mutnomen/>). Mutations were confirmed by using bi-directional sequencing before they were evaluated in the 300 chromosomes of 150 control individuals.

**Results**

Two heterozygous mutations in KIF21A were identified in the two families, including a novel c.3000\_3002delTGA (p.Asp1001del) and a recurrent c.2861G>A (p.Arg954Gln), mutation, respectively (Fig. 1A and D). The c.3000\_3002delTGA variant results in a loss of aspartic acid at codon 1001, which is located in the third coiled-coil domain of the KIF21A protein. This acidic residue is highly conserved throughout evolution in different species (Fig. 1C). The c.2861G>A variant is the second most common mutation in KIF21A and it has been identified in Caucasian, Venezuelan, Turkish and Chinese families (4,9,12). These two mutations were not present in the 150 control individuals.

The 4 patients in the two families showed typical signs of CFEOM1: bilateral congenital non-progressive ophthalmoplegia, ptosis, infraducted primary position of both eyes without the ability to raise the eye above the midline, and a backward tilt of the head. The proband in QT198 was a six year-old boy with CFEOM1, but without a family history. His best visual acuity was 0.1 (OD) and 0.2 (OS). Ocular movement was completely restricted. He had a normal cornea, iris, lens, and fundus appearance. In the other family, QT742, three patients across two generations were identified (Fig. 2). The proband (III:2) was a twelve year-old boy who suffered from bilateral ptosis after birth. His best visual acuity was 0.4 (OD) and 0.5 (OS). The palpebral fissure size was measured as 4 mm in both eyes and raised arched eyebrows to compensate for ptosis were

noted. The movement of both eyes was completely restrictive. The sister and mother of the proband also presented typical signs of CFEOM1 (Fig. 2). Bilateral palpebral fissure sizes were about 2 mm for the mother and 6 mm for the sister. CFEOM phenotype and KIF21A mutation were not observed in the normal family member (II:1). All 3 patients in the QT742 family had normal corneas, irises, and lenses, but 2 patients had hypoplasia of the optic disc (Fig. 2).

## Discussion

In this study, we identified a novel deletion (c.3000\_3002delTGA, p.Asp1001del) and a reported missense mutation (c.2861G>A, p.Arg954Gln) in KIF21A in 2 Chinese families with CFEOM1. These mutations were co-segregated with the disease in the families and were absent in 300 control chromosomes. All 4 patients with KIF21A mutation had typical phenotypes of CFEOM1.

KIF21A consists of 38 exons with alternative splicing of exon12 and exon29-31. It is predicted to encode a plus-end kinesin motor protein KIF21A that ATP-dependently transports cargo forming a dimer along microtubules and is important to the development of the oculomotor nerve and extraocular muscles (4,13). The KIF21 protein is made up of 3 parts: the high conserved kinesin motor domain interacting with microtubules in the N-terminus, the central stalk with 4 coiled-coil regions important for dimer formation, and the tail domain with WD40 repeats in the C-terminus that is responsible for cargo loading. Only 12 missense mutations in KIF21A have been reported (4,5,8-10): two of them are located in the motor domain (C28W located in exon2 and M356T located in exon8) and the rest are clustered in the third coiled-coil region (E944Q, M947V, M947R, M947I and M947T in exon20 and R954W, R954Q, R954L, A1008P and I1010T in exon21). Substitution changes may affect the dimer formation of KIF21A (14,15). By contrast, p.Asp1001del was predicted to change the structure of the following heptad repeat units with a subsequent impact on the electrostatic potential, which may be the cause of the CFEOM phenotype in the QT742 family. This finding adds to our understanding of the etiology of CFEOM caused by the KIF21A mutation.

Optic nerve hypoplasia or optic disc colobomas were noticed in Caucasian and Arab patients with CFEOM, and a few of them carried the R954W mutation in KIF21A (16,17). Several studies about KIF21A screening in Chinese patients with CFEOM have been published in recent years (12,18-20), but none of them describe the optic abnormalities in CFEOM patients. In the QT742 family, in addition to the classical phenotypes, optic nerve hypoplasia was observed in patients II:3 and III:1, but not in the proband with the same mutation. Our finding supports the optical phenotype abnormality that exists in some patients with CFEOM1 caused by the KIF21A mutation in different populations. Careful fundus examination should be performed in patients with suspected CFEOM mutations, which may improve diagnosis and patient care.

In summary, we identified a novel deletion mutation c.3000\_3002delTGA (p.Asp1001del) and a recurrent missense mutation c.2861G>A (p.Arg954Gln) in KIF21A in two families with CFEOM1. Our study expands the KIF21 mutation spectrum.

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