

# Congenital fibrosis of extraocular muscle type 1A due to *KIF21A* mutation: first case report from Hong Kong

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With the advancement of ophthalmological genetics, the molecular basis for more and more eye diseases can be elucidated. Congenital fibrosis of extraocular muscle (CFEOM) is an example. It is characterised by a congenital non-progressive restrictive ophthalmoplegia and ptosis. It is an autosomal dominant disease, caused by mutations of the *KIF21A* gene. With positive family history and typical ophthalmological findings, mutational analysis of *KIF21A* gene should be performed, not only to confirming the diagnosis, but also to offer a prognosis, for genetic counselling, and the possibility of prenatal diagnosis. Here we report the first *KIF21A* mutation associated with CFEOM1A in Hong Kong.

## Introduction

Congenital fibrosis of extraocular muscle (CFEOM) was first described by HW Brown in 1950, as a kind of congenital strabismus syndrome.<sup>1</sup> In 2002, it was re-classified under the category 'congenital cranial dysinnervation disorder' that was caused by primary neurological maldevelopment of ocular muscle and facial innervation.<sup>2</sup> To date, seven subtypes of CFEOM are recognised; namely CFEOM 1A, 1B, 2, 3A, 3B, 3C, and Tukel syndrome. Among these, CFEOM1 (OMIM #135700) is the most common and is characterised by congenital non-progressive restrictive ophthalmoplegia and ptosis. The upward gaze of affected individuals is severe; the eyes are completely or partially fixed in an infraducted and strabismic position. Typically, the patient has a chin-up head position to compensate for ptosis and ophthalmoplegia. It is an autosomal dominant disorder, which is subdivided into types 1A and 1B based on the underlying genetic defects. In 2003 and 2010, *KIF21A* and *TUBB3* were identified as the disease-causing genes for CFEOM1A<sup>3</sup> and CFEOM 1B,<sup>4</sup> respectively. Although CFEOM1 has been reported in many parts of the world, it is rare; the reported prevalence (mostly in western populations) is around 1 in 230 000.<sup>5</sup>

## Case report

The proband was a 7-year-old boy who presented to us in July 2011 for bilateral ptosis and restrictive ophthalmoplegia since birth. He was the first child of a non-consanguineous Chinese couple, born at full term with a birth weight of 3.1 kg. The perinatal history was unremarkable. His mother also had congenital non-progressive bilateral ptosis and restrictive ophthalmoplegia but had not sought medical advice.

The parents noticed that their son had bilateral droopy eyelids since birth. Otherwise his feeding and growth were satisfactory and there were no other systemic symptoms. He was referred to the regional paediatric and ophthalmology unit by the Maternal and Child Health Centre at 2 months of age. Eye assessment showed bilateral ptosis and left convergent squint. As both of his visual axes were blocked by the eyelids and affected his visual development, surgical correction for his congenital ptosis by frontalis slings was performed when he was 6 months old. On follow-up, apart from residual ptosis, he was noted to have impaired vertical eye movement.

Further investigations for underlying causes were performed and included the tensilon test, anti-acetylcholine receptor antibody, metabolic investigations including serum lactate and pyruvate levels were all non-contributory. Computed tomography of the eyes and brain were normal. Magnetic resonance imaging showed mild thinning of superior oblique muscles bilaterally, but without structural brain abnormalities.

Apart from the left eye amblyopia that required occlusion therapy and mild astigmatism, his ophthalmoplegia and ptosis were static and did not deteriorate over the last few years. There was no other systemic symptom and he enjoyed normal growth and development. In view of the positive family history of similar ocular abnormalities, the proband and his mother were referred to our clinical genetic service for evaluation.

### Key words

Chinese; DNA mutational analysis; Oculomotor muscles; Ophthalmoplegia; Pedigree

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Physical examination showed normal growth parameters without craniofacial dysmorphism apart from bilateral ptosis. His forehead muscles were constantly contracted and he adopted a 'chin-up' head position for compensation. He had no lid lag and no lagophthalmos. In the primary gaze position, the left eye was esotropic and infraducted. Regarding extraocular eye movements, his vertical gaze was severely impaired with an inability to raise both eyes above the horizontal midline. On attempted upward gaze, aberrant eye movements displayed synergistic convergence. For horizontal movement, there was mild limitation in adduction of the right eye. For the left eye, however, both abduction and adduction were significantly impaired. No globe retraction was noted during eye closure. There was no Marcus Gunn jaw-winking phenomenon. Pupil reactions were normal.

### KIF21A基因變異引致先天性眼外肌纖維化 1A型：香港首宗病例報告

隨着眼科遺傳學的進步，越來越多眼疾的分子基礎已被闡明。先天性眼外肌纖維化（CFEOM）是其中一個例子，此病的特點是先天性眼外肌麻痺和眼瞼下垂。CFEOM1A是由於KIF21A基因變異而引致的一種常染色體顯性遺傳病。在有家族史和典型眼科臨床症狀的情況下，患者應進行KIF21A基因分析。這樣不但可以確診此症，而且對遺傳諮詢有幫助，並可預測復發風險及作產前診斷。這是香港利用分子檢測先天性眼外肌纖維化的首宗確診病例。

Fundoscopy examination showed no pigmentary retinopathy or optic atrophy. Other cranial nerves and the rest of the neurological examination were normal. No fatigability could be demonstrated. Cardiovascular,

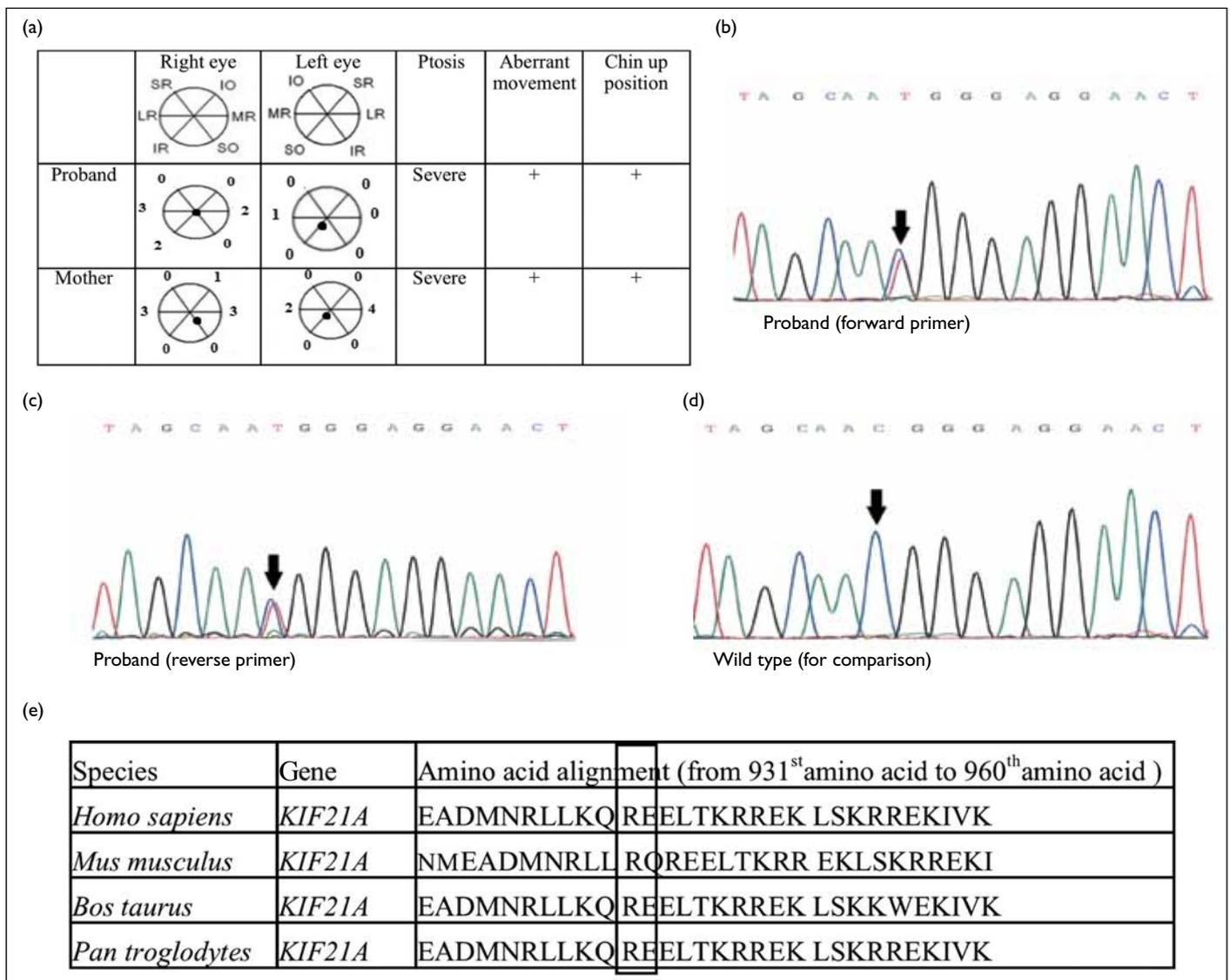


FIG. (a) Summary of the ocular features of our proband and his mother. The second and third columns document the range of extraocular movements in each eye and the position of the eyes in primary position. Extraocular movements are rated on a scale from 0 to 4, with 0 indicating no movement in that direction and 4 indicating normal movement in that direction. The black dot indicates the position of the pupil within the orbit in primary gaze. SR = superior rectus; IR = inferior rectus; LR = lateral rectus; MR = medial rectus; SO = superior oblique; IO = inferior oblique. (b-d) DNA sequence chromatographs of our patients. (e) The alignment of amino acids around Arg941 of *KIF21A* revealed evolutionary conservation of this residue from *Pan troglodytes*, *Bos taurus*, *Mus musculus* to *Homo sapiens*

respiratory, and abdominal examinations were also normal. The eye examination findings of his mother were similar, except that she had less severe ptosis and relatively normal horizontal eye movement. The ocular findings of proband and his mother are summarised in Figure a.

Because of familial congenital non-progressive restrictive ophthalmoplegia and ptosis without significant positive investigation results, the clinical diagnosis of autosomal dominant CFEOM was suspected. Accordingly, *KIF21A* mutational analysis was then performed.

Informed consent was obtained from parents for molecular study. Genomic DNA was extracted from peripheral blood using a QIAmp DNA minikit (Qiagen, Germany) as per the instruction of the manufacturer. The coding sequence of exons 8, 20 and 21 of the *KIF21A* gene {NM\_017641.3} and flanking intron-exon boundaries were amplified by polymerase chain reaction. The amplicons were then subjected to bi-directional direct DNA sequencing on an ABI PRISM 310 automated DNA sequencer (Applied Biosystems, Foster City [CA], US).

A heterozygous c.2821C>T mutation was identified in exon 21 of the *KIF21A* gene for both the proband and his mother (Fig b-d). This is a missense mutation that changes the 941st amino acid of the KIF21A protein product from Arginine to Tryptophan (Arg941Trp), and is a common mutation also reported in other patients/families with CFEOM1A.<sup>3,6</sup> The diagnosis of autosomal dominant CFEOM1A was thus confirmed. Since other family members of the maternal side are living in China and contact has been lost for many years, extended family screening was not performed.

## Discussion

Our patient had typical ophthalmological features and a positive family history suggestive of autosomal dominant CFEOM. After excluding the other causes of ptosis and ophthalmoplegia such as myasthenia gravis, mitochondrial disease and structural problems, mutational analysis for *KIF21A* gene should be considered. Mutations of this gene account for more than 90% of CFEOM1 cases.<sup>7</sup>

The *KIF21A* gene consists of 38 exons encoding a 1674 amino acid protein. The KIF21A protein belongs to the kinesin superfamily. Its major role appears to be to transport membranous organelles and protein complexes in a microtubule,<sup>8</sup> which is essential for neuronal morphogenesis and functioning. The structure of the KIF21A protein is characterised by an NH2 terminal kinesin motor domain that interacts with microtubules, a coiled-coil domain for dimer formation,<sup>9</sup> and a tail with seven WD40 repeats that is assumed to interact with the presently unidentified

cargo.<sup>10</sup> Despite the large size of the *KIF21A* gene, up to now only 13 missense mutations have been reported to cause CFEOM1A,<sup>3,9,11,12</sup> and they are all located in exons 2, 8, 20, and 21. The strategy for genetic testing of CFEOM1 should start with targeted analysis of exons 2, 8, 20, and 21. If the result is negative, sequencing of the remaining 34 exons and TUBB3 should be pursued if the clinical phenotype is compatible. Otherwise an alternative diagnosis should be considered.

The missense heterozygous mutation of Arg941Trp that was detected in exon 21 of the *KIF21A* gene in our patients is a common mutation reported in the literature.<sup>3,6</sup> It involves the coiled-coil region of the KIF21A protein that is evolutionarily conserved among different species (Fig e). Functional characterisation studies have been done by various researchers and showed that such mutations enhanced the translocation of Kank1 protein, a binding partner of KIF21A, to the membrane fraction. The main function of Kank1 protein is to inhibit actin polymerisation at the cell periphery and cell migration. Therefore, Arg941Trp mutation of the *KIF21A* gene is expected to result in abrogation of neuronal development.<sup>6</sup> Based on the above considerations, this mutation is predicted to be pathogenic.

By far, most of the mutations for CFEOM1 were located in exon 21. The hypothesis for such observation was that exon 21 is particularly rich in CpG dinucleotides, which provide a hypermutable template for transitional mutations to occur.<sup>13</sup> Mutations of the coiled-coil region may abolish dimerisation of the KIF21A protein and affect normal innervation of the extraocular muscles.

The clinical features and characteristics of CFEOM are summarised in the Table.<sup>2,13,14</sup> Apart from ocular features, extraocular manifestations like craniofacial dysmorphism, developmental delay and neuropathy may occur in CFEOM3 while oligosyndactyly may ensue in the Tugel syndrome.

So far, no genotype-phenotype correlation has been demonstrated in CFEOM. One of the reasons is that the number of patients reported with molecular confirmation is small. Thus, this disease may be underdiagnosed and the full spectrum of gene mutations and associated phenotypes is not yet well understood. Therefore, the mutation cannot predict visual acuity, risk of amblyopia, and the rate of progression to visual impairment. The management of CFEOM is just like in other squint and ptosis syndromes. Notably, early treatment is expected to yield better ophthalmic outcomes.

Genetic counselling of autosomal dominant CFEOM1A is straightforward in familial cases; the recurrence risk for offspring of affected individuals is 50%. For apparently de-novo cases, germline mosaicism cannot be excluded. Some experts quoted a recurrence risk of up to 5% for subsequent siblings

TABLE. Clinical features and characteristics of congenital fibrosis of extraocular muscle<sup>2,13,14</sup>

Feature/characteristic	Type 1	Type 2	Type 3 (A, B, C)
Clinical features	Bilateral ptosis	Bilateral ptosis	Bilateral or unilateral ptosis
	Infraducted primary vertical eye position	Normal or infraducted/supraducted primary vertical eye position	Infraducted or normal primary vertical eye position
	Restricted upgaze above the horizontal midline	Severely restricted upgaze	Variable restricted upgaze
	Horizontal strabismus	Horizontal strabismus	Horizontal strabismus
	Normal to severely restricted horizontal eye movements	Severely restricted horizontal eye movements	Normal to severely restricted horizontal eye movements
	Aberrant eye movements	Aberrant eye movements	May or may not have aberrant eye movements
	Positive forced duction test	Positive forced duction test	Positive forced duction test
	Absent binocular vision/refractive errors/amblyopia	Absent binocular vision/refractive errors/amblyopia	May or may not have refractive errors/absent binocular vision
	Normal pupils	Small and sluggishly reactive pupils	Normal pupils
	-	-	3A-neuropathy, developmental delay and MRI abnormalities; 3C-dysmorphism, developmental delay
Pathogenesis	Absence of superior division of oculomotor nerve. Abnormalities of the levator palpebrae superior and rectus superior	Absence of the motor neuron in oculomotor/trochlear nuclei and its innervated muscles	Variable developmental anomaly of the oculomotor nerve (superior > inferior branch)
Gene	1A- <i>KIF21A</i> 1B- <i>TUBB3</i>	<i>PHOX2A</i>	3A- <i>TUBB3</i> 3B- <i>KIF21A</i> 3C-unknown
Inheritance	AD with full penetrance and variable expression	AR	AD with incomplete penetrance and variable expression

\* MRI denotes magnetic resonance imaging, AD autosomal dominant, and AR autosomal recessive

of an apparently de-novo case.<sup>15</sup> Therefore, if expertise and resources are available, all clinically suspected CFEOM1 patients should undergo *KIF21A* mutational

analysis. Apart from confirming the diagnosis, this could also facilitate offering a prognosis, genetic counselling, and prenatal diagnosis.

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