Prenatal Akinesia/Arthrogryposis Panel Sequence Analysis and Exon-Level Deletion/Duplication Testing

PANEL GENE LIST

ACTA1, CHRNA1, CHRND, CHRNE, CHRNG, CNTN1, CNTNAP1, DOK7, ECEL1, FKRP, GBE1, GLE1, KLHL40, LMOD3, MAGEL2, MUSK, MYBPC1, MYH3, PIEZO2, PLEC, RAPSN, RIPK4, TNNI2**, TNNT3, TPM2, ZC4H2, ZMPSTE24*

*Sequence analysis only for FKRP

** Only whole gene deletions and duplications may be detected for TNNI2

Clinical Features

Arthrogryposis, also known as arthrogryposis multiplex congenita (AMC), describes the presence of multiple non-progressive congenital joint contractures in at least two different areas of the body, leading to restricted movement of the affected joints.[1,2] It generally results from fetal akinesia and has been noted as a clinical finding in more than 400 disorders. Fetal akinesia presents prenatally as abnormal joint positioning and/or lack of fetal movement on ultrasound.[3,4] The most common manifestations include talipes equinovarus (club foot) and flexion deformities of the wrists, however involvement of the jaw, neck, or spine may be observed.[1] Other ultrasound findings include, craniofacial anomalies (micrognathia, retrognathia, cleft palate), polyhydramnios, cerebral ventriculomegaly, intrauterine growth retardation (IUGR), increased nuchal translucency, cystic hygroma, and fetal hydrops.[3-5] Although fetal akinesia can be observed by ultrasound as early as 12 weeks' gestation, most cases are diagnosed during the second or third trimesters of pregnancy by ultrasound or maternal recognition of reduced fetal mobility.[5] The use of 4D ultrasound can improve identification of reduced fetal movement at an earlier time point.[6] Fetal akinesia and arthrogryposis can be caused by a variety of primary defects including disorders of the central or peripheral nervous systems, neuromuscular junction, musculature, connective tissue, or metabolic pathways.[1,7] Central nervous system and neuromuscular junction diseases are the most common cause of fetal akinesia/severe arthrogryposis, which are associated with several prenatal or neonatal lethal conditions.[8] Due to genetic heterogeneity and overlapping phenotypes, the specific diagnosis cannot be determined accurately with imaging alone. When available, genetic testing can aid in determining the precise diagnosis after the differential has been established by imaging.[9]

GENETICS

Fetal akinesia and arthrogryposis can be genetic or environmental in nature. Genetic forms of these disorders are inherited in an autosomal dominant, autosomal recessive, or X-linked manner, or they may be the result of a de novo variant. Pathogenic variants have been identified in genes that function in the central or peripheral nervous system, neuromuscular junctions, skeletal muscle, connective tissue, or skin.[1,8]

TEST METHODS

Using genomic DNA extracted from the submitted specimen, the complete coding regions and splice site junctions of the genes tested are enriched using a proprietary targeted capture system developed by GeneDx for next-generation sequencing with CNV calling (NGS-CNV). The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. After gene specific filtering, data are analyzed to identify sequence variants and most deletions and duplications involving coding exons; however, technical limitations and inherent sequence properties effectively reduce this resolution for some genes. Alternative sequencing or copy number detection methods are used to analyze or confirm regions with

inadequate sequence or copy number data by NGS. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported but are available upon request.

The technical sensitivity of sequencing is estimated to be >99% at detecting single nucleotide events. It will not reliably detect deletions greater than 20 base pairs, insertions or rearrangements greater than 10 base pairs, or low-level mosaicism. The copy number assessment methods used with this test cannot reliably detect copy number variants of less than 500 base pairs or mosaicism and cannot identify balanced chromosome aberrations. Assessment of exon-level copy number events is dependent on the inherent sequence properties of the targeted regions, including shared homology and exon size. Gene specific exclusions for exon-level deletion/duplication testing for this panel are: FKRP gene, no copy number testing, TNNI2 gene only whole gene deletions or duplications may be detected.

Additionally, genotype analysis of maternal and fetal DNA for several polymorphic markers to test for maternal cell contamination is performed. Therefore, in all prenatal cases, a maternal sample should accompany the fetal sample.

CLINICAL SENSITIVITY

The clinical sensitivity of sequencing and deletion/duplication analysis of the genes included in this panel depends in part on the patient's clinical phenotype. Specific information about the diagnostic yield for each gene in selected populations is summarized in the table below.

DIAGNOSTIC YIELD OF PRENATAL AKINESIA/ARTHROGRYPOSIS PANEL GENES IN SELECTED POPULATIONS

Gene	Inheritance	Disease Associations	Diagnostic Yield in Selected
ACTA1	AD/AR	Congenital fiber-type disproportion myopathy; Nemaline myopathy(NM)	15-25% of NM;[10] Rare for AMC[11,12]
CHRNA1	AD/AR	Congenital myasthenic syndrome(CMS); LMPS	<1% for CMS;[13] ~3% for FADS[14]
CHRND	AD/AR	CMS; LMPS	<1% for CMS;[13] ~3% for FADS[14]
CHRNE	AD/AR	CMS	49% for CMS;[13] Rare for AMC[11]
CHRNG	AR	LMPS; Escobar syndrome (EVMPS)	~27% of EVMPS;[15] 5-8% patients with LMPS/FADS;[14,15] 7-10% for AMC[16,17]
CNTN1	AR	Compton-North congenital myopathy	Rare for AMC[11]
CNTNAP1	AR	Congenital hypomyelinating neuropathy;LCCS 7	~13% for AMC[17]
DOK7	AR	DOK7-related CMS; FADS	10-21% for CMS;[18,19] ~7% for lethal MPS/FADS without *CHRNG, CHRNA1, CHRNB1, CHRND*, or *RAPSN*[20]

Gene	Inheritance	Disease Associations	Diagnostic Yield in Selected
ECEL1	AR	Distal arthrogryposis type 5D (DA5D)	~70% DA5D; [21] ~3-6% for AMC[16,17]
FKRP	AR	Walker–Warburg syndrome; congenital muscular dystrophy (CMD) with/without intellectual disability and microcephaly; Limbgirdle muscular dystrophy (LGMD) type 2	~6% of AR LGMD;[22] ~9% of the alphadystroglycanopathies;[23] rare for AMC[11]
GBE1	AR	Glycogen storage disease IV	3% of glycogen storage disease;[24] ~2% for AMC[11]
GLE1	AR	LCSS 1/Lethal arthrogryposis with anterior horn cell disease	Unknown[25]
KLHL40	AR	NM	~20% of severe NM;[26] rare for AMC[11]
LMOD3	AR	Nemaline myopathy	Unknown[7,27]
MAGEL2	AD	Schaaf-Yang syndrome	Unknown[28]
MUSK	AR	CMS;FADS	Rare for CMS;[18] unknown for AMC, founder mutation (p.Ile575Thr) causing FADS among the Dutch[29]
MYBPC1	AD/AR	DA type 1B(AD);LCCS4	~13% for DA1 family [30]
MYH3	AD	DA1;DA2A/FSS,DA2B/SHS;DA8	~8% for DA1;[31] ~90% for DA2A/FSS;[32,33] 11-32% for DA2B/SHS;[31,32] rare for DA8;[34] ~3% for AMC[17]
PIEZO2	AD/AR	DA3/Gordon syndrome & DA5	~83% for DA3 and ~82% for DA5[35]
PLEC	AD/AR	Epidermolysis bullosa simplex with muscular dystrophy (EBS-MD); Limb-girdle muscular dystrophy 2Q (LGMD2Q)	Rare[36]
RAPSN	AR	CMS;FADS	~1.5% for FADS;[14] ~3% for AMC[17]
RIPK4	AR	Lethal popliteal pterygium syndrome/Bartsocas-Papas type	Rare[11,37]
TNNI2	AD	DA1&2B	~10% of DA2B and 6% for DA1[31]
TNNT3	AD	DA1&2B	~8% for DA1 and ~7% for DA2B;[31] ~3% for AMC[17]

Gene	Inheritance	Disease Associations	Diagnostic Yield in Selected
TPM2	AD	DA 1&2B; NM	~6% for DA1 and ~10% for DA2B;[31] ~2% for AMC[11]
ZC4H2	XLR	Wieacker-Wolff syndrome	Rare[38]
ZMPSTE24	AR	Mandibuloacral dysplasia with type B lipodystrophy; Lethal restrictive dermopathy	~75% for restrictive dermopathy[39]

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