

Ataxia *Xpanded*® Panel

A Targeted Test for Genetic Causes of Ataxia Using a Trio Approach

OVERVIEW

The hereditary ataxias are a clinically and genetically heterogeneous group of neurodegenerative disorders, characterized by poor coordination of movement, a wide-based unsteady gait, and often associated with poor coordination of limbs, eye movements, and speech (PMID: 20301317). Due to the heterogeneous nature of hereditary ataxias, it can be challenging to determine the specific form of ataxia or predict the disease-causing gene based on clinical features alone. It is often necessary to perform testing of multiple genes to identify the underlying genetic cause of ataxia in an individual. Moreover, new genes associated with ataxia are being discovered regularly, making it challenging for clinical laboratories to keep traditional testing panels up-to-date. Additionally, interpretation of the clinical significance of variants in these newly discovered genes is often difficult in the absence of parental testing to clarify the inheritance of suspicious variants.

The Ataxia *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 ataxia in an individual. Depending on the family structure, family history of ataxia, 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 Ataxia *Xpanded*® Panel. The Ataxia *Xpanded*® Panel is based on whole exome capture (WEC), NextGeneration sequencing (NGS), and targeted analysis of a comprehensive list of approximately 1000 genes currently associated with ataxia. The design of the panel allows for a comprehensive, dynamic gene list that is updated regularly to ensure inclusion of genes recently associated with ataxia.

INHERITANCE PATTERN/GENETICS

Ataxia can be either genetic or acquired in nature. Non-genetic causes include, but are not limited to, alcoholism, vitamin deficiencies, multiple sclerosis, vascular disease, tumors, and paraneoplastic diseases (PMID: 20301317). Genetic forms of ataxia include the cerebellar ataxias, episodic ataxias, spastic paraplegias, pontocerebellar hypoplasia, and neuropathies. The inheritance pattern of hereditary ataxias can be autosomal dominant, autosomal recessive, X-linked, or mitochondrial (PMID: 20301317, 23538602). The prevalence of the hereditary ataxias varies by population and is estimated to be 1-9:100,000 (PMID: 19650351, 25055202, 20723845, 20069237). Pathogenic variants in a certain gene may be associated with a wide range of phenotypes (clinical heterogeneity), and conversely, pathogenic variants in different genes can cause the same phenotype (genetic heterogeneity). In some instances, molecular confirmation of a clinical diagnosis of ataxia may have implications for treatment and management of the specific form of disease.

TEST METHODS

Using genomic DNA from the submitted specimen(s), DNA is enriched for the complete coding regions and splice junctions of most genes of the human genome using a proprietary 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. Data are filtered and analyzed to identify sequence variants and most deletions and duplications involving three or more coding exons in the selected genes or regions of interest. Smaller deletions or duplications may not be reliably identified. Reported variants are confirmed, if necessary, by an appropriate orthogonal method in the proband and, if submitted, in selected relatives. Sequence variants are reported according to the Human Genome Variation Society (HGVS) guidelines. Copy number variants are reported based on the probe coordinates, the coordinates of the exons involved, or precise breakpoints when known. Reportable variants include pathogenic variants and likely pathogenic variants. Variants of uncertain significance, likely benign and benign variants, if present, are not routinely reported.

Please note that while the Ataxia *Xpanded*[®] panel captures and sequences the whole exome, analysis is targeted to the specific phenotype-driven gene list for this panel. The Ataxia *Xpanded*[®] Panel gene list includes approximately 1000 genes. The list was developed by searching for genes associated with ataxia in multiple sources, including OMIM, HGMD, and Human Phenotype Ontology (HPO) terms. Additionally, genes were added to the list using GeneDx data from clinical whole exome sequencing done on patients with ataxia. The current gene list is available on our website.

RESULT REPORTING

The Ataxia *Xpanded*[®] 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 ataxia in 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 are not routinely reported, only at our discretion. Variants that are considered to be benign or likely benign will not be reported. As the Ataxia *Xpanded*[®] Panel includes approximately 1000 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 increased risk 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. The 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 Ataxia *Xpanded*[®] Panel depends in part on the patient's clinical phenotype. Diagnosis by sequencing after negative repeat expansion analysis varies from 8.3% in late onset patients to 40% in adolescents (PMID: 24030952). Overall, patients with a clinical indication that includes ataxia have a

32.5% positive rate by whole exome sequencing (WES). When a trio is submitted (concurrent sequencing of parents and proband) the positive rate increases to 37.5%, whereas the positive rate is significantly lower (23.9%) for singleton testing (sequencing of the proband only) (Sastry et al. Exome Sequencing Provides a Broad Evaluation and High Diagnostic Rate for Ataxia-Related Disorders [abstract and platform presentation ACMG 2017]). The sensitivity of this test is expected to be comparable to trio-based whole exome sequencing since it uses a trio approach to test a comprehensive list of genes known to be associated with ataxia. The clinical sensitivity is expected to be significantly lower for alternative testing strategies that do not include both biological parents (PMID: 26633542).

The average coverage of all genes on the panel is greater than 99% at 10X (with a depth of 10 or more reads). Several genes with a high clinical sensitivity have an average coverage of less than 90% at 10X, including NEB (87%), MED27 (73%), and VAMPI (82%). Note that these numbers represent the average coverage for the genes on the panel, derived by combining data from a large number of patients. 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

The hereditary ataxias encompass a variety of different disorders, including the common spinocerebellar ataxias. These disorders are typically caused by nucleotide repeat expansions in a number of different genes. Nucleotide repeat expansion would not be detected by the Ataxia *Xpanded*[®] Panel. Specifically, repeat expansions in the ATXN1, ATXN2, ATXN3, CACNA1A, ATXN7, ATXN8, ATXN10, BEAN1, NOP56, PPP2R2B, and TBP genes would not be identified by this test. Other disorders associated with nucleotide repeat expansions and contractions would not be identified by this test, including ARX-related disorders (ARX), Unverricht-Lundborg disease (CSTB), fragile X tremor-ataxia syndrome (FMRI), Friedreich ataxia (FXN), Huntington chorea (HTT), Dentatorubro-pallidoluysian atrophy (ATN1), and cerebellar ataxia, neuropathy, vestibular areflexia syndrome (RFC1).

Additionally, small sections of a few individual genes may have inherent sequence properties that yield suboptimal data causing variants in those regions to not be reliably detected. Specifically, abnormal methylation of UBE3A causing Angelman syndrome would not be detectable by this Ataxia *Xpanded*[®] Panel. Mitochondrial genome sequencing is not performed as part of the Ataxia *Xpanded*[®] Panel.

The scientific knowledge available about the function of all genes in the human genome is incomplete at this time. It is possible that the Ataxia *Xpanded*[®] 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 Ataxia *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.