

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 speech¹. 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 diseases¹. 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^{1,3}. The prevalence of the hereditary ataxias varies by population and is estimated to be 1-9:100,000^{2,4,5,6}. 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), the exonic regions and flanking splice junctions of the genome were captured using a proprietary system developed by GeneDx and sequenced by massively parallel (NextGen) sequencing on an Illumina sequencing system with 100bp or greater paired-end reads. Reads are aligned to human genome build GRCh37/UCSC hg19, and analyzed for sequence variants in targeted genes using a custom-developed analysis tool (Xome Analyzer). Capillary sequencing or another appropriate method is used to confirm all potentially pathogenic variants identified in this individual and relative samples, if submitted. Sequence and copy number alterations are reported according to the Human Genome Variation Society (HGVS) and International System for Human Cytogenetic Nomenclature (ISCN) guidelines, respectively.

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 gene list is systematically updated at least quarterly. 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 adolescents⁸. 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)⁹. 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 parents¹⁰.

The average coverage of all genes on the panel is greater than 98% at 10X (with a depth of 10 or more reads), and approximately 88% of the genes on the panel have an average coverage of greater than 99.0% coverage at 10X. Several genes with a high clinical sensitivity have an average coverage of less than 90% at 10X, including NEFL (80.3%), VAMP1 (83.5), and COX20 (86.8%). 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 (FMR1), Friedreich ataxia (FXN), Huntington chorea (HTT) and Dentatorubro-pallidoluysian atrophy (ATN1).

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

REFERENCES:

1. Bird T. Hereditary Ataxia Overview. 1998 Oct 28 [Updated 2016 Nov 3]. In: Pagon RA, Adam MP, Ardinger HH, et al., editors. Gene Reviews [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2016. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1138/>
2. Finsterer et al. (2009) *Can J Neurol Sci* 36 (4):409-28 (PMID: 19650351)
3. Jayadev et al. (2013) *Genet. Med.* 15 (9):673-83 (PMID: 23538602)
4. Sandford et al. (2014) *Genes (Basel)* 5 (3):586-603 (PMID: 25055202)
5. Durr et al. (2010) *Lancet Neurol* 9 (9):885-94 (PMID: 20723845)
6. Embiruçu et al. (2009) *Arq Neuropsiquiatr* 67 (4):1143-56 (PMID: 20069237)
7. Kalia et al. (2016) *Genet. Med.*: (PMID: 27854360)
8. Nemeth et al. (2013) *Brain* 136 (Pt 10):3106-18 (PMID: 24030952)
9. Sastry et al. Exome Sequencing Provides a Broad Evaluation and High Diagnostic Rate for Ataxia-Related Disorders [abstract and platform presentation] To be presented at the 2017 ACMG Annual Clinical Genetics Meeting, March 21-25, 2016 Phoenix, AZ
10. Retterer et al. (2015) *Genet. Med.*: (PMID: 26633542)