Title: An Autosomal Dominant TUBB3 Mutation Associated with Congenital Fibrosis of the Extraocular Muscles Type 3 in an Iranian Family

Running Title: A TUBB3 variant causes CFEOM3A.

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Abstract

Congenital fibrosis of the extraocular muscles type 3 (CFEOM3) is a congenital cranial dysinnervation disorder marked by variable ophthalmoplegia and ptosis with considerable phenotypic heterogeneity. We report a large multigenerational Iranian family with autosomal dominant CFEOM3. Affected individuals underwent comprehensive ophthalmologic evaluation. Whole-exome sequencing in the proband, followed by Sanger sequencing in ten affected and four unaffected relatives, identified a heterozygous missense variant, c.784C>T (p.Arg262Cys), in exon 4 of *TUBB3* (16q24.3), which co-segregated with the disease phenotype.

Cranial magnetic resonance imaging (MRI) in two affected individuals revealed asymmetric basal ganglia morphology, predominantly affecting the caudate nuclei and putamen. At the level of the anterior commissure, the bilateral CFEOM3 patient lacked a visible commissure, whereas the unilateral patient exhibited a thin but identifiable structure. Midline sagittal imaging demonstrated corpus callosum dysgenesis in both individuals, with slightly greater involvement of the corpus callosum body in the unilateral case, though this difference was not radiologically significant. Despite differences in ocular phenotype, overall cerebral involvement was largely comparable.

These findings confirm the pathogenic role of the *TUBB3* p.Arg262Cys variant in CFEOM3A and extend the spectrum of ophthalmologic and neuroimaging abnormalities within an extended family. The results highlight the complex genotype–phenotype relationships in *TUBB3*-related disease and underscore the importance of integrated clinical and molecular evaluation for precise diagnosis.

Keywords: CFEOM3A, *TUBB3*, Ophthalmoplegia, Neuroimaging, R262C, Variable Expression.

Highlights

- TUBB3 R262C segregates with CFEOM3A in a large Iranian family.
- Brain MRI shows asymmetric basal ganglia and corpus callosum dysgenesis.
- Anterior commissure absent in bilateral, thin but visible in unilateral case.
- Ophthalmoplegia severity varies among affected individuals.
- Our findings expand the genotype–phenotype spectrum of TUBB3-related CFEOM3A.

Plain Language Summary

Some people are born with a condition called congenital fibrosis of the extraocular muscles type 3 (CFEOM3), which limits eye movement and may cause drooping eyelids. The severity of eye problems can differ even among family members. We studied a large Iranian family with several members affected by CFEOM3A. Medical exams, eye tests, brain imaging, and DNA analysis were performed. We found a specific change in the *TUBB3* gene, called c.784C>T, present in all affected members but not in healthy relatives. This change likely disrupts normal eye and nerve development, explaining the observed eye problems. Brain imaging showed that this gene change can also alter brain structures, such as the basal ganglia and connections between the brain's two halves. Family members with more severe eye problems had more noticeable brain changes, helping explain the variability in symptoms. These findings improve understanding of how a single genetic change can affect both eye and brain development. This knowledge can aid diagnosis, guide families, and may eventually help develop better strategies to manage congenital eye movement disorders.

Introduction

Congenital fibrosis of the extraocular muscles (CFEOM) is a congenital cranial dysinnervation disorder (CCDD) characterized by non-progressive ophthalmoplegia and ptosis resulting from abnormal development of the oculomotor or trochlear nerves (Wang et al., 1998; Yamada et al., 2004). Among the CFEOM phenotypes, CFEOM type 3 (CFEOM3) is a rare, dominantly inherited form of congenital restrictive ophthalmoplegia. CFEOM3 represents a clinically prominent and genetically heterogeneous form, notable for marked phenotypic variability, asymmetric neurological and anatomical involvement resulting in complicating clinical classification (EC, 2002; Traboulsi, Lee, Mousawi, Khamis, & Engle, 2000; Yamada et al., 2003). In some pedigrees, the phenotype segregates with full penetrance (Mackey et al., 2002), whereas in others it may show probable incomplete penetrance (Doherty et al., 1999).

Early clinical descriptions emphasized congenital limitation of eye movements associated with variable ptosis, frequent interocular asymmetry, and relative preservation of horizontal gaze in some affected individuals (Gillies, Harris, Brooks, Rivers, & Wolfe, 1995). Linkage studies subsequently mapped the disease locus to chromosome 16q24.3 (Doherty et al., 1999; Mackey et al., 2002). Subsequent molecular studies of CFEOM3A identified mutations in *TUBB3*, which encodes the neuron-specific β-tubulin isotype III, a critical component of microtubules involved in axon guidance, neuronal connectivity, and cranial nerve development (Chew et al., 2013; Tischfield et al., 2010). Neuroimaging and experimental investigations subsequently revealed abnormalities of oculomotor nerve development and broader axon guidance defects, placing CFEOM3 within the wider group of CCDDs rather than a purely ocular motility disorder (Tischfield et al., 2010).

Pathogenic *TUBB3* variants are associated with a wide phenotypic spectrum, ranging from isolated congenital ophthalmoplegia to combined ocular and neurodevelopmental abnormalities with variable neuroimaging findings. Notably, base substitutions affecting conserved residues, including p.Arg62Gln, p.Arg262Cys, p.Ala302Thr, and p.Asp417Asn, have been shown to produce distinct yet overlapping clinical manifestations, underscoring the variable genotype–phenotype correlations characteristic of TUBB3-related CFEOM3 (Chew et al., 2013; Tischfield et al., 2010).

In this study, we report an Iranian family with autosomal dominant CFEOM3A in which a heterozygous *TUBB3* missense variant, c.784C>T (p.Arg262Cys (R262C)), was identified and demonstrated to co-segregate with the disease phenotype. This report provides, to our knowledge, the first detailed clinical, genetic, and neuroimaging characterization of a TUBB3-related CFEOM3 family from Iran, which confirms the phenotypic spectrum associated with the c.784C>T variant.

Materials and Methods

Subjects and clinical assessment

A large multigenerational family originating from the central region of Iran, exhibiting a hereditary disorder of extraocular eye movement, was ascertained for this study. In total, 28 family members demonstrated clinical manifestations of the condition. Both affected individuals and available unaffected relatives from each generation were enrolled, and demographic information, including age, sex, and detailed clinical features, were systematically documented.

The study was conducted in accordance with the principles of the Declaration of Helsinki and received approval from the Ethics Committee of the Neuroscience Research Center (Approval No. IR.SBMU.PHNS.REC.1400.172). Written informed consent was obtained from all participants before their inclusion. Comprehensive ophthalmologic assessments were performed at Shahid Labbafi Nedjad Educational Hospital, whereas genetic analyses and related investigations were carried out at the Neuroscience and Cognitive Sciences Research Center, Shahid Beheshti University of Medical Sciences.

Ophthalmologic and neuroimaging evaluation

Comprehensive ophthalmologic examinations were performed in all affected family members. These evaluations included assessment of refractive errors, amblyopia, eyelid position and function, pupillary light reflexes, extraocular muscle motility, and the type and severity of strabismus. Forced duction testing was conducted to assess mechanical restriction, and levator

palpebrae superioris muscle function was specifically evaluated in individuals presenting with ptosis. Ptosis was observed in all affected participants.

Cranial magnetic resonance imaging (MRI) was obtained for two affected individuals, one with bilateral CFEOM3A and one with unilateral CFEOM3A, to further characterize associated neuroanatomical features. MRI protocols included acquisition of coronal and axial T1-weighted sequences as well as axial T2-weighted images, enabling detailed assessment of the basal ganglia, commissural pathways, and midline brain anatomy. Due to logistical constraints, additional family members were not imaged.

All MRI studies were conducted at the National Brain Mapping Laboratory (NBML) on a 3-Tesla MRI scanner using standardized clinical imaging protocols. Neuroimaging evaluation focused on the morphology of the basal ganglia, visualization of the anterior commissure at its expected anatomical level, and assessment of corpus callosum development. Comparative analyses were performed between the two imaged individuals to evaluate inter-individual variability in neuroanatomical findings in relation to unilateral versus bilateral CFEOM involvement.

Genetic analysis

Peripheral blood samples were collected in EDTA-containing tubes, and genomic DNA was extracted from leukocytes using the standard salting-out method (Miller, Dykes, & Polesky, 1988). Whole-exome sequencing (WES) was performed on genomic DNA obtained from the proband using the Illumina HiSeq 2500 platform (Illumina, San Diego, CA, USA).

Bioinformatic analysis focused on chromosomal regions and genes previously implicated in congenital cranial dysinnervation disorders, identified c.784C>T (R262C) in exon 4 of *TUBB3* (reference transcript NM_006086).

The candidate variant was validated by polymerase chain reaction (PCR) amplification followed by Sanger sequencing using specific primers (forward: 5′-

GGAGTCACCACCTCCTTGC-3′; reverse: 5′-CCATCATGTTCTTGGCATCG-3′). Segregation analysis was performed in available family members to assess co-segregation of the variant with the disease phenotype.

Results

Clinical findings

Comprehensive ophthalmologic evaluation of affected family members revealed some common characteristics of CFEOMs, including ptosis (unilateral in some individuals and bilateral in others) and hypotropia with restricted upgaze, chin up posture, exotropia with restricted horizontal gaze. Affected individuals were unable to elevate one or both eyes above the horizontal midline and exhibited limited function of the lateral and inferior rectus muscles (Fig. 1).

Based on ophthalmologic findings alone, the clinical presentation showed substantial overlap between clinical features traditionally attributed to CFEOM1 and CFEOM3, making reliable clinical distinction between these subtypes challenging. However, limited supraduction movement observed in some of the patients, is compatible with CFEOM3. No progressive worsening of ocular manifestations was reported by any of the patients.

[Insert Fig. 1 here]

Genetic analysis

Pedigree analysis demonstrated an autosomal dominant inheritance pattern for the disease phenotype (Fig. 2). Whole-exome sequencing performed on the proband identified a heterozygous missense variant, c.784C>T (R262C), in exon 4 of the *TUBB3* gene located on chromosome 16q24.3, corresponding to the known CFEOM3A locus. Segregation analysis using Sanger sequencing confirmed the presence of this variant in all affected family members and its absence in all tested unaffected members of the family (Fig. 3).

[Insert Fig. 2,3 here]

Neuroimaging findings

Brain magnetic resonance imaging (MRI) was performed in two closely related affected individuals: Patient 1 with bilateral CFEOM3A and Patient 2 with unilateral CFEOM3A, revealing variable but largely overlapping neuroanatomical findings.

Both patients demonstrated asymmetric morphology of the basal ganglia, predominantly involving the caudate nucleus and putamen. These alterations were bilateral and more pronounced in the left hemisphere in both patients (Figs. 4A1, 4B1, 4C1, 4A2, 4B2, 4C2).

Evaluation at the expected anatomical level of the anterior commissure showed absence of a visible anterior commissure in the bilateral CFEOM3A case, whereas a thin but identifiable anterior commissure was present in the unilateral CFEOM3A case (Figs. 4D1, 4E1, 4D2, 4E2).

Midline sagittal imaging revealed corpus callosum dysgenesis in both patients. The unilateral CFEOM3A patient exhibited slightly greater involvement of the body of the corpus callosum compared with the bilateral case; however, this difference was not considered radiologically significant. Notably, despite the differences in ocular phenotype, no meaningful differences in overall cerebral involvement were observed (Figs. 4F1, 4F2).

[Insert Fig. 4 here].

Discussion

Congenital fibrosis of the extraocular muscles type 3 (CFEOM3) is a genetically and phenotypically heterogeneous form of congenital cranial dysinnervation disorder (CCDD) most commonly associated with heterozygous missense variants in the *TUBB3* gene, which encodes the neuron-specific β-tubulin isotype III (Demer et al., 2010; Tischfield et al., 2010). βIII-tubulin plays a critical role in microtubule assembly and dynamics, axon guidance, and

neuronal migration during neurodevelopment (Tischfield et al., 2010). Pathogenic *TUBB3* variants disrupt microtubule function, occasionally impair interactions with kinesin motor proteins, and result in abnormal cranial nerve development and defective extraocular muscle innervation (Tischfield et al., 2010).

In the present study, we identified a heterozygous *TUBB3* variant, c.784C>T (R262C), segregating with disease in an autosomal dominant manner across multiple generations in a large Iranian family. Clinically, affected individuals exhibited congenital ptosis, severe vertical gaze limitation, and variable involvement ranging from unilateral to bilateral CFEOM3A, consistent with the broad phenotypic spectrum previously reported for *TUBB3*-related CFEOM3(Ceylan et al., ٢٠١٧; Chew et al., ٢٠١٣; Tischfield et al., ٢٠١٠))).

The penetrance of the disease allele could not be formally evaluated due to the lack of systematic assessment of all unaffected family members. However, the biochemical consequences of cysteine replacing arginine at position 262 (p.Arg262Cys) are expected to disrupt the electrostatic and structural properties of βIII-tubulin, potentially impairing microtubule dynamics and axon guidance and contributing to the observed disease phenotype. This interpretation is supported by the loss of a positively charged arginine residue and the introduction of a reactive thiol group by cysteine, changes predicted to adversely affect tubulin folding and function. Collectively, these considerations suggest that the c.784C>T (R262C) variant is likely to exhibit high, and possibly complete, penetrance.

Neuroimaging in our cohort further supported the diagnosis of CFEOM3A and demonstrated variable central nervous system involvement. In both unilateral and bilateral patients examined by neuroimaging, basal ganglia involvement was observed, and abnormalities of the anterior commissure were present despite differences in ocular phenotype. These largely overlapping neuroanatomical findings suggest that central nervous system perturbations are a consistent

feature of *TUBB3*-related CFEOM3A. Such observations are in line with prior reports describing corpus callosum dysgenesis, basal ganglia abnormalities, and commissural defects in *TUBB3*-associated disordersChew et al., ۲۰۱۳; Poirier et al., ۲۰۱۰; Tischfield et al., ۲۰۱۰;) (Tischfield & Engle, ۲۰۱۰), and further support the concept of variable expressivity in this condition.

Taken together, our findings expand the phenotypic and neuroimaging spectrum associated with the *TUBB3* variant (c.784C>T (R262C)) and highlight the variable expressivity observed in CFEOM3A. This study underscores the critical value of integrating detailed molecular genetic analysis with comprehensive clinical and neuroimaging assessments to achieve accurate diagnosis and inform effective genetic counseling in families affected by congenital cranial dysinnervation disorders.

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Ceylan, A. C., Gursoy, H., Yildirim, N., Basmak, H., Erol, N., & Cilingir, O. (2017). Clinical heterogeneity associated with TUBB3 gene mutation in a Turkish family with congenital fibrosis of the extraocular muscles. *Ophthalmic Genet, 38*(3), 288-290. doi:10.1080/13816810.2016.1193881

Chew, S., Balasubramanian, R., Chan, W.-M., Kang, P. B., Andrews, C., Webb, B. D., . . . Crawford, T. O. J. B. (2013). A novel syndrome caused by the E410K amino acid substitution in the neuronal β-tubulin isotype 3. *136*(2), 522-535.

Doherty, E. J., Macy, M. E., Wang, S. M., Dykeman, C. P., Melanson, M. T., Engle, E. C. J. I. o., & science, v. (1999). CFEOM3: a new extraocular congenital fibrosis syndrome that maps to 16q24. 2-q24. 3. *40*(8), 1687-1694.

EC, E. J. B. G. (2002). CFEOM1, the classic familial form of congenital fibrosis of the extraocular muscles, is genetically heterogeneous but does not result from mutations in ARIX. *3*, 3.

Gillies, W. E., Harris, A. J., Brooks, A. M., Rivers, M. R., & Wolfe, R. J. J. O. (1995). Congenital fibrosis of the vertically acting extraocular muscles: a new group of dominantly inherited ocular fibrosis with radiologic findings. *102*(4), 607-612.

Mackey, D. A., Chan, W.-M., Chan, C., Gillies, W., Brooks, A. M., O'Day, J., & Engle, E. C. J. H. g. (2002). Congenital fibrosis of the vertically acting extraocular muscles maps to the FEOM3 locus. *110*(5), 510-512.

Miller, S. A., Dykes, D. D., & Polesky, H. J. N. a. r. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *16*(3), 1215.

Poirier, K., Saillour, Y., Bahi-Buisson, N., Jaglin, X. H., Fallet-Bianco, C., Nabbout, R., . . . Desguerre, I. J. H. m. g. (2010). Mutations in the neuronal β-tubulin subunit TUBB3 result in malformation of cortical development and neuronal migration defects. *19*(22), 4462-4473.

Tischfield, M. A., Baris, H. N., Wu, C., Rudolph, G., Van Maldergem, L., He, W., . . . Robertson, R. L. J. C. (2010). Human TUBB3 mutations perturb microtubule dynamics, kinesin interactions, and axon guidance. *140*(1), 74-87.

Tischfield, M. A., & Engle, E. C. J. B. r. (2010). Distinct α-and β-tubulin isotypes are required for the positioning, differentiation and survival of neurons: new support for the 'multi-tubulin'hypothesis. *30*(5), 319-330.

Traboulsi, E. I., Lee, B. A., Mousawi, A., Khamis, A., & Engle, E. C. J. A. j. o. O. (2000). Evidence of genetic heterogeneity in autosomal recessive congenital fibrosis of the extraocular muscles. *129*(5), 658-662.

Wang, S., Zwaan, J., Mullaney, P., Jabak, M., Al-Awad, A., Beggs, A., & Engle, E. J. T. A. J. o. H. G. (1998). Congenital fibrosis of the extraocular muscles type 2, an inherited exotropic strabismus fixus, maps to distal 11q13. *63*(2), 517-525.

Yamada, K., Andrews, C., Chan, W.-M., McKeown, C. A., Magli, A., De Berardinis, T., . . . Letson, R. J. N. g. (2003). Heterozygous mutations of the kinesin KIF21A in congenital fibrosis of the extraocular muscles type 1 (CFEOM1). *35*(4), 318-321.

Yamada, K., Chan, W.-M., Andrews, C., Bosley, T. M., Sener, E. C., Zwaan, J. T., . . . science, v. (2004). Identification of KIF21A mutations as a rare cause of congenital fibrosis of the extraocular muscles type 3 (CFEOM3). *45*(7), 2218-2223.

Figures legend:

Figure 1. Clinical photographs of affected family members with the c.784C>T variant demonstrating the phenotypic spectrum of CFEOM3A.

The c.784C>T variant can cause bilateral ptosis and severe CFEOM3A with an infraducted and abducted resting eye position. (A, B, D, F), moderate unilateral CFEOM3A (C, H), and mild CFEOM3A (E, G). Images demonstrate congenital ptosis and ophthalmoplegia with variable severity across individuals, consistent with a congenital non-progressive phenotype. All photographs were obtained in primary gaze.

Figure 2. Pedigree of the family affected by CFEOM3A. Pedigree analysis suggested an autosomal dominant pattern of inheritance, characterized by vertical transmission across successive generations. The arrow marks the proband subjected to whole-exome sequencing, and asterisks (*) indicate individuals analyzed by Sanger sequencing.

Figure 3. Sanger sequencing confirmation of the *TUBB3* variant. Electropherograms show a heterozygous c.784C>T (p.Arg262Cys) variant in exon 4 of the *TUBB3* gene (16q24.3) in affected individuals. The variant is indicated by overlapping C/T peaks, consistent with a heterozygous state. This variant segregated with the disease phenotype and was absent in unaffected family members.

Figure 4. Brain MRI findings in two patients with *TUBB3*-associated CFEOM3A, including one patient with bilateral CFEOM3A and one patient with unilateral CFEOM3A.

(A1–C1) Coronal and axial T1-weighted images of the patient with bilateral CFEOM3A show asymmetric involvement of the basal ganglia bilaterally, predominantly affecting the caudate nuclei and putamen (arrows).

(A2–C2) Coronal and axial T1-weighted images of the patient with unilateral CFEOM3A demonstrate unilateral, less pronounced asymmetry of the basal ganglia, primarily involving the left caudate nucleus and putamen (arrows).

(D1, E1) Coronal and axial T1-weighted images at the expected anatomical level of the anterior commissure in the bilateral CFEOM3A patient reveal absence of a visible anterior commissure, with arrows indicating the anticipated location.

(D2, E2) Coronal T1-weighted and axial T2-weighted images of the unilateral CFEOM3A patient depict a thin but clearly identifiable anterior commissure (arrows).

(F1, F2) Midline sagittal T1-weighted images demonstrate corpus callosum dysgenesis in both patients; involvement of the body of the corpus callosum is slightly more pronounced in the unilateral CFEOM3A patient (L), although this difference is not considered radiologically significant.

MRI, magnetic resonance imaging; T1, T1-weighted; T2, T2-weighted.