Case Report

Identification of a Novel Frameshift Variant in MYF5 Leading to External Ophthalmoplegia with Rib and Vertebral Anomalies

Paulina Ocieczek 1,2, Ngozi Oluonye 1,3, Cécile Mélécase 2,4, Elena Schiff 1, Vijay Tailor 1,2 and Mariya Moosajee 1,2,3,4,*

1 Moorfields Eye Hospital NHS Foundation Trust, London EC1V 9EL, UK
2 UCL Institute of Ophthalmology, London EC1V 9EL, UK
3 Great Ormond Street Hospital for Children NHS Foundation Trust, London WC1N 3JH, UK
4 Francis Crick Institute, London NW1 1AT, UK
* Correspondence: m.moosajee@ucl.ac.uk

Abstract: Myogenic transcription factors with a basic helix–loop–helix (bHLH) such as MYOD, myogenin, MRF4, and MYF5 contribute to muscle differentiation and regulation. The MYF5 gene located on chromosome 12 encodes for myogenic factor 5 (MYF5), which has a role in skeletal and extraocular muscle development and rib formation. Variants in MYF5 were found to cause external ophthalmoplegia with rib and vertebral anomalies (EORVA), a rare recessive condition. To date, three homozygous variants in MYF5 have been reported to cause EORVA in six members of four unrelated families. Here, we present a novel homozygous MYF5 frameshift variant, c.596dupA p. (Asn199Lysfs*49), causing premature protein termination and presenting with external ophthalmoplegia, ptosis, and scoliosis in three siblings from a consanguineous family of Pakistani origin. With four MYF5 variants now discovered, genetic testing and paediatric assessment for extra-ocular features should be considered in all cases of congenital ophthalmoplegia.

Keywords: MYF5; myogenic factor 5; external ophthalmoplegia with vertebral and rib anomalies (EORVA); external ophthalmoplegia; scoliosis; rib anomalies; myogenic transcription factors

1. Introduction

During mammalian development, skeletal muscle differentiation is regionalised with skeletal muscles of the trunk, limbs, diaphragm, and tongue derived from somites, while the craniofacial muscles, including extraocular muscles (EOM), originate from prechordal and paraxial mesoderm [1]. In the case of disease or injury, skeletal muscles can be regenerated by myogenic precursor cells called satellite cells [2,3]. EOM are more complex than limb skeletal muscles with smaller motor units, higher mitochondrial content, increased blood flow from a dense vascular bed, and co-expression of several myosin isoforms. They are the fastest contracting muscles in the human body and are highly resistant to fatigue [4,5].

Embryonic and post-natal myogenesis of skeletal muscle and EOM is regulated by four basic helix–loop–helix (bHLH) transcription factors: MYF5, MYOD, MYOG (also called myogenin), and MRF4 (also called MYF6 or herculin) [6–10]. MYF5 is the first myogenic factor to be expressed; in mice, Myf5 expression occurs around embryonic day 8 (E8), preceding somite differentiation into the dermis, axial muscles, vertebrae, and ribs [11]. Myf5 or Mrf4 activates myogenesis via MyoD expression, which initiates MyoG expression [12–16] (Figure 1).
Figure 1. The role of myogenic transcription factors in extraocular myogenesis in mice. Extraocular muscles (EOM) are derived from cranial mesoderm progenitors. Expression of either Myf5 or Mrf4 is required for EOM progenitor cells to acquire their myogenic fate. Myf5 or Mrf4 activates MyoD, which in turn activates MyoG and EOM differentiation. Created with Biorender.

Disruption of the Myf5 gene in mice causes abnormal development of the distal parts of the ribs and postnatal death due to respiratory distress [15]. Studies on mice carrying heterozygous variants in Myf5 in trans with a second heterozygous variant in Mrf4 (Myf5+/m1 Mrf4+/bh1) showed severe rib anomalies and undetectable myotome formation, similar to Myf5 knockout models [17]. Homozygous Mrf4 mutants also exhibit rib defects, but the most severe rib anomalies are present in Myf5 null models [17,18]. Myf5 mutant mice with normal expression of Myod1, MyoG, and Mrf4 develop normal skeletal muscles, but are lethal due to an inability to breathe [15]. Inserting MyoG cDNA into the Myf5 locus via homologous recombination leads to partial phenotypic rescue, with development of a normal rib cage in MyoG knock-in mice [19]. In mice lacking both MYF5 and MYOD transcription factors, no skeletal muscles were formed, and mice died postnatally within minutes from birth. The presence of a healthy copy of either MyoD or Myf5 in mutant mice led to partial or full skeletal muscle development [7].

Congenital fibrosis of extraocular muscles (CFEOM) characterises a non-progressive congenital ophthalmoplegia with or without ptosis. It is a development disorder primarily affecting cranial nerves (a cranial innervation disorder) and results in fibrosis and hypoplasia of innervated EOMs. It can be inherited in an autosomal dominant or autosomal recessive manner and there are five types; types 1 and 3 are autosomal dominant, whilst types 2, 4, and 5 are inherited recessively. Six genes are known to be associated with this condition: COL25A1, KIF21A2, PHOX2A, TUBA1A, TUBB2B, and TUBB3. Systemic features can present as neurodevelopmental, brain, or limb anomalies, including reduced numbers of fingers or toes (oligodactyly).

External ophthalmoplegia with rib and vertebral abnormalities (EORVA) is a rare autosomal recessive disease associated with MYF5 variants and is characterised by congenital, non-progressive ophthalmoplegia and ptosis, with vertebral and rib anomalies, scoliosis, and torticollis. First described in 2018, Di Goia et al. reported three families presenting with this condition caused by biallelic mutations in the MYF5 gene (OMIM # 159990) located on 12q21.31 [20]. Unlike CFEOM, the primary pathology is not neurological but originates in the muscle. The characteristic ocular features are similar in both EORVA and CFEOM. Overlooking mild systemic features that differ in both these conditions can lead to misdiagnosis and suboptimal management (Table 1). Ocular management in EORVA and CFEOM include monitoring and correction of refractive error and amblyopia; in some cases, surgery to correct ptosis or extraocular muscles alignment should be considered. Management of systemic complications will vary depending on presenting features and include multidisciplinary management and the involvement of a paediatrician.

Table 1. Similarities and differences between CFEOM and EORVA. CFEOM: congenital fibrosis of extraocular muscles; EORVA: external ophthalmoplegia with rib and vertebral anomalies. Phenotypic differences between CFEOM and EORVA are highlighted in bold.

<table>
<thead>
<tr>
<th>Condition</th>
<th>CFEOM</th>
<th>EORVA</th>
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<tbody>
<tr>
<td>Pathophysiology</td>
<td>Cranial innervation disorder</td>
<td>Muscle disorder</td>
</tr>
<tr>
<td>Ocular features</td>
<td>Non-progressive ophthalmoplegia +/- ptosis</td>
<td>Non-progressive ophthalmoplegia</td>
</tr>
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</table>
Herein, we describe a novel homozygous c.596dupA variant in the MYF5 gene associated with EORVA in three siblings from a consanguineous family of Pakistani origin.

### 2. Case Description

Two brothers aged 8 (IV-1) and 7 years (IV-2), and their younger sister aged 4 years (IV-3) from a consanguineous family of Pakistani origin were referred to the genetic eye disease clinic at Moorfields Eye Hospital (MEH) with a diagnosis of CFEOM. Their parents were unaffected (Figure 2).

Patient IV-1 first presented to a general paediatric ophthalmology service aged six months with left exotropia, left hypertropia, and ophthalmoplegia. Magnetic resonance imaging (MRI) of the head and orbits showed smaller left medial rectus, superior rectus and superior oblique muscles compared to the contralateral side, leading to a presumed diagnosis of CFEOM (Figure 3J). At most recent presentation, age 8 years, his ophthalmic examination showed a best corrected visual acuity (BCVA) of 0.00 LogMAR in the right and 0.30 LogMAR in the left eye (Table 2). Orthoptic assessment showed constant left exotropia, bilateral ophthalmoplegia, chin elevation, and mild bilateral ptosis. Anterior and posterior segment examination showed no abnormalities. Optos imaging and optical coherence tomography (OCT) of the macula showed no abnormalities (Figure 3M).

#### Table 2. Orthoptic and ophthalmic examination of Patient IV-1, Patient IV-2, and Patient IV-3. Abbreviations: M: months; Y: years; BC-RVA: best corrected right visual acuity (logMAR); BC-LVA: best corrected left visual acuity (logMAR); R: right eye; L: left eye; LXT: left exotropia; R/AXT: right/alternating exotropia; r/o: restriction of; B/L: bilateral.
Patient IV-2 presented to a general paediatric ophthalmology clinic at 23 months with ophthalmoplegia. There were no notable concerns regarding intellect or development. At age 7 years, his ocular examination showed BCVA of 0.00 LogMAR in the right and 0.08 LogMAR in the left eye, a constant right alternating exotropia, bilateral ophthalmoplegia, and mild bilateral ptosis (Table 2). Anterior and posterior segment examination was within normal limits.

Patient IV-3 was originally found to have restricted eye movements aged four months. She had normal intellect but there were some minor concerns about clumsiness; her neurological examination was unremarkable but mild in-toeing was noted. At age 4 years BCVA was 0.24 LogMAR in each eye. An intermittent distance left exotropia was detected with bilateral ophthalmoplegia and mild right-sided ptosis (Table 2). Anterior and posterior segment examination was normal, Optos imaging and OCT scans were within normal limits.

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**Figure 2.** Family pedigree—three siblings from a consanguineous Pakistani family are affected with external ophthalmoplegia, with vertebral and rib anomalies. Square symbols indicate males; circles indicate females.
indicate females. Diamonds represent either gender. The number inside the shape is the number of individuals. Filled symbols are affected individuals. The black arrow indicates the proband.

Previously reported variants in *MYF5* were known to cause external ophthalmoplegia, with vertebral and rib anomalies [18]. Therefore, following the genetic results, patients IV-1, IV-2, and IV-3 were assessed by a developmental paediatrician to look for syndromic features, in particular torticollis, scoliosis, spinal, and rib cage or chest abnormalities, none of which were found on physical examination. Neurological examination and growth indices were within normal limits. Patients were referred for spinal X-rays to investigate for radiological evidence of rib cage and spinal abnormalities. Varying degrees of thoracic, thoracolumbar, and lumbar scoliosis were reported in all three patients (Figures 3C and 3D).

**Figure 3.** (A) Facial photo; (B) dental photo; (C) Patient IV-1 skeletal X-ray, antero-posterior view; mild thoracic scoliosis centred at T6-T7 concave to the left; (D) Patient IV-1 skeletal X-ray, lateral (L) view; gaze position photos (N.B. not all positions of gaze were obtained due to ptosis obscuring eye position); (E) dextro-elevation; (F) direct elevation; (G) laevo elevation; (H) primary position; (I) laevo version; (J) MRI orbits Patient IV-1: left medial rectus, left superior rectus, and left superior oblique muscles smaller comparing to respective EOMs in the right eye; (K) colour fundus photo; (L) autofluorescence photo; (M) macular OCT.
3. Genetic Testing

Informed consent was obtained from all subjects involved in the study through the Genetic Study of Inherited Eye Disease (REC reference 12/LO/0141). A clinical exome (Agilent SureSelect Focused Exome +1 capture) for Patient IV-1 was performed on the Illumina NextSeq 500 platform, with sequence data generated across the full capture region of greater than 5000 genes. Next-generation sequencing analysis was then performed for a virtual panel of coding exons (+/- 20bp) of 14 genes associated with eye movement disorders (EMD_v2 panel, North East Thames Regional Genetics Laboratory: CHN1, COL25A1, DCC, FRMD7, HOXA1, HOXB1, KIF21A, MAFB, PHOX2A, ROBO3, SALL1, SALL4, TUBB2B, TUBB3). Larger insertion/deletion mutations and copy variants were analysed using ExomeDepth. Variants were filtered according to minor allele frequency (>2%) from 1000G, ExAC or EVS databases.). No pathogenic or likely pathogenic variants were identified. Variants in non-coding regions that could affect gene expression could not be excluded.

Both brothers IV-1 and IV-2 were subsequently recruited to the Genomics England 100,000 Genomes Project together with their unaffected parents for whole genome sequencing as previously described [21,22]. Both brothers were found to be homozygous, and the unaffected parents heterozygous carriers for a novel duplication c.596dupA in the MYF5 gene resulting in a frameshift variant p.(Asn199Lysfs*49). Targeted sequence analysis of the MYF5 variant confirmed the 100,000 Genome Project findings. The third affected sibling, IV-3, underwent familial MYF5 testing and was found to be homozygous for the same variant. In the gnomAD population database, this variant was found at heterozygous state in two individuals (f = 0.00000124) and has not been described before at homozygous state.

According to ACMG variant classification guidelines, MYF5 c.596dupA is likely pathogenic. It is a null variant in a gene where loss of function is the presumed mechanism of disease and the variant results in a reduction of more than 10% of the protein (PVS1 strong). The variant is present in only two alleles (no homozygotes) in the gnomAD v4.0 database (PM2 moderate). In vitro studies would help confirm if any residual function of the truncated protein is present and would help to upgrade the classification.

4. Discussion

Here we present the three siblings with EORVA (non-progressive ophthalmoplegia, ptosis, and scoliosis), without vertebral anomalies and torticollis, caused by a novel homozygous MYF5 frameshift variant, c.596dupA, p.(Asn199Lysfs*49), in exon 3. Three MYF5 variants have been previously reported to be associated with EORVA in six members of four unrelated families: (i) deletion c.23_32del p.(Gln8Leufs*86); (ii) deletion c.191del p.(Ala64Valfs*33); and (iii) missense variant c.283C>T p.(Arg95Cys), all located in exon 1 (Figure 4, Table 3) [18,21].
Figure 4. Mutational spectrum of MYF5 related external ophthalmoplegia, with vertebral and rib anomalies. (A) Variants previously described depicted across exon 1 of MYF5 gene (NM_005593.3), while the novel disease-causing variant reported in this study is located in exon 3. (B) Amino acid changes mapped across MYF5 transcription factor (NP_005584, UniProt P13349); bHLH-basic helix-loop–helix protein domain (amino acid residue 83 to 134) binding DNA. The novel disease-causing variant reported in this study is indicated in bold. Asterisk (*) indicates premature termination (stop) codon.

Table 3. Genotype–phenotype correlation in MYF5 variants. Abbreviations: EOM: extraocular muscles; R: right eye; L: left eye; XT: exotropia; HoT: hypotropia; HT: hypertropia; +: mild; ++: moderate; +++: severe; ND: no data; ✓: present; ✘: absent.
The homozygous 10bp deletion, c.23_32del, in exon 1 was reported in a brother, age 9 years, and his sister, age 19 years, of Turkish descent from unaffected consanguineous first cousins. Both exhibited external ophthalmoplegia, ptosis, squint, scoliosis, torticollis, and dysmorphic hypoplastic ribs. The same variant c.23_32del was discovered in another 16-year-old male from the same village, with similar ocular and vertebral features, and pectus carinatum [20]. The c.23_32del variant appears to exert a more severe extra-ocular phenotype compared to our reported c.596dup variant, where scoliosis was the only skeletal feature detected on X-ray imaging. The c.191del variant in exon 1 was found in an 8-year-old Chinese boy with paternal uniparental disomy. Extra-ocular features included scoliosis and hypoplastic ribs. This patient had a milder ocular phenotype with only ptosis reported; however, the ocular features were not provided in detail [23].

The two frameshift variants in exon 1, c.23_32del p.(Gln8Leufs*86) and c.191del p.(Ala64Valfs*33), introduce a premature termination codon (PTC) at least 280 nucleotides after the start codon. These PTCs are predicted to be sensitive to nonsense-mediated decay (NMD) [24,25], leading to degradation of mRNA and an absence of the MYF5 protein.

The missense variant p.(Arg95Cys) forms a full-length protein and has been associated with EORVA in two members of one Yemeni family [20,26]. In vitro and in silico assays reported that mutant MYF5 is mislocalised to the cytoplasm and has lost its DNA binding ability [20]. Even if the consequence of the mutation on the protein differs between null or truncated protein with an alternative C-terminus, only a few EORVA patients have been described and a larger cohort of patients are required for more significant genotype–phenotype correlation.

Several mouse models have shown that rib morphogenesis is indirectly affected by Mrf4 and Myf5 expression via fibroblast growth factor (Fgf) and platelet-derived growth factor (Pdgf) mediation in myotome–sclerotome interactions. Myf5/Mrf4 activation in hypaxial myotome signals to the adjacent sclerotome using Pdgf and Fgf to promote rib and vertebral development [14,27,28]. Mice lacking Pdgfra, the gene encoding the Pdgf receptor, have severe rib anomalies [29]. In addition, insertion of Pdgfra cDNA into the Myf5 locus results in increased rib and vertebral development [14]. The genes encoding MRF4 and MYF5 are closely linked, with approximately 8.5kb separating their coding sequences on human chromosome 12 [30–32], and cis-acting interaction of MRF4 negatively impacts expression of adjacent MYF5 [17,18]. A Mrf4 knockout mouse model exhibits rib defects and lacks Myf5 expression. Rib anomalies are more severe in compound heterozygote Myf5/Mrf4 than Mrf4 knockout models, and the most severe are in Myf5 homozygous mutants [15,17,33] (Table 4). These mouse models did not examine the influence of Myf5/Mrf4 on extraocular muscle function. Rib development is highly sensitive to quantitative difference in MYF5 function [15,17].

Table 4. Genotype–phenotype correlation in mice models with rib morphogenesis defects; ND—no data; wt—wild type.

<table>
<thead>
<tr>
<th>Genotype Mice Model</th>
<th>Rib Cage Defects</th>
<th>Vertebrae Defects</th>
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</thead>
<tbody>
<tr>
<td>Mrf4−/− [34]</td>
<td>Ribs not attached to the sternum</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Truncation of ribs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bifurcation and fusion of adjacent ribs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Irregular sternum ossification</td>
<td></td>
</tr>
<tr>
<td>Mrf4−/Myf5−/− [17]</td>
<td>Ribs not attached to the sternum</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Truncation of ribs: shorter vs wt/Mrf4−/−; longer vs. Myf5−/−</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bifurcation and fusion of adjacent ribs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Irregular sternum ossification</td>
<td></td>
</tr>
<tr>
<td>Myf5−/− [15]</td>
<td>Absence of the distant parts of the ribs</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Complete ossification of the sternum</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lethal immediately postnatally (inability to breath)</td>
<td></td>
</tr>
<tr>
<td>Pdgfra&lt;sup&gt;+&lt;/sup&gt; [29]</td>
<td>Ribs mostly attached to the sternum</td>
<td>Structural anomalies of cervical and thoracic vertebrae</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------------------------------</td>
<td>-------------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>Bifurcation and fusion of adjacent ribs</td>
<td>Spina bifida</td>
</tr>
<tr>
<td></td>
<td>Irregular and shorter sternum</td>
<td>Anomalies of spinal column curvature</td>
</tr>
</tbody>
</table>

After a genetic diagnosis, detailed phenotyping is important to assess for features that can aid diagnosis and provide clinical evidence in support of variant pathogenicity. Our cases presented with a presumptive diagnosis of CFEOM due to characteristic features of ophthalmoplegia and ptosis, however, radiology revealed scoliosis in all three patients, which revised the diagnosis to EORVA. Recognition of these additional features allows for a multi-disciplinary approach to provide the best possible care for the patients in the long-term.

5. Conclusions

In conclusion, we report three siblings of consanguineous parents with a novel homozygous variant c.596dupA p.(Asn199Lys*49) in exon 3 of MYF5 associated with EORVA, a newly recognised syndrome easily mistaken for CFEOM, which has a different pathogenesis and systemic implications. With four EORVA-associated variants now discovered, it is important to perform genetic testing on patients with external ophthalmoplegia with and without extra-ocular features. Our family had no extra-ocular abnormalities on clinical examination, but genetic results prompted further radiological investigation, revealing scoliosis in all affected members. Patients with signs of ocular fibrosis, especially with a family history of ophthalmoplegia should undergo genetic testing and be referred to paediatric services for a full work up.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of MEH and the Northwest London Research Ethics Committee (REC reference 12/LO/0141, approval date 30/09/2022).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study through the Genetic Study of Inherited Eye Disease (REC reference 12/LO/0141).

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflicts of interest.

References


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