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REVISED VERSION

Germline mutation in *DOK7* associated with Foetal Akinesia Deformation Sequence

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Abstract

Foetal akinesia deformation sequence syndrome (FADS) is a heterogenous disorder characterised by foetal akinesia and developmental defects including, in some case, pterygia. Multiple pterygium syndromes (MPS) are traditionally divided into prenatally lethal and non-lethal (such as Escobar) types. Previously, we and others reported that homozygous mutations in the foetal acetylcholine receptor gamma subunit (*CHRNG*) can cause both lethal and non-lethal MPS, demonstrating that pterygia resulted from foetal akinesia, and that mutations in the acetylcholine receptor subunits *CHRNA1*, *CHRND*, and Rapsyn (*RAPSN*) can also result in a MPS/FADS phenotype. We hypothesised that mutations in other acetylcholine receptor-related genes may interfere with neurotransmission at the neuromuscular junction and so we analysed 14 cases of lethal MPS/FADS without *CHRNG*, *CHRNA1*, *CHRNBI*, *CHRND*, or *RAPSN* mutations for mutations in *DOK7*. A homozygous *DOK7* splice site mutation, c.331+1G>T, was identified in a family with three children affected with lethal FADS. Previously *DOK7* mutations have been reported to underlie a congenital myasthenic syndrome with a characteristic 'limb girdle' pattern of muscle weakness. This finding is consistent with the hypothesis that whereas incomplete loss of *DOK7* function may cause congenital myasthenia, more severe loss of function can result in a lethal foetal akinesia phenotype.

Key words

Multiple pterygium syndrome, Foetal akinesia, *DOK7* mutations

Foetal akinesia deformation sequence syndrome (FADS) is characterised by a variable combination of foetal akinesia, intrauterine growth retardation, developmental defects (e.g. cystic hygroma, lung hypoplasia, cleft palate, cryptorchidism, intestinal malrotation, cardiac defects), arthrogryposis and, in some cases, limb pterygia (1,2). FADS is clinically and genetically heterogeneous. For example, different multiple pterygium syndrome may be inherited as an autosomal recessive (most common), autosomal dominant or X-linked trait (3-5). Although a diagnosis of a specific primary myopathy, metabolic or neurodevelopmental disorder can be made in a few cases of multiple pterygium syndrome, until recently, the underlying aetiology was unknown in most cases (6.). However, we and others have reported that germline mutations in *CHRNA1* (which encodes the foetal gamma subunit of the acetylcholine receptor) account for ~30% of lethal and non-lethal (Escobar variant) multiple pterygium syndrome (LMPS and EVMPS) (7, 8). Subsequently mutations in genes that encode other subunits that make up the foetal acetylcholine receptor (*CHRNB1*, *CHRND*) or the crucial AChR clustering protein rapsyn (*RAPSN*) have been detected in FADS/LMPS (9,10). Previously, mutations in *CHRNB1*, *CHRND*, and *RAPSN* had been described in congenital myasthenia syndrome (CMS), a disorder that is characterised by muscle fatigability and, rarely, arthrogryposis (11-13). In the light of this, we hypothesised that mutations in *DOK7*, a gene that has been implicated in CMS and foetal acetylcholine receptor function (14), might also cause a FADS/LMPS phenotype.

Fourteen families with features of FADS/LMPS (see Table 1) and no evidence of *CHRNA1*, *CHRNB1*, *CHRND* and *RAPSN* mutations were examined for germline mutations in the exons and flanking intronic sequences of *DOK7* by direct sequencing on an ABI 3730 DNA Analyser (primers details are available on request). All families gave

informed consent and the study was approved by the South Birmingham Research Ethics Committee.

A homozygous *DOK7* splice site mutation (c.331+1G>T) was identified in a consanguineous Bengali family with three children affected with the foetal akinesia deformation sequence (see Fig 1). Both parents and the two unaffected siblings were heterozygous for the mutation. The mutation was not detected in 378 ethnically matched control chromosomes. Because this nucleotide substitution affects the consensus donor splice site motif, it is likely to abolish correct RNA splicing and an *in silico* splice site prediction programme (http://www.fruitfly.org/seq_tools/splice.html) suggested that the consensus donor splice site was lost in the mutant sequence. As no foetal tissue from which RNA could be extracted was available and *DOK7* expression was not detected in blood (data not shown), it was not possible to directly examine the effect of the mutation on RNA splicing. However, this mutation would be predicted to result in exon 3 skipping of an in frame deletion of 77 amino acids from the PH domain of *DOK7*. A recent report confirms this in frame skipping of exon 3 and showed that *Dok-7* harbouring this mutation impaired MuSK activation in cultured cells (14).

Review of the clinical records revealed that the first foetus was stillborn at 32 weeks gestation and had signs of a neuromuscular developmental abnormality. The second affected foetus miscarried spontaneously at 22 weeks of gestation. Post mortem examination revealed that the foetus had downslanting palpebral fissures, a small jaw and a short neck. The limbs had extended extremities, overlapping fingers, normal palmar creases, bilateral talipes and rockerbottom feet with reduced muscle bulk. Bilateral hydrothoraces and severe generalised oedema was present. Histopathological examination detected features of a muscle denervation type lesion. Thus the muscle appeared immature, with irregularly shaped muscle cells, and there was a disparity in the slow to fast muscle density with patchy foci of slow

fibres and atrophy of the fast fibres. The third affected foetus was detected by ultrasonography at 24 weeks gestation when no foetal movements were observed. None of the affected fetuses had evidence of pterygia.

DOK7 (Downstream of kinase) encodes a 55-kD (504 amino acid) protein (15). Like the other Dok family proteins, Dok-7 contains a pleckstrin homology (PH) and phosphotyrosine binding (PTB) domain in the N-terminal region and Src homology 2 (SH2) domain target motifs in the C-terminal portion. In C2C12 mouse myotubes Dok-7 binds to the MuSK phosphotyrosine binding domain target motif through its PTB domain and induces the tyrosine phosphorylation of MuSK, resulting in numerous differentiated AChR clusters (15). Homozygous mutations in Dok-7 have previously been found in autosomal recessive congenital myasthenic syndrome (CMS) (16-18). *DOK7* mutations in CMS patients have been associated with small neuromuscular junctions with reduced postsynaptic folding (19) but with normal AChR density and function..Patients with *DOK7* mutations, almost exclusively, have at least one allele harbouring a mutation in the C-terminal region, frequently the frameshift mutation 1124_1127dupTGCC. Truncating mutations in the C terminal domain of Dok-7 have been shown to result in an impaired ability to induce phosphorylation of MuSK and to form the specialization of the postsynaptic structures (16) and it has been suggested that they have little effect on the initial synapse formation (CMS patients homozygous for such mutations do not usually present at birth but in early childhood), but may impair maturation and maintenance of synaptic structure (16). The phenotype of CMS patients with *DOK7* mutations although variable in severity has characteristic features. Typically they have a limb girdle pattern of muscle weakness with the muscles of the limbs and trunk mainly affected. Facial and bulbar weakness is frequent and although ptosis is commonly observed, eye movements are usually unaffected. For some, respiratory function may be impaired and deteriorate over time necessitating nocturnal

ventilation, whilst for others respiratory weakness and impairment may require a period of assisted ventilation (17). However, the congenital joint contractures seen in CMS patients with *RAPSN* mutations are not a feature of *DOK7*-associated CMS (18). We found that homozygosity for an intron 3 splice site mutation was lethal *in utero*. This finding is consistent with the hypothesis that whereas incomplete loss of *Dok-7* function will cause a CMS phenotype, complete loss of function is lethal. A similar model of disease pathogenesis has been suggested for *RAPSN* mutations (9). Although the development of neuromuscular junction differs in mice and humans (in mice the switch from foetal to adult acetylcholine receptors takes place in the first two weeks of life, but in humans the switch occurs earlier and is apparently complete by 31 weeks gestation (20, 21), the phenotype of *Chrng*, *Rapsn* and *Dok7* knockout mice are similar. Thus *Dok7* deficient mice were unable to breathe or move and died shortly after birth, suggesting a severe neuromuscular transmission defect, whereas their heterozygous littermates appeared normal. The *Dok7* deficient mice had no detectable AChR clusters in the endplate region of the diaphragmatic muscle, consistent with *Dok-7* having a critical role in neuromuscular synaptogenesis (15).

The findings of *DOK7* mutations in FADS further develop the concept that a spectrum of disorders, ranging from FADS/MPS to CMS may be caused by mutations in genes that influence the function of foetal and adult acetylcholine receptors with the severity of disease reflecting the effect of specific mutations on neuromuscular transmission.

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REFERENCES

1. Hall JG, Reed SD, Rosenbaum KN, Gershanik J, Chen H, Wilson KM. Limb pterygium syndromes: a review and report of eleven patients. *Am J Med Genet* 1982;12:377-379
2. Moessinger AC. Fetal alinesia deformation sequence: an animal model. *Pediatrics* 1983;72:857-63
3. McKeown CM and Harris R. An autosomal dominant multiple pterygium syndrome. *J Med.Genet* 1988; 25:96-103
4. Prontera P, Sensi A, Merlo L, Garani G, Cocchi G, Calzolari E. Familial occurrence of multiple pterygium syndrome: expression in a heterozygote of the recessive form or variability of the dominant form? *Am J Med.Genet A* 2006;140:2227-2230.
5. Tolmie JL, Patrick A, and Yates JR. A lethal multiple pterygium syndrome with apparent X-linked recessive inheritance. *Am J Med.Genet* 1987;27:913-919.
6. Cox PM, Brueton LA, Bjelogrljic P, Pomroy P, Sewry CA. Diversity of neuromuscular pathology in lethal multiple pterygium syndrome. *Pediatr Dev Pathol* 2003;6:59-68
7. Morgan NV, Brueton LA, Cox P, greally MT, Tolmie J, Pasha S, Aligianis IA, van Bokhoven H, Marton T, Al-Gazali L, Morton JEV, Oley C, Johnson CA, Trembath RC, Brunner HG, Maher ER. Mutations in the embryonal subunit of the acetylcholine receptor (CHRNG) cause lethal and Escobar variants of multiple pterygium syndrome. *Am J Hum.Genet* 2006;79:390-395
8. Hoffmann K, Muller JS, Stricker S, Megarbane A, Rajab A, Lindner TH, Cohen M, Chouery E, Adaimy L, Ghanem I, Delague V, Boltshauser E, Talim B, Horvath R, Robinson PN, Lochmuller H, Hubner C, Mundlos S. Escobar syndrome is a prenatal myasthenia caused by disruption of the acetylcholine receptor fetal gamma subunit. *Am J Hum Genet* 2006;79:303-12
9. Vogt J, Harrison BJ, Spearman H, Cossins J, Vermeer S, ten Cate LN, Morgan NV, Beeson D, Maher ER. Mutation analysis of CHRNA1, CHRNB1, CHRND, and RAPSN genes in multiple pterygium syndrome/fetal akinesia patients. *Am J Hum Genet* 2008;82:222-7
10. Michalk A, Stricker S, Becker J, Rupps R, Pantzar T, Miertus J, Botta G, Naretto VG, Janetzki C, Yaqoob N, Ott CE, Seelow D, Wiczorek D, Fiebig B, Wirth B, Hoopmann M, Walther M, Körber F, Blankenburg M, Mundlos S, Heller R,

- Hoffmann K. Acetylcholine receptor pathway mutations explain the fetal akinesia deformation sequence. *Am J Hum Genet* 2008;82:464-476
11. Brownlow S, Webster R, Croxson R, Brydson M, Neville B, Lin JP, Vincent A, Newsom-Davis J, Beeson D. Acetylcholine receptor δ subunit mutations underlie a fast-channel myasthenic syndrome and arthrogryposis multiplex congenita. *J Clin Invest* 2001;108:125-130
 12. Burke G, Cossins J, Maxwell S, Owens G, Vincent A, Robb S, Nicolle M, Hilton-Jones D, Newsom-Davis J, Palace J, Beeson D. Rapsyn mutations in hereditary myasthenia: distinct early- and late-onset phenotypes. *Neurology* 2003;23;61:826-8
 13. Beeson D, Hantai D, Lochmuller H, Engel AG. 126 International Workshop: congenital myasthenic syndromes, 24-26 September 2004, Naaden, the Netherlands. *Neuromuscul Disord* 2005;15:498-512
 14. Selcen D, Milone M, Shen XM, Harper CM, Stans AA, Wieben ED, Engel AG. Dok-7 myasthenia: Phenotypic and molecular genetic studies in 16 patients. *Ann Neurol* 2008;64:71-87
 15. Okada K, Inoue A, Okada M, Murata Y, Kakuta S, Jigami T, Kubo S, Shiraishi H, Eguchi K, Motomura M, Akiyama T, Iwakura Y, Higuchi O, Yamanashi Y. The muscle protein Dok-7 is essential for neuromuscular synaptogenesis. *Science* 2006;312:1802-1805
 16. Beeson D, Higuchi O, Palace J, Cossins J, Spearman H, Maxwell S, Newsom-Davis J, Burke G, Fawcett P, Motomura M, Muller JS, Lochmuller H, Slater C, Vincent A, Yamanashi Y. Dok-7 mutations underlie a neuromuscular junction synaptopathy. *Science* 2006;313:1975-1978
 17. Muller JS, Herczegfalvi A, Vilchez JJ, Colomer J, Bachinski LL, Mihaylova V, Santos M, Schara U, Deschauer M, Shevell M, Poulin C, Dias A, Soudo A, Hietala M, Äärimaa T, Krahe R, Karcagi V, Huebner A, Beeson D, Abicht A, Lochmüller H. Phenotypical spectrum of DOK7 mutations in congenital myasthenic syndromes. *Brain* 2007;130:1497-1506
 18. Palace J, Lashley D, Newsom-Davis J, Cossins J, Maxwell S, Kennett R, Jayawant S, Yamanashi Y, Beeson D. Clinical features of the DOK7 neuromuscular junction synaptopathy. *Brain* 2007;130:1507-1515
 19. Slater CR, Fawcett PRW, Walls TJ, Lyons PR, Bailey SJ, Beeson D, Young C, Gardner-Medwin D. Pre- and post synaptic abnormalities associated with impaired

neuromuscular transmission in a group of patients with 'limb-girdle myasthenia'.
Brain 2006;129, 2061-2076

20. Kues WA, Sakmann B, Witzemann V. Differential expression patterns of five acetylcholine receptor subunit genes in rat muscle during development. *Eur J Neurosci* 1995;7:1376-1385
21. Hesselmans LF, Jennekens FG, Van den Oord CJ, Veldman H, Vincent A. Development of innervation of skeletal muscle fibres in man: relation to acetylcholine receptors. *Anat Rec* 1993;236:553-562

Table 1: Details of the 14 families with fetal akinesia syndrome/lethal multiple pterygium syndrome phenotype and no evidence of *CHRNA1*, *CHRNA1*, *CHRNA1*, *CHRNA1* and *RAPSN* mutations that were examined for germline mutations in the exons and flanking intronic sequences of *DOK*.

LMPS= lethal multiple pterygium syndrome

Family ID	Ethnic Origin	Parental Consanguinity	Phenotype
MPS012	Bengali	Yes	No pterygia
MPS013	Pakistani	Yes	LMPS
MPS016	White	No	LMPS
MPS017	White	No	LMPS
MPS019	Indian	No	LMPS
MPS020	White	No	LMPS
MPS021	White	Yes	LMPS
MPS022	Afrikaner	No	LMPS
MPS023	White	No	LMPS
MPS027	Afrikaner Afro	No	LMPS
MPS028	Caribbean	No	LMPS
MPS010	Pakistani	Yes	No pterygia
MPS009	Pakistani	Yes	LMPS
MPS029	Turkish	Yes	LMPS

Figure 1: Sequence traces for normal control, homozygote and heterozygote carrier of *DOK7* splice site mutation (c.331+1G>T)

