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Case report

Lethal multiple pterygium syndrome: A severe phenotype associated with a novel mutation in the nebulin gene

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Abstract

Fetal akinesia deformation sequence is a clinically and genetically heterogeneous disorder characterized by a variable combination of fetal akinesia, intrauterine growth restriction, developmental abnormalities such as cystic hygroma, hydrops fetalis, pulmonary hypoplasia, occasional arthrogryposis, and pterygia. The pathogenetic mechanisms of fetal akinesia deformation sequence include neuropathy, muscular disorders, neuromuscular junction disorders, maternal myasthenia gravis, restrictive dermopathy and others. We here report an Egyptian family presenting with recurrent lethal multiple pterygium syndrome. The diagnosis was based on antenatal sonographic demonstration of complete fetal akinesia and a large cystic hygroma with severe limb contractures evident on postmortem examination. Next generation sequencing performed on the second affected fetus identified a novel homozygous essential splice-site variant in the nebulin gene. In conclusion, our report adds further evidence for the involvement of the nebulin gene in the etiology of fetal akinesia deformation sequence/lethal multiple pterygium syndrome.

Keywords: Fetal akinesia; Lethal multiple pterygium syndrome; Nebulin gene

1. Introduction

Fetal akinesia deformation sequence (FADS) or Pena Shokeir syndrome is characterized by intrauterine growth retardation, contractures, craniofacial anomalies, limb anomalies, pulmonary hypoplasia and polyhydramnios and results from reduced movement *in utero*. A number of other fetal akinesia syndromes overlap phenotypically with FADS, including the lethal congenital contracture syndromes, multiple pterygium syndromes and arthrogryposis multiplex congenita. The clinical findings are dependent upon the time of onset of the akinesia, with earlier onset being associated with a more severe phenotype [1–3]. The unifying feature in all these disorders is reduced or absent fetal movement. Multiple joint contractures and subsequent pterygia are secondary consequences of diminished fetal movement, independent of the primary insult [2,4]. Fetal akinesias can result from primary defects involving any point along the motor system: motor neurons, peripheral nerves, neuromuscular junction and the skeletal muscle regulatory and contractile apparatus, and also the connective tissue [1].

Lethal multiple pterygium syndrome (LMPS; OMIM #253290) is a rare autosomal recessive genetic disorder with less than 30 families reported to date [5]. It is characterized by intrauterine growth retardation, multiple pterygia and flexion contractures causing severe arthrogryposis, and fetal akinesia. In severe cases, affected fetuses may develop subcutaneous edema, hydrops fetalis and cystic hygroma. Structural defects including cleft palate, cryptorchidism, pulmonary hypoplasia, diaphragmatic hernia, microcephaly, cerebellar and pontine hypoplasia can also be present [6].

The etiology of fetal akinesia deformation sequence/lethal multiple pterygium syndrome (FADS/LMPS) is heterogeneous. It is thought that more than half of the causes of fetal akinesia are of neuromuscular origin [7]. At least 30 causative genes have been identified, involving all points along the neuromuscular axis [8]. Defects in the embryonal acetylcholine receptor (AChR) were discovered to account for a significant

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proportion of patients with LMPS and fetal akinesia [9]. More recently, mutations of the nebulin gene (*NEB*) have also been associated with LMPS [10,11]. Among the other genes involved in the etiology of neuromuscular fetal akinesias are *RYR1* [12], *GBE1* [13], *RAPSN* [14], *CHRND* and *CHRNA1* [15].

We here report the identification of a novel essential splice-site variant in the nebulin gene in a consanguineous Egyptian family presenting with recurrent FADS/LMPS.

2. Case report

An Egyptian pregnant female, married to her first cousin, presented with an abnormal second-trimester scan, and a history of a previous male fetus therapeutically aborted due to hydrops fetalis at 20 weeks of gestation. Postmortem clinical pictures were kept for the first fetus (Fig. 1A–C), while no DNA sample was available. The current pregnancy was also complicated by polyhydramnios and recurrence of the same fetal abnormalities. A detailed malformation scan, performed at 18 weeks of gestation, revealed a male fetus with growth restriction, fetal akinesia, a huge cystic hygroma, generalized skin edema, pericardial effusion and hydrothorax (Fig. 2). Flexion deformity of fetal extremities with lack of movements of the large joints was also observed. On the basis of the poor fetal prognosis, the parents opted for termination of the pregnancy. They consented for a fetal blood sample but declined muscle biopsies. Karyotyping showed a normal 46XY karyotype.

Postmortem clinical examination revealed a male fetus with growth retardation, huge cystic hygroma, generalized skin edema, particularly severe scalp edema, and all extremities were kept in a flexed position. The elbows were abnormally flexed and the hands showed camptodactyly with ulnar deviation of fingers. Multiple pterygia could be seen across all major joints and was predominantly striking at the neck to the extent that the chin was obscured by the marked anterior (chin to sternum) webbing. Dysmorphic facial features, including hypertelorism, downslanting palpebral fissures, long philtrum, and low set ears, were also noted (Fig. 1D–F).

A clinical diagnosis of fetal akinesia deformation sequence/lethal multiple pterygium syndrome was made.

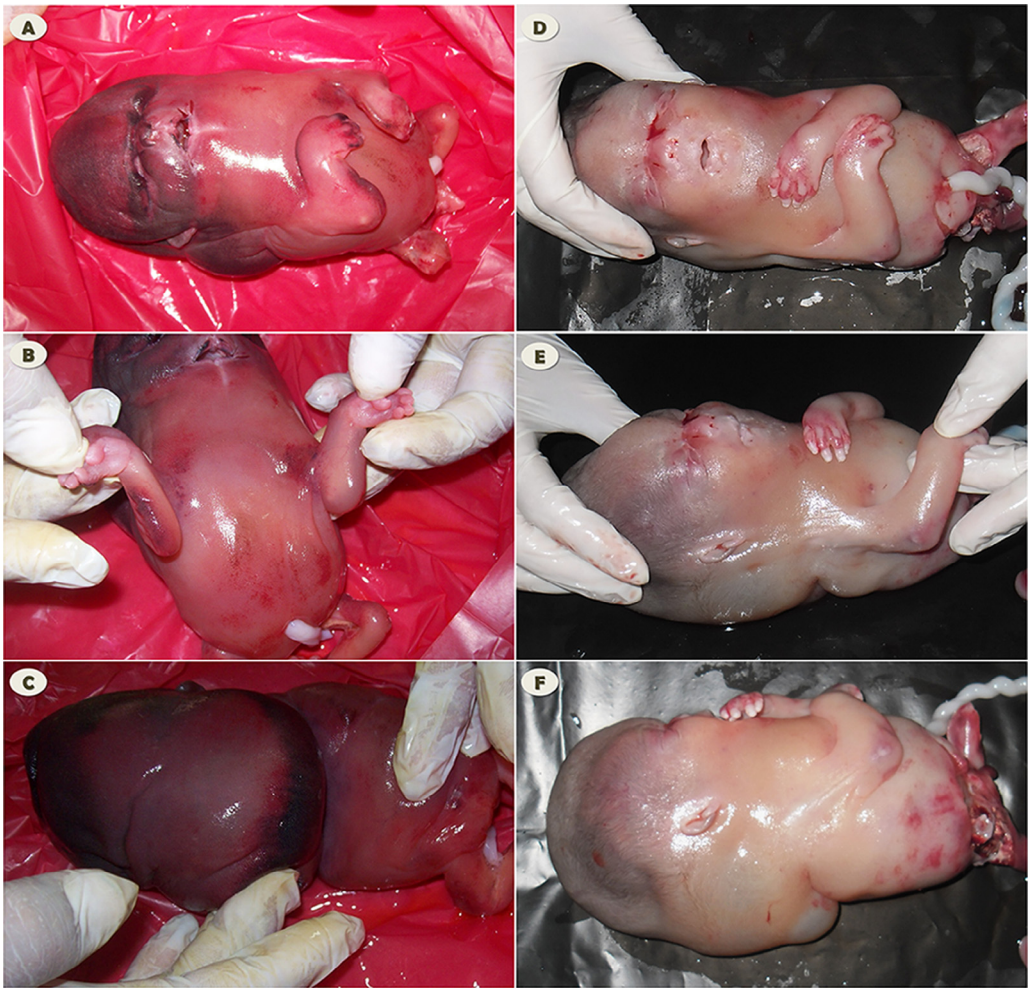

Fig. 1. Postmortem clinical photographs demonstrating the severe consequences of prolonged fetal akinesia in two sibling fetuses with lethal multiple pterygium syndrome. Dysmorphic facial features, including hypertelorism, downslanting palpebral fissures and long philtrum, striking anterior and lateral neck webbing, clenched hands with ulnar deviation of fingers, abnormally flexed elbows (A and D), multiple pterygia seen across all major joints (B and E), severe scalp edema and huge cystic hygroma (C and F).

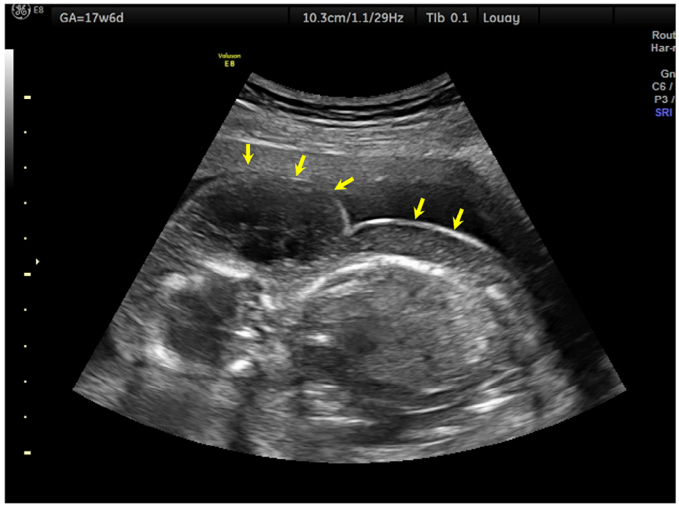

Fig. 2. Mid trimester scan of the second affected fetus (GA: 17w+6d) showing diffuse subcutaneous edema involving the whole fetal body (arrows).

Molecular genetic studies

Exome enrichment was performed on DNA from the second affected fetus with the Ampliseq Whole Exome Kit (ThermoFisher Scientific) and sequencing was performed using an Ion Proton sequencer, as previously described [16]. After mapping, annotation and filtering, 176 coding rare variants that were called as homozygous remained. Of these the only variant within a known neurogenetic disease gene was *NEB*. We used a list of 463 known neurogenetic disease genes to interrogate the exome data, this included all known genes for neuromuscular fetal akinesias, arthrogryposes, congenital myopathies, myasthenia syndromes and dystrophies.

Sequencing identified a homozygous essential splice-site variant in *NEB* (ENST00000427231.2, exon74, c.10872+1G>T). Sanger sequencing confirmed this variant was present in a homozygous state in the proband and showed that both parents were carriers (Fig. 3).

3. Discussion

The nebulin gene (*NEB*), with its 183 exons, encodes one of the biggest proteins in the muscle sarcomere (600–900 kDa) [17]. In this report, we describe a novel essential splice-site variant in the nebulin gene associated with FADS/LMPS in an Egyptian family. The mutation was identified, by next generation sequencing, in their second affected fetus. Sanger sequencing confirmed that the fetus was homozygous, and that both parents were heterozygous for the mutation. This mutation was not found in the recent *NEB* mutation update [10].

In 1997, *NEB* was localized to the linkage region for the autosomal recessive nemaline myopathy (NM), leading to the identification of the first mutations in *NEB* associated with this rare muscle disorder [18]. Later, mutations in this gigantic gene

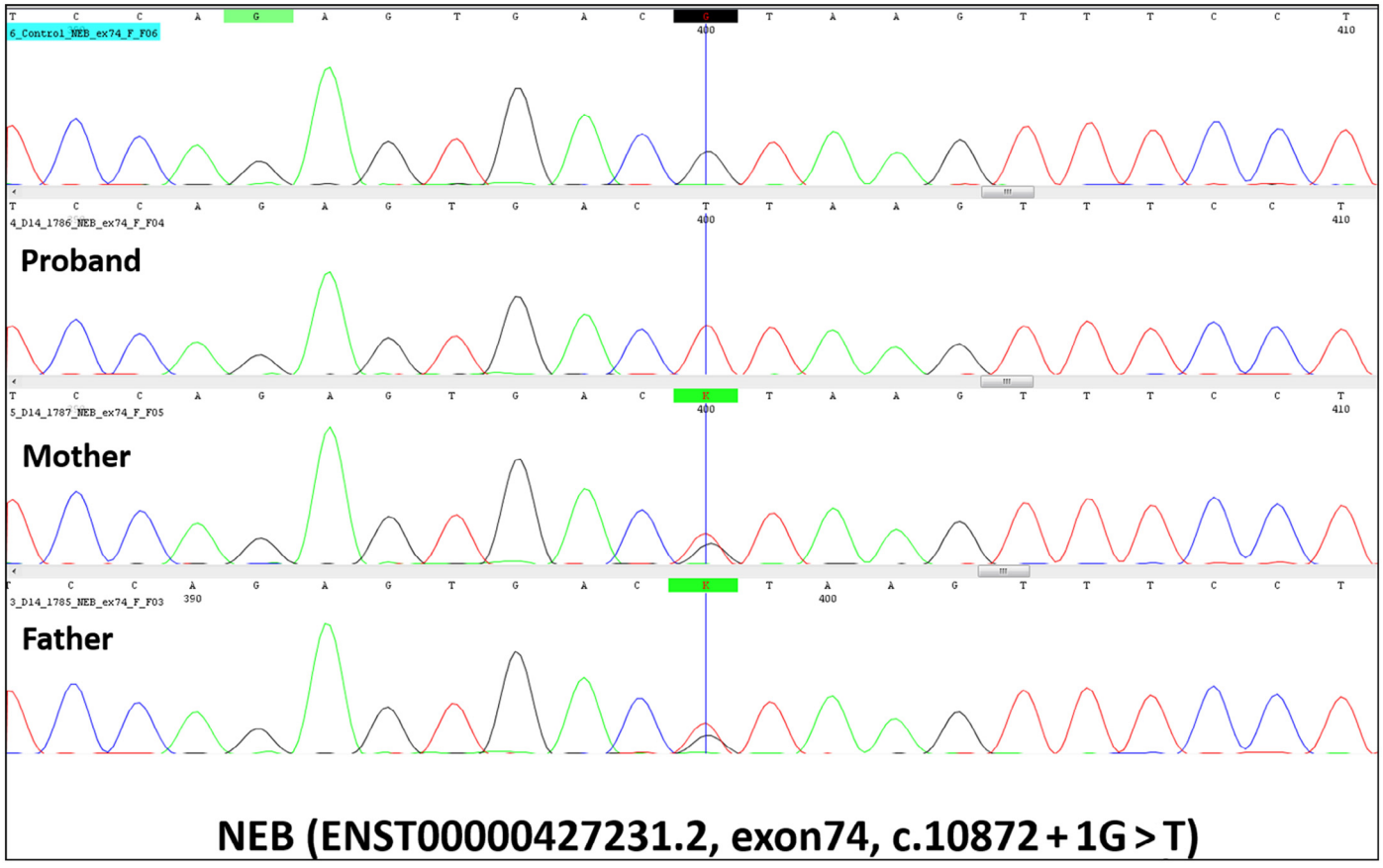

Fig. 3. Partial sequence chromatogram showing a splice-site variant (c.10872+1G>T) in exon 74 of *NEB* for the proband and his parents.

have been associated with other disorders: core-rod myopathy [19], early-onset distal myopathy without nemaline bodies [20], a distal form of NM [21], and a childhood-onset distal myopathy with rods and cores [22].

Recently, Lehtokari et al. identified *NEB* variants in fetuses with FADS/LMPS [10]. The muscle biopsy performed on the fetuses showed almost complete replacement of muscle with adipose tissue. Todd et al. and Laquérriere et al. also reported homozygous mutations in *NEB* gene in consanguineous cases of FADS/LMPS [11,23]. In the absence of a muscle biopsy finding, we are unable to determine whether our cases had nemaline myopathy. Ideally, the diagnosis of fetal akinesia must combine clinical evaluation, muscle morphology and confirmation by genetic testing.

Up to 2014, 222 different mutations have been identified in *NEB*; all pathogenic variants have been recessive [10]. The most common types of variants were splice-site mutations followed by frameshift mutations caused by small (<20 bp) deletions or insertions and nonsense mutations, which are predicted to cause mRNA instability or premature truncation of nebulin [17,24]. Missense mutations were less common, while large deletions and duplications were rare [10]. Our study, thus, agrees with previous reports as the revealed mutation was a recessive splice-site variant. It also adds to the heterogeneity of *NEB* mutations since the variant identified in our proband was not included in the most recent *NEB* mutation update [10].

Although *NEB* was initially described as a candidate gene for the typical form of autosomal recessive nemaline myopathy [18], analyses of additional families provided further evidence that *NEB* mutations can indeed cause the severe forms associated with fetal akinesia [10,11,23]. A similar situation exists with the acetylcholine receptor γ subunit gene (*CHRNG*), which represents a major cause of both severe and mild (Escobar-variant) multiple pterygium syndrome [9,15]. Recently, *RAPSN* mutations were also observed to cause a spectrum of phenotypes ranging from later onset myasthenia with "mild mutations" through neonatal congenital myasthenic syndrome with arthrogryposis, to severe fetal akinesia with lethality associated with homozygosity for truncating mutations [14]. Another recent study also indicated that LMPS can be considered as the extreme end of the RYR1-related neonatal myopathy spectrum [25]. Accordingly, it is becoming increasingly clear that FADS/LMPS represents the severe end of the spectra of some of the recognized neuromuscular disorders [9,11,12,14,16,25].

In conclusion, our report adds further evidence to the contribution of *NEB* mutations in the etiology of FADS/LMPS. This study also highlights the usefulness of next generation sequencing to determine the genetically heterogeneous etiology of fetal akinesia.

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