Congenital HypotoniaXpanded PanelA targeted test for monogenic causes of hypotoniausing a trio approach

CLINICAL FEATURES:

Hypotonia is a general term that refers to low muscle tone or reduced resistance to passive movement, not to be confused with muscle weakness, which is defined as reduced muscle strength and power.[1] Hypotoniacan be used to describe muscle groups throughout the body and is a feature of hundreds of neonatal disorders and diseases.[2,3] Central hypotonia is due to defects in the upper motor neuron, whereas peripheral hypotonia is due to defects ofthe lower motor neuron, neuromuscular junction, or skeletal muscle.2Central hypotonia is commonly associated with syndromic disorders of the CNS and presents with normal serum creatine kinase, hyperreflexia, cognitive delay, and/or seizures.2Infants with peripheral hypotonia often have elevated serum creatine kinase, significant muscle weakness, absent anti-gravity movements, head lag, joint contractures, and normal cognition.[1,3] A large number of the disorders that present congenitally with hypotonia have a genetic etiology that can guide future testing, treatment, and medical management.[1-3] The cause of hypotonia can be difficult to discern as it is a feature of many, sometimes heterogeneous,disorders.[2,4] It is often necessary to perform testing of multiple genes (concurrently or as reflex tests) to identify an underlying genetic cause in an individual. Moreover, new genes known to cause disorders that present with hypotonia are being discovered regularly, making it challenging for clinical laboratories to keep traditional testing panels updated. Additionally, interpretation of the clinical significance of variants in these newly discovered genes is often difficult in the absence of parental testing to clarify which variants are de novoor inherited.The Congenital Hypotonia Xpanded Panel uses a trio approach that includes concurrent analysis of the affected proband and both parents, which increases the likelihood of identifying a definitive genetic explanation for the hypotonia. Depending on the family structure, family history, and the availability of both parents, other family members of the affected individual may be evaluated in conjunction with the proband. Please contact GeneDx for prior approval when both parents are not available to submit samples for the Congenital HypotoniaXpanded Panel. The Congenital Hypotonia Xpanded Panel is based on exome capture, Next Generation sequencing (NGS), and targeted analysis of a comprehensive list of 1400+ genes currently associated with congenital hypotonia. The design of the panel allows for a comprehensive, dynamic gene list that is updated regularly to ensure inclusion of genes recently associated with hypotonia.

INHERITANCE PATTERN/GENETICS:

Hypotonia usually occursas part of a genetic syndrome, but may be the result of clinical or environmental risk factors including,but not limited to,prenatal drug exposure, prematurity, congenital anomalies, and infections.[1,3] Central hypotonia accounts for 60-80% of congenitalhypotonia, the remaining case are peripheral or unknown.[1,4] A genetic etiology, including chromosomal abnormalities, may be identified in up to 60% of cases, after acquired causes have been ruled out.[1,4] The inheritance pattern can be autosomal dominant, autosomal recessive, X-linked, or mitochondrial. Additionally, multiple studies have reported a high frequency of de novovariants in patients with neurodevelopmental disorders, including those resulting in central hypotonia, highlighting the importance ofa trio approach including the affected proband and both parents when performing genetic testing.5Confirmation of the molecular genetic cause of hypotonia may have implications for medical management, surveillance for associated complications or other organ systems involvement, eligibility for needed services, and treatment.[2,3]

TEST METHODS:

Using genomic DNA from the submitted specimen, the complete coding regions and splice site junctions are enriched for most genes of the human genome using a proprietary capture system developed by GeneDx for next-generation sequencing. The enriched targetsare 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. Using a custom-developed analysis tool

(XomeAnalyzer), data are filtered and analyzed to identify sequence variants. Reported clinically significant variants are confirmed by an appropriate orthogonal method in the proband and, if submitted, in selected relatives as necessary. Reportable variants include pathogenic variants and likely pathogenic variants. Variants of uncertain significance, likely benign and benign variants, if present, are not routinely reported. A list of additional variants not included in the report is availableupon request.Please note that while the Congenital HypotoniaXpanded Panel captures and sequences the whole exome, analysis is targeted to the specific phenotype-driven gene list for this panel. The Congenital Hypotonia Xpanded Panel gene list includes 1400+ genes. The list was developed by searching for genes associated with hypotoniain multiple sources, including OMIM, HGMD, and Human Phenotype Ontology (HPO) terms. This list undergoes continual review and curation by GeneDx experts. During this review, genes are added to the list using GeneDx data from clinical exome sequencing (ES)done on patients with congenital hypotonia. Additionally, genes may be removed from the panel if they are found to be weakly or questionably associated with congenital hypotonia. In rare situations, genes are removed from the panel if they are expected to be low yield for this phenotype,but contain an inherent high risk for incidental findings. The gene list is routinely updated.

RESULT REPORTING:

The Congenital HypotoniaXpanded Panel is performed on the proband and the parental samples (and/or additional relatives) when submitted together for analysis. A single report will be issued on the affected proband in the family. A separate report will not be issued for unaffected parents or other family members who may also have submitted a specimen for the purpose of allowing better interpretation of the results from the affected individual. If additional reports are requested for other affected family members, extra fees will apply.The report that is issued for the affected individual will include reportable variants in genes that have been previously associated with hypotoniain the published or emerging literature. Pathogenic/likely pathogenic variants in genes responsible for the phenotype of the patient will be reported; however, because this is a phenotype-driven test of a large number of genes, variants of uncertain significance (VUS) are not routinely reported, only at our discretion. Variants that are considered to be benign or likely benign will not be reported. As the Congenital HypotoniaXpanded Panel includes 1400+ genes, the report will not include a comprehensive list of all observed variants. If desired, clinicians can request a separate file containing a list of identified variants that will be provided upon completion of testing. In rare instances, this test may reveal a pathogenic variant that is not directly related to the test indication. For example, pathogenic variants in genes associated with an increasedrisk for cancer, cardiac abnormalities, or metabolic defects could be identified. In the event that an incidental finding is identified, this information will be disclosed to the ordering health care provider if it is likely to impact medical care.7The absence of reportable incidental findings for any particular gene does not rule out the possibility of pathogenic variants in that gene.

TEST SENSITIVITY:

The clinical sensitivity of the Congenital HypotoniaXpanded Panel depends in part on the patient's clinical phenotype. Previous ES studies have reported identification of a definitive pathogenic variant in 27% of individuals with developmental disorders and 37% for individuals with congenital hypotonia or childhood onset weakness.5,8It has been demonstrated that the yield of ES testing is higher with a Trio approach compared to a Proband-only approach.6The sensitivity of this test is expected to be comparable to trio-based exome sequencing since it uses a trio approach to test a comprehensive list of genes previously associated with congenital hypotonia. The clinical sensitivity is expected to be significantly lower for singleton testing when only the affected proband is tested. The average coverage of all genes on the panel is greater than 99% at 10X (with a depth of 10or more reads), and approximately 92% of the genes on the panel have an average coverage of greater than 99% at 10X. Some genes with a relatively high clinical sensitivity have an average coverage of less than 90% at 10X, including POMGNT1(89.1%), NEB(87.3%), POMT2(87.1%), OCLN(84.0%), CHRNA7(83.3%), UBA5(82.7%), VAMP1(82.2%) and KIF5C(10.1%). The coverage of each gene on the panel for a specific patient may vary, and the actual coverage for each gene is included in the final report.

LIMITATIONS:

Some types of genetic disorders, such as those due to nucleotide repeat expansion/contraction, abnormal DNA methylation, and other mechanisms may not be detectable with this test. Additionally, small sections of a few individual genes have inherent sequence properties that yield suboptimal data and variants in those regions may not be reliably detected. For example, repeat expansions in FMR1and DMPKcausing fragile X syndrome and myotonic dystrophy type 1, the polyalanine repeat expansions in ARX, and abnormal methylation ofSNRPNcausing Prader-Willi syndromewould not be detectable by thisCongenital Hypotonia Xpanded panel.Additionally, this test includes screening for homozygous loss of exon 8 (formerly exon 7) of the SMN1gene. If a high clinical suspicion of SMN1-related SMA is present, diagnostic testing for SMN1/2should be completed. The scientific knowledge available about the function of all genes in the human genome is incomplete at this time. It is possible that the Congenital HypotoniaXpanded Panel may identify the presence of a variant in the sequence of an affected individual, but that we will not recognize it as the cause of their disease due to insufficient knowledge about the gene and its function. Even if the Congenital Hypotonia Xpanded Panel identifies the underlying genetic cause of a disorder in an affected individual, it is possible that such a diagnosis will not permit an accurate prediction of the prognosis or severity of the disease. While there is a possibility that identifying the genetic cause may help direct management and treatment of the disease, it is also possible that this knowledge will not change management or treatment.

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