

REVIEW

Genetics of neuromuscular fetal akinesia in the genomics era

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ABSTRACT

Fetal hypokinesia or akinesia encompasses a broad spectrum of disorders, united by impaired movement in utero. Often, the underlying aetiology is genetic in origin, affecting part of the neuromuscular system. The affordable and high-throughput nature of next-generation DNA sequencing has led to an explosion in disease gene discovery across rare diseases, including fetal akinesias. A genetic diagnosis has clinical utility as it may affect management and prognosis and informs recurrence risk, facilitating family planning decisions. More broadly, knowledge of disease genes increasingly allows population-based preconception carrier screening, which has reduced the incidence of recessive diseases in several populations. Despite gains in knowledge of the genetics of fetal akinesia, many families lack a genetic diagnosis. In this review, we describe the developments in Mendelian genetics of neuromuscular fetal akinesia in the genomics era. We examine genetic diagnoses with neuromuscular causes, specifically including the lower motor neuron, peripheral nerve, neuromuscular junction and muscle.

INTRODUCTION

Fetal hypokinesia or akinesia encompasses a broad spectrum of disorders with the unifying feature of reduced or absent fetal movement, which results in a variety of secondary defects.¹ For simplicity, we will from here on refer to these diseases as fetal akinesias. This group contains several overlapping entities, ranging in severity from distal arthrogryposis, multiple pterygium syndrome and arthrogryposis multiplex congenita (AMC), to the most severe, fetal akinesia deformation sequence (FADS).¹ Features vary depending on when fetal movement was impaired. The phenotype may be further complicated by abnormalities associated with the underlying cause of the akinesia. However, common features include subcutaneous oedema, fetal hydrops, lung hypoplasia, rocker-bottom feet, craniofacial anomalies (particularly cleft palate, retromicrognathia), intrauterine growth restriction and poor muscle bulk.¹ Landmark studies demonstrated reduced fetal movement is responsible for the main clinical features, highlighting the need for adequate fetal movement in normal development.^{2,3}

The underlying defect can be genetic or environmental. The aetiology is generally categorised into muscular disorders, neurological disorders (central and/or peripheral, and neurometabolic), connective tissue disorders, fetal vascular compromise, uterine

space limitations and maternal diseases or drug use.¹ This review is an update from Ravenscroft *et al*¹ covering discoveries in monogenic fetal akinesia disorders with neuromuscular causes.¹ Specifically, we focus on conditions affecting the lower motor neuron, peripheral nerves, neuromuscular junction (NMJ) and skeletal muscles. As per the 2011 review, restrictive dermopathies and primary brain abnormalities are excluded. However, it is important to note that brain abnormalities are often difficult to distinguish from primary neuromuscular causes of fetal akinesia because the clinical features can overlap considerably. Genes recently associated with central forms of fetal akinesia include *PI4KA*, *PDHA1* and *SLC6A9*.^{4–6} Table 1 summarises new discoveries since the 2011 review.

Since 2011, there has been an explosion in disease gene discovery, powered by affordable massively parallel next-generation sequencing (NGS). New genotype–phenotype correlations are blurring the boundaries between what were once considered distinct entities.^{7,8} This trend extends beyond fetal akinesia, across neuromuscular diseases as a whole.⁸ Defining the genetic and phenotypic spectra of fetal akinesias is significant for patients and clinicians, as it facilitates a genetic diagnosis.^{8,9} This is vital, as clinical diagnosis of fetal akinesia disorders mid-gestation is especially challenging; ultrasonography has limited diagnostic utility, and clinical information such as intellectual ability is not available.^{10–12} Post-natal diagnosis is hampered by the clinical heterogeneity of the fetal akinesia spectrum.¹⁰ Beyond immediate clinical utility, genetic diagnosis clarifies family genetic implications and allows consideration of reproductive options in ‘high risk’ cases (ie, preimplantation genetic diagnosis, prenatal genetic diagnosis). Paediatric precision medicine has the potential to resolve heterogeneity, limit unnecessary testing and spare parents and patients from the diagnostic odyssey.¹³ Rapid NGS-based testing for genetic diseases showed significant success in neonatal and paediatric intensive care units, allowing reduction of morbidity and mortality via precision interventions.^{13,14} However, this requires large quantities of high-quality DNA from the affected offspring. Access to sufficient quantity/quality of material is often limited, particularly in cases of fetal demise.¹² An alternative approach is parental exome sequencing, which searches for rare, heterozygous variants occurring in the same gene in both parents. Despite being unable to identify de novo variants, this method had an overall success rate of



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Table 1 Summary of neuromuscular fetal akinesia genetics, 2011 and 2018

Gene symbol	MIM	Mode of inheritance	Fetal disease entity
Genes involved in motor neuron development and survival			
<i>SMN1</i>	600354	AR	SMA, FADS
<i>ERBB3</i>	190151	AR	LCCS2
<i>GLE1</i>	603371	AR	LCCS1, LAAHD, non-lethal arthrogryposis
<i>PIP5K1C</i>	606102	AR	LCCS3
<i>UBE1</i>	314370	XL	XL-SMA
<i>ERGIC1</i>	NA	AD	AMC, neuropathic type
<i>TUBB2B</i>	612850	AD	Cortical dysplasia, complex, with other brain malformations 7
Genes involved in combined central and peripheral nervous system defects			
<i>ASCC1 and TRIP4</i>	614215, 604501	AR	Congenital bone fractures 1 and 2
<i>CNTNAP1</i>	602346	AR	LCCS7
<i>LGII4</i>	608303	AR	AMC, neurogenic with myelin defect
Genes encoding components of the peripheral nervous system			
<i>ADCY6</i>	600294	AR	LCCS8
<i>ADGRG6 (GPR126)</i>	612243	AR	LCCS9
<i>ECEL1</i>	605896	AR	DA5D
<i>GLDN</i>	608603	AR	LCCS11 (not always lethal)
<i>PIEZO2</i>	613629	AD, AR*	MWKS, DA3, DA5, DAIPT
Genes encoding components of the neuromuscular junction			
<i>CHRNA1</i>	100690	AR	Lethal multiple pterygium syndrome
<i>CHRND</i>	100720	AR	AMC/CMS with fetal akinesia, FADS
<i>CHRNA1</i>	100730	AR	Lethal and EV MPS
<i>CNTN1</i>	600016	AR	CM with fetal akinesia
<i>DOK7</i>	610285	AR	FADS
<i>SYNE1</i>	608441	AR	AMC with fetal akinesia
<i>RAPSN</i>	601592	AR	AMC, FADS
<i>MUSK</i>	601296	AR	CMS9, FADS
<i>SLC18A3</i>	600336	AR	CMS21, FADS
<i>UNC50</i>	NA	AR	Lethal AMC
Genes encoding adult skeletal muscle proteins			
<i>ACTA1</i>	102610	AD	FADS
<i>BIN1</i>	601248	AR	CNM with fetal akinesia
<i>DMPK</i>	605377	AD	FADS/DM
<i>FKRP</i>	606596	AR	VWS with fetal akinesia
<i>LMNA</i>	150330	AR	LGMD1B with fetal akinesia
<i>MTM1</i>	300415	XL	MTM with fetal akinesia
<i>NEB</i>	161650	AR	FADS
<i>RYR1</i>	180901	AD, AR*	FADS, CRM with fetal akinesia, MPS
<i>TPM2</i>	190990	AD, AR*	EV MPS, DA1, DA2B, NEM 4, cap myopathy 2
<i>TNNI2</i>	191043	AD	DA1, DA2B
<i>TNNI3</i>	600692	AD	DA1, DA2B
<i>CACNA1S</i>	114208	AD, AR	Congenital myopathy, fetal akinesia
<i>DNM2</i>	602378	AD, AR*	LCCS5
<i>FKTN</i>	607440	AR	MDDGA4
<i>SCN4A</i>	603967	AD, AR*	Congenital myopathy, fetal hypokinesia
<i>VMA21</i>	300913	XL	XMEA
Genes encoding fetally expressed myostructural proteins			
<i>MYH3</i>	160270	AD	DA2A, DA2B, DA8, MPS
<i>MYH8</i>	160741	AD	CC-DA7, DA7
<i>UTRN</i>	128240		Arthrogryposis with fetal akinesia
<i>KLHL40</i>	615340	AR	NEM8, fetal akinesia
<i>KLHL41</i>	607701	AR	NEM9, fetal akinesia
<i>LMOD3</i>	616112	AR	NEM10, fetal akinesia
<i>MYOD1</i>	159970	AR	Lethal fetal akinesia
<i>MYBPC1</i>	160794	AD, AR*	DA1B, LCCS4
<i>TTN</i>	188840	AD, AR*	Cardiomyopathy, muscular dystrophy, fetal akinesia

Continued

Table 1 Continued

Gene symbol	MIM	Mode of inheritance	Fetal disease entity
ZBTB42	613915	AR	LCCS6
Other genes			
<i>FGFR2</i>	176943	AD	FADS
<i>GBE1</i>	607839	AR	GSD-IV/FADS
DPAGT1	191350	AR	Congenital disorder of glycosylation, type Ij

Plain text indicates information from 2011 review.

Bold text indicates new information from 2018 review. Genes with asterisks manifest severe fetal akinesia phenotypes only with recessive inheritance.

AD, autosomal dominant; AMC, arthrogryposis multiplex congenita; AR, autosomal recessive; CC, Carney complex; CM, congenital myopathy; CMS, congenital myasthenic syndrome; CNM, centronuclear myopathy; CRM, core-rod myopathy; DA, distal arthrogryposis; DAIPT, arthrogryposis, distal, with impaired proprioception and touch; DM, myotonic dystrophy; EV, Escobar variant; FADS, fetal akinesia deformation sequence; GSD-IV, glycogen storage disease type IV; LAAHD, lethal arthrogryposis with anterior horn cell disease; LCCS, lethal congenital contracture syndrome; LGMD1B, limb-girdle muscular dystrophy type 1B; MDDG, muscular dystrophy-dystroglycanopathy; MPS, multiple pterygium syndrome; MTM, myotubular myopathy; MWKS, Marden-Walker syndrome; NA, not available; NEM, nemaline myopathy; SMA, spinal muscular atrophy; WWS, Walker-Warburg syndrome; XL, X linked; XMEA, X linked myopathy with excessive autophagy.

52% in a cohort of 50 couples.¹² Although various sequencing approaches offer hope of rapid and non-invasive diagnosis, cost, feasibility and ethical issues require consideration before routine implementation of these in a diagnostic setting.¹⁵

GENES INVOLVED IN MOTOR NEURON DEVELOPMENT AND SURVIVAL

ERGIC1

In 2017, Reinstein *et al* performed a genetic investigation of a large Israeli Arab family clinically described by Lebenthal *et al*.^{16 17} Forty affected individuals were examined and diagnosed with AMC neurogenic type (MIM 208100) specifically affecting the lower motor neurons.¹⁷ Key features included congenital equinovarus and flexion contractures at the knees and elbows with muscle hypotrophy around the involved joints. One patient had severe mental retardation. A neuropathic cause was supported by electrophysiological studies and neurological examination. Exome sequencing revealed a homozygous missense mutation in *ERGIC1* (endoplasmic reticulum-Golgi intermediate compartment 1).¹⁷ *ERGIC1* encodes a putative transmembrane protein with a role in transport between the endoplasmic reticulum and the Golgi apparatus.¹⁷

GLE1

Biallelic *GLE1* (RNA export mediator; MIM 603371) mutations were initially shown to cause two lethal diseases in the Finnish population (lethal congenital contracture syndrome 1 (LCCS1), MIM 253310; and lethal arthrogryposis with anterior horn cell disease (LAAHD), MIM 611890).¹ Previously, *GLE1* mutations were described almost exclusively in Finnish patients. Homozygosity for the Fin_{major} mutation caused LCCS1, while compound heterozygosity for Fin_{major} and one of two common missense mutations caused the slightly milder LAAHD.¹⁸ However, recent reports describe four non-Finnish patients surviving into childhood, prompting debate if these cases constitute a new clinical entity or a phenotypic expansion.^{18–20} One patient died of respiratory insufficiency at age 4; three patients are alive at 26 months, 4 years and 12 years.^{18–20} Finding that patients without the 'Finnish' mutations can survive beyond infancy suggests disease severity is mutation-dependent.¹⁸

TUBB2B

TUBB2B (MIM 612850) encodes beta-2B tubulin, a microtubule component.²¹ Dominant mutations cause complex cortical dysplasia with other brain malformations (MIM 610031). Laquerrière *et al*²² added *de novo* dominant FADS as a severe manifestation of this phenotypic spectrum. Ultrasound at 14

weeks' gestation revealed complete fetal akinesia; pregnancy was terminated at 15 weeks. The fetus showed multiple brain abnormalities, cleft palate, lung hypoplasia, dilated renal pelvis, global amyotrophy and severe spinal cord hypoplasia. Motoneurons were almost undetectable in the anterior and posterior horns.²²

GENES INVOLVED IN COMBINED CENTRAL AND PERIPHERAL NERVOUS SYSTEM DEFECTS

ASCC1 and TRIP4

The tetrameric ASC-1 transcriptional cointegrator complex comprised four subunits: subunit 1 (*ASCC1*; MIM 614215), subunit 2 (*ASCC2*; MIM 614216), subunit 3 (*ASCC3*; MIM 614217) and thyroid hormone receptor interactor 4 (*TRIP4*; MIM 604501).²³ This complex may act in neuromuscular unit development via myogenic differentiation and growth regulation.^{23 24} In 2016, four families with congenital bone fractures 1 (MIM 616866) were described with recessive loss-of-function *TRIP4* mutations, while one family with congenital bone fractures 2 (MIM 616867) showed biallelic *ASCC1* loss-of-function mutations.^{23 24} Consistent features included fetal hypokinesia, lack of muscle contraction on electrical nerve stimulation, axonal neuropathy and requirement for ventilator assistance. Muscle biopsy showed features suggestive of spinal muscular atrophy. One *TRIP4* patient showed apoptotic alpha-motoneurons, while sural nerve biopsy of two *ASCC1* patients showed unmyelinated axon loss. All patients died of respiratory distress before the age of 2.²³ In 2017, a second *ASCC1* case was described, supporting this as a human disease gene.²⁵

CNTNAP1

CNTNAP1 (contactin-associated protein 1; MIM 602346) encodes the contactin-associated protein CASPR, which is involved in the correct formation of paranodal junctions in myelinated axons.^{26 27} Laquerrière *et al*²⁷ reported homozygous frameshift *CNTNAP1* mutations in seven patients from four consanguineous families. Five of the patients had fetal akinesia. At birth, patients had severe motor paralysis, leading to death within 2 months. Features included markedly decreased nerve conduction velocity, widening of the nodes of Ranvier and thin myelin sheaths in the sciatic nerve.²⁷ This entity was designated lethal congenital contracture syndrome 7 (MIM 616286). Later, Hengel *et al*²⁶ described five patients from two families with biallelic truncating *CNTNAP1* mutations causing severe hypomyelinating leucodystrophy with peripheral neuropathy.

LGI4

LGI4 regulates peripheral nerve myelination.²⁸ Xue *et al*²⁸ described nine patients with AMC from four families with biallelic loss-of-function *LGI4* mutations (leucine-rich repeat LGI family member 4; MIM 608303). This entity is defined as arthrogryposis multiplex congenita, neurogenic, with myelin defect (MIM 617468). Features included clubfoot, camptodactyly, retrognathia and pulmonary hypoplasia. One patient survived past infancy. At age 4, he has dysmorphic features, verbal developmental delay, hypotonia, scoliosis and seizures. Muscle biopsy was suggestive of spinal anterior horn cell dysgenesis, but the sciatic nerve of one patient showed hypomyelination.²⁸ *Lgi4*-deficient mice also display arthrogryposis and peripheral hypomyelination.²⁸

GENES ENCODING COMPONENTS OF THE PERIPHERAL NERVOUS SYSTEM

ADCY6

Recessive *ADCY6* mutations (adenylyl cyclase 6; MIM 600294) cause lethal congenital contracture syndrome 8 (LCCS8; MIM 616287). Laquerrière *et al* described two consanguineous siblings with reduced fetal movements and distal joint contractures. Postnatally, the infants had areflexia, severe motor paralysis and respiratory distress leading to death within 3 months of birth.²⁷ No response was found on motor nerve conduction velocity testing. Electron microscopy showed no myelinated axons despite normal Schwann cell distribution.²⁷ *ADCY6* synthesises cyclic AMP, although its role in myelin protein expression in the peripheral nervous system is not fully understood.²⁷ Knockdown in zebrafish showed absence of myelin basic protein from the peripheral nervous system.²⁷

ADGRG6 (GPR126)

Ravenscroft *et al* described four individuals from three consanguineous families variably displaying polyhydramnios, ulnar deviation of hands, diaphragmatic atrophy and pterygia. Ultrasound findings were abnormal from as early as 16 weeks' gestation.²⁹ The phenotype was labelled lethal congenital contracture syndrome 9 (LCCS9; MIM 616503). All patients harboured homozygous loss-of-function mutations in *ADGRG6* (adhesion G protein-coupled receptor G6; MIM 612243). *Gpr126*^{-/-} mice also display a lethal arthrogryposis phenotype.³⁰ *ADGRG6* is critical for myelination of the peripheral nervous system.³⁰ Patients' peripheral nerves showed almost complete absence of myelin basic protein.²⁹

ECEL1

Biallelic mutations in *ECEL1* (endothelin-converting enzyme-like 1; MIM 605896) cause distal arthrogryposis type 5D (DA5D; MIM 615065).^{31 32} McMillin *et al* differentiated this from DA5 (see *PIEZO2* below) on the basis of the *ECEL1* cohort having ptosis but not ophthalmoplegia.³¹ The consensus phenotype includes hand and foot camptodactyly, wrist and knee contractures, clubfoot, short neck, round-shaped face, and bulbous nose.³³ Variable features include micrognathia, tongue atrophy, astigmatism and hip dislocation.³³ Muscle biopsies from two affected siblings showed features suggestive of centronuclear myopathy, although further studies are needed to confirm this finding.³³ Mouse models demonstrated that *Dine* (*ECEL1* homologue) is required for normal arborisation of motor axons after innervation, and subsequent NMJ formation.^{34 35} Additionally, disruption of *Dine* impaired differentiation of immature Schwann cells.³⁴

GLDN

Maluenda *et al*³⁶ identified six individuals with AMC, marked polyhydramnios and fetal akinesia at 27–32 weeks' gestation, despite unremarkable earlier ultrasounds. Features variably included intrauterine growth retardation, knee and elbow contractures, camptodactyly and retrognathia.³⁶ Electron microscopy of one patient's sciatic nerve showed reduced numbers of myelinated fibres and a significant increase in nodal length. Biallelic mutations were identified in the gene encoding gliomedin (*GLDN*; MIM 608603), which is an adhesion molecule involved in the formation of the nodes of Ranvier and development of the peripheral nervous system.³⁶ This disorder is designated lethal congenital contracture arthrogryposis 11 (LCCS11; MIM 617194). However, a 2017 study found four patients with recessive *GLDN* mutations who survived into late adolescence with intensive care, showing LCCS11 is not necessarily congenitally lethal.³⁷

PIEZO2

PIEZO2 is a mechanically activated non-selective cation channel essential for mechanotransduction in certain tissues.³⁸ *PIEZO2* (Piezo-type mechanosensitive ion channel component 2; MIM 613629) mutations have been implicated in both dominant and recessive disease. Dominant mutations cause three overlapping phenotypes: Marden-Walker syndrome (MIM 248700), distal arthrogryposis type 3 (Gordon syndrome; MIM 114300) and distal arthrogryposis type 5 (DA5; MIM 108145).^{38–40} These entities are distinguished by mental retardation, cleft palate and ocular abnormalities, respectively.³⁹ However, these may be variable presentations of the same disorder.⁴⁰ A dominant negative mechanism has been proposed, with mutants impairing multimer formation.⁴¹ Recessive loss-of-function mutations in *PIEZO2* cause autosomal recessive DA with impaired proprioception and touch (MIM 613629).^{41–43} Key features include dysmetria, muscle atrophy, progressive contractures and impaired mechanosensation causing ataxia. Reduced fetal movements and congenital hip dysplasia have also been described.⁴² Although *Piezo2* knockout is perinatal lethal in mice, human life expectancy is normal. It has been suggested that humans with homozygous loss-of-function alleles may produce some functional *PIEZO2* through alternative splicing.⁴⁴ This may explain the relatively mild phenotype compared with mice.

GENES ENCODING COMPONENTS OF THE NMJ

MUSK

MUSK (muscle associated receptor tyrosine kinase; MIM 601296) encodes a muscle-specific receptor tyrosine kinase (MuSK) critical for formation and maintenance of postsynaptic NMJs.⁴⁵ Recessive *MUSK* mutations cause postsynaptic congenital myasthenic syndrome (CMS), with severe cases resulting in fetal akinesia. A founder effect was discovered within a Dutch genetic isolate of 10 families, revealing a novel homozygous p.Ile575Thr substitution in all 14 cases (carrier frequency 8%).⁴⁵ The mutation causes abnormal acetylcholine receptor clustering and synaptogenesis.⁴⁵ Wilbe *et al*⁴⁶ discovered a second founder effect in Sweden. One Swedish couple had five affected fetuses that all harboured a homozygous truncating mutation.⁴⁶

SLC18A3

SLC18A3 (solute carrier family 18 member A3; MIM 600336) encodes vesicular acetylcholine transporter (VACHT), which packages acetylcholine into presynaptic vesicles.⁴⁷ Biallelic mutations cause presynaptic CMS, with severe presentations

including fetal akinesia.⁴⁷ Aran *et al* described two brothers with retrognathia, severe hypotonia, bilateral dislocated hips, bilateral undescended testes, micropenis and marked hirsutism. The first sibling died 5 days postnatally due to respiratory insufficiency. The second sibling has profound global developmental disability, delayed brain myelination, extreme hypotonia and progressive microcephaly at 4.5 years old. Western blot suggested the mutant VACHT protein undergoes post-translation degradation. This loss-of-function might explain the severe phenotype in this family.⁴⁷

UNC50

A single lethal AMC case with a homozygous frameshift deletion in *UNC50* (inner nuclear membrane RNA-binding protein; MIM 617826) has been described.⁴⁸ Ultrasound showed AMC and subcutaneous oedema before a stillborn male infant was spontaneously delivered at 28 weeks with pulmonary hypoplasia and limb muscular atrophy.⁴⁸ Muscle biopsy showed some atrophic fibres and areas of myofibrillar disorganisation. Although publication of a second family is required for confirmation of pathogenicity, *UNC50* is a candidate for similar cases.⁴⁸ *UNC50* is involved in acetylcholine receptor trafficking. Introduction of an equivalent mutation in *Caenorhabditis elegans* caused loss of acetylcholine receptor expression at the NMJ.⁴⁸

GENES ENCODING ADULT SKELETAL MUSCLE PROTEINS

CACNA1S

Dominant mutations in L-type voltage-dependent Ca^{2+} channel, alpha-1a subunit (*CACNA1S*; MIM 114208) are well known to cause hypokalaemic periodic paralysis, type 1 (MIM 170400) and susceptibility to malignant hyperthermia (MIM 601887).⁴⁹ Recently, dominant and recessive *CACNA1S* mutations were shown to cause congenital myopathy with particular morphological hallmarks, with fetal akinesia being the most severe manifestation.⁴⁹ The described 11 patients presented with congenital or early-onset hypotonia, facial weakness, delayed motor milestones and progressive muscle weakness. Respiratory function ranged from severe impairment to normal. Muscle biopsy showed sarcoplasmic reticulum dilatation, internal nuclei and areas of myofibrillar disorganisation in some samples. Three patients had decreased fetal movement and breech presentation. Of these, one had a heterozygous *de novo* mutation and two were compound heterozygous. Regardless of inheritance pattern, western blot and RT-PCR studies suggested reduced protein levels, possibly through reduced stability or inability to form complexes.⁴⁹

DNM2

Heterozygous *DNM2* mutations (MIM 602378) cause comparatively mild disorders including Charcot-Marie-Tooth disease and centronuclear myopathy.^{50–53} Koutsopoulos *et al* recently identified a novel homozygous mutation in three consanguineous patients with fetal hypokinesia, polyhydramnios, multiple contractures, brain and retinal haemorrhages, and thin bones.⁵³ Postnatally, the patients showed respiratory insufficiency and absent reflexes. Electromyogram in one case showed possible myopathic or lower motor neuron signs. The phenotype is labelled lethal congenital contracture syndrome 5 (MIM 615368). *DNM2* acts in muscle and axonal maintenance.⁵³ Morpholino knockdown of *dnm2* in zebrafish showed mild misalignment of myofibres and altered blood vessel morphology, but muscle innervation was normal. The authors concluded the aetiology was not neuropathic.

FKTN

FKTN (*fukutin*, MIM 607440) encodes a putative glycosyltransferase enzyme of the same name.^{54–55} Recessive mutations cause a range of phenotypes via abnormal O-glycosylation of α -dystroglycan, which reduces binding to extracellular matrix proteins.^{54–56} Three forms of muscular dystrophy-dystroglycanopathy have been attributed to *FKTN*: the most severe congenital form with structural brain and eye anomalies (MDDGA4; MIM 253800); a less severe congenital form without mental disability (MDDGB4; MIM 613152); and the milder limb-girdle form (MDDGC4; MIM 611588).⁵⁵ Dilated cardiomyopathy with adult-onset muscle weakness and normal cognition has also been described (dilated cardiomyopathy 1X; MIM 611615).⁵⁷ Severe MDDGA4 cases onset prenatally, such as those described by Chang *et al*.⁵⁴ They describe a muscle biopsy from an MDDGA4-affected fetus, terminated at 15 weeks' gestation due to brain and heart abnormalities. The muscle was dystrophic, suggestive of α -dystroglycan hypoglycosylation, and severe secondary merosin deficiency.⁵⁴

SCN4A

SCN4A (MIM 603967) encodes the alpha subunit of the skeletal muscle voltage-gated sodium channel ($\text{Na}_v1.4$), which is critical for generation and propagation of the muscle action potential.⁵⁸ Dominant gain-of-function mutations cause myotonia/paramyotonia or periodic paralysis.⁵⁹ Recessive loss-of-function mutations were more recently shown to cause congenital myopathy, with severity ranging from severe fetal hypokinesia to 'classical' congenital myopathy.⁵⁹ Eleven individuals from six unrelated families were recently described.⁵⁹ Seven patients presented with severe fetal hypokinesia, contractures, polyhydramnios and marked muscle hypoplasia. Of these patients, one survived for 5 hours neonatally, while all others died before or during birth (one pregnancy was terminated at 28 weeks). Each patient carried at least one mutation that completely inactivated the $\text{Na}_v1.4$ channel.⁵⁹ Muscle biopsies confirmed a myopathic process without specific structural abnormalities.⁵⁹

VMA21

VMA21 (MIM 300913) encodes vacuolar ATPase (V-ATPase) assembly integral membrane protein VMA21, required for assembly and function of the V0 complex of the V-ATPase in the endoplasmic reticulum.⁶⁰ Mutations in *VMA21* cause X linked recessive myopathy with excessive autophagy (MIM 310440). Severe manifestations have congenital or prenatal onset.^{61–62} Five of seven affected males with congenital onset described by Yan *et al* did not survive infancy.⁶¹ However, no abnormalities were noted during pregnancy. A later case report expanded the phenotype to include micrognathia, short limbs and arthrogryposis detected on ultrasound.⁶⁰ Sequencing revealed a novel in-frame deletion in the transmembrane domain.⁶⁰

GENES ENCODING FETALLY EXPRESSED MYOSTRUCTURAL PROTEINS

KLHL40

Recessive mutations in *KLHL40* (kelch-like 40; MIM 615340) cause severe nemaline myopathy (NEM8; MIM 615348), with features including fetal akinesia, contractures, fractures, respiratory failure and swallowing difficulties at birth.⁶³ The cohort described by Ravenscroft *et al* had an average survival time of 5 months.⁶³ Consistent with a loss-of-function mechanism, muscle biopsies showed absence of *KLHL40* even in patients with two missense mutations.⁶³ Knockdown of the

zebrafish orthologues (*Klhl40a/Klhl40b*) caused disrupted muscle patterning, gaps between myofibres and widened Z-discs.⁶³ Mouse knockouts echoed human nemaline myopathy features.⁶⁴ *KLHL40* was shown to bind, stabilise and maintain expression of nebulin and LMOD3.⁶⁴ Absence of *KLHL40* reduces the amount of these thin filament proteins, likely leading to the sarcomeric disarray and impaired muscle contractility.⁶⁴

KLHL41

Gupta *et al* identified biallelic mutations in *KLHL41* (kelch-like 41; MIM 607701) in five unrelated children with nemaline myopathy 9 (NEM9; MIM 615731).⁶⁵ *KLHL41* is a key protein in myofibre development.⁶⁵ NEM9 severity ranges from neonatal death to progressive distal weakness with ambulation preserved into late childhood.⁶⁵ Western blot showed reduced *KLHL41* in patients compared with controls. *klhl41* knockout in zebrafish caused myofibril disorganisation with nemaline body formation and impaired motor function.⁶⁵ Mouse knockouts showed a marked reduction in nebulin and lesser reduction in other sarcomeric proteins.⁶⁶ *KLHL41* stabilises nebulin, acting as a polyubiquitin-dependent chaperone that prevents nebulin aggregation.⁶⁶

LMOD3

Yuen *et al* identified 21 patients from 14 families with severe congenital nemaline myopathy.⁶⁷ Consistent features were significant generalised hypotonia at birth, respiratory insufficiency, feeding difficulties and bulbar weakness. Most patients died of respiratory failure shortly after birth. Yuen *et al* identified biallelic nonsense or frameshift mutations in leiomodlin 3 (*LMOD3*; MIM 616112), which is expressed in both the skeletal and cardiac muscles.⁶⁷ Biopsies showed nemaline bodies and atrophic myofibres, and absence of *LMOD3* protein in all but one patient.⁶⁷ Some patients' muscle showed shortening and disorganisation of thin filaments while others had no discernible sarcomeric structure.⁶⁷ Mouse knockouts showed sarcomeric disarray visible by histological staining.⁶⁸ Yuen *et al* suggested *LMOD3* is pivotal for sarcomeric thin filament stability and regulation of thin filament length.⁶⁷ Cenik *et al* showed *LMOD3* is involved in regulation of sarcomeric assembly and skeletal muscle function.⁶⁸

MYBPC1

Dominant *MYBPC1* (myosin-binding protein C, slow type; MIM 160794) missense mutations cause mild distal arthrogryposis 1B (MIM 614335).^{1,69,70} Recessive mutations cause lethal congenital contracture syndrome 4 (MIM 614915).⁷⁰ Two consanguineous families from a Bedouin tribe in Israel harboured a homozygous *MYBPC1* founder mutation. *MYBPC1* provides thick filament stability and modulates contraction through its interactions with myosin and actin.⁷¹

MYH3

Mutations in the head and neck domains of *MYH3* (myosin heavy chain, embryonic; MIM 160720) cause dominant distal arthrogryposis 2A and 2B (MIM 193700, DA2B; MIM 601680).¹ Chong *et al* expanded the phenotype to include distal arthrogryposis 8 (MIM 178110), predominantly caused by mutations in the tail region of *MYH3*.⁷² Features include pterygia, camptodactyly, vertebral fusions and scoliosis.⁷²

MYOD1

MYOD1 (MIM 159970) encodes MyoD, a major regulator of precursor cell commitment to the myogenic differentiation pathway and repair of damaged muscle.⁷³ Watson and colleagues⁷⁴ described three patients from a consanguineous family with lethal fetal akinesia. Consistent features included third-trimester polyhydramnios, respiratory insufficiency, cleft palate and dysmorphic facial features. Variable features included pulmonary hypoplasia and diaphragmatic eventration. Exome sequencing revealed a homozygous nonsense *MYOD1* variant that likely causes nonsense-mediated RNA decay.⁷⁵ Mutations of *MYOD1* seem to be a rare cause of fetal akinesia, since additional families are yet to be described.

TTN

TTN (MIM 188840) encodes the giant muscle protein titin, spanning from Z-disc to M-line and playing a crucial role in cardiac and skeletal muscle sarcomere assembly, structure and force transmission.⁷⁶ Chauveau and colleagues⁷⁷ first described *TTN*-related fetal akinesia caused by recessive *TTN* mutations. They reported a female patient with distal AMC, congenital muscle weakness, neonatal cardiac failure and bilateral camptodactyly. Muscle biopsy at 15 months was consistent with multiminiore disease. Terminal heart failure required a transplant at age 4. Fernández-Marmiesse *et al* described fetal hypokinesia caused by a homozygous truncating mutation in exon 197.⁷⁶ This exon is absent from adult *TTN* isoforms, but present in the fetal 364-exon inferred complete isoform, also known as the *TTN* 'metatranscript' (reference sequence NM_001267550.2).^{76,78} The authors suggest cardiac muscle was spared because exon 197 is absent from the cardiac titin isoforms.⁷⁶ Features included fetal hypokinesia, distal arthrogryposis, delayed motor milestones, high-arched palate and weak suction. Muscle biopsy showed persistent amyoplasia.⁷⁶ A later cohort study of 30 patients with recessive congenital titinopathy showed 10 also had a mutation within *TTN* metatranscript-only exons.⁷⁸ These mutations were suggested to affect as-yet uncharacterised developmental *TTN* isoforms.⁷⁸

ZBTB42

Patel *et al* described *ZBTB42* (zinc finger and BTB domain containing protein 42; MIM 616248) to cause lethal congenital contracture syndrome 6 (MIM 616248).⁷⁹ A consanguineous family had three infants harbouring a homozygous *ZBTB42* missense mutation.⁷⁹ Zebrafish studies showed *ZBTB42* is necessary for normal skeletal muscle development, with deficiency causing sarcomeric disorganisation and NMJ abnormalities.⁷⁹ Zebrafish with the mutation recapitulated the patient phenotype, confirming its pathogenicity.⁷⁹

OTHER

DPAGT1

Genital disorders of glycosylation are increasingly recognised as a cause of complex neurogenetic malformations.⁸⁰ *DPAGT1* (dolichyl-phosphate N-acetylglucosamine phosphotransferase) initiates the biosynthetic pathway needed for protein N-glycosylation.⁸¹ Recessive mutations in *DPAGT1* (MIM 191350) cause a range of phenotypes, from severe neonatal neurogenetic disease to muscular dystrophy.⁸⁰ One patient was reported with fetal akinesia.⁸⁰ Features included central respiratory depression, delayed brain myelination and hypoplasia of the inferior cerebellar vermis.⁸⁰ She also had bilateral cataracts and arachnodactyly.⁸⁰

DISCUSSION

The fetal akinesia disease spectrum encompasses several aetiologies unified by reduced or absent fetal movement. We grouped these diseases by affected tissue. In the 2011 review, no definitive peripheral nerve-related fetal akinesia genes were described. In the intervening years, eight genes have been described. Impaired axoglial function is the most common pathway, with four genes implicated (*ADCY6*, *ADGRG6*, *GLDN*, *LGI4*). Impaired muscle contractility caused by sarcomeric disarray is strongly represented in the muscle genes. Severe muscle channelopathies are a recent addition to the fetal akinesia family of genes (*CACNA1S*, *SCN4A*). Although both genes are associated with comparatively mild dominant disease, loss-of-function causes a severe phenotype, echoing *DNM2*, *MYBPC1*, *RYR1*, *TPM2* and *TTN*. Many new fetal akinesia genes encode interacting partners of genes described in the 2011 review. It seems reasonable that additional genes in these pathways will become associated with neuromuscular disease.

Within rare diseases, unbiased NGS is blurring the boundaries between what were previously considered distinct clinical entities. In 2013, Boycott *et al* predicted many 'novel' diseases would be phenotypic expansions of known disease genes, which is a recurring theme in fetal akinesias.⁸² These novel genotype-phenotype correlations are often surprising, and can be difficult to classify without additional cases or functional studies to confirm pathogenicity. The rise of genetic 'match-making' services such as Matchmaker Exchange⁸³ greatly assists by providing additional cases to corroborate findings and justify further investigation. In the case of *GLDN* and *GLE1*, phenotypic expansions demonstrated mutations in these genes are not universally fatal, radically altering the prognostic landscape. The implications are far-reaching, raising questions about the universal fatality of other fetal akinesia disease genes.

Despite the power of current genetic diagnostics, variant interpretation remains challenging, even within known disease genes. Variants that cannot be definitively classified as either pathogenic or benign (variants of unknown significance) leave many patients without a genetic diagnosis. Diffusion of NGS from research into the clinic has blurred the distinction between these two realms.⁸² This porous boundary necessitates diagnostic labs to collaborate with researchers for functional validation. However, as diagnostic genomics integrates into mainstream healthcare, ad hoc research follow-up will become increasingly unfeasible. High-throughput functional assays will need to be developed by researchers and implemented into diagnostic pipelines for pathogenicity validation. This diagnostic functional validation should be funded as part of a 'whole of service' charge for the diagnostic testing.

An example of a successful centralised functional genomic collaboration is the Canadian Care4Rare initiative (previously Finding of Rare Disease Genes in Canada (FORGE Canada) Consortium), which established a portal to connect rare disease researchers and clinicians with fundamental biologists that have interest and particular strength in a candidate disease gene or functional assay.^{8 84} As our ability to discover variants increases, we may need high-throughput functional assays to assist with variant interpretation on a similar scale. Methods include saturation mutagenesis or multiplex assays of variant effect, which may provide functional insight into the vast depths of possible pathogenic variants.⁸⁵ However, conferring clinical meaning to variants based on these assays may require additional computational

approaches such as machine learning.⁸⁵ Regardless of the avenue of genetic investigation, banking of patient material and/or cell lines should be performed whenever possible to facilitate follow-up functional studies. Use of patient cells or tissue for investigation is often much faster and more cost-effective than other methods, for example generating CRISPR cell lines harbouring the variant of interest or studies in model organisms.⁸⁶

Other reasons many cases lack candidate variants likely include that further disease mechanisms and genes remain undiscovered or underexplored. Variants that are difficult to detect with current NGS technology may explain a proportion of unsolved cases. Genetic mosaicism contributes to monogenic disease, but the full extent is uncertain due to the difficulties in detection.⁸² Exome-based and short-read technology struggle to detect structural variants, duplications, deletions and repeat sequences. Although whole genome sequencing and long-read technologies can detect these variants, they are currently unaffordable for many groups. As they become more accessible, detection of this class of variants will improve. Beyond improved detection, the ability to interpret variant pathogenicity is vital. Transcriptomics can identify splicing alternations that would not have been predicted from DNA sequencing alone. This also hints at the contribution of non-coding mutations in Mendelian disease. Recent papers identified splicing defects in gene promoters and introns through combining DNA and RNA sequencing.^{87–89} This approach provided an answer for approximately one-third of patients in a recent study.⁸⁷ However, full understanding of the role of the vast non-coding genome in Mendelian disease will take years.⁸ Therefore, the impact of non-exonic mutations in human disease remains somewhat controversial.

The prenatal onset in fetal akinesia combined with the high incidence of severe structural defects limits therapeutic options.⁸² Additionally, detection of fetal akinesia by ultrasound is poor.¹⁰ Thus, there is increasing interest in preventing severe or lethal congenital conditions via preconception carrier screening. This detects couples at risk of having a child with a severe recessive disorder before pregnancy, facilitating family planning.⁹⁰ Although broad implementation of preconception carrier screening requires careful ethical and practical consideration, it is gaining credence as a viable preventative measure.⁹⁰

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