THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Genetic Testing for Ataxia

Ataxias are a heterogeneous group of neurological disorders affecting individuals of all age groups and are characterized by the incoordination of voluntary movements. Features can include cerebellar dysfunction involving disturbance of stance, gait, eye movements, muscle tone, skilled movements and speech [1].

Genetic testing for hereditary ataxias can present challenges in daily clinical practice due to significant clinical and genetic heterogeneity. Ataxia can be isolated or part of a multisystemic syndromic presentation, age of onset and severity of symptoms is highly variable, and inheritance can be autosomal dominant, autosomal recessive, X-linked, or mitochondrial. The most common hereditary forms of ataxia include the autosomal dominant spinocerebellar ataxias (SCAs) and the autosomal recessive Friedreich ataxia which are caused by trinucleotide repeat expansions [2]. Fragile X-associated tremor/ataxia syndrome (FXTAS), caused by a premutation in the *FMR1* gene, is the most common X-linked cause of cerebellar ataxia [3]. A biallelic intronic expansion in the *RFC1* gene has recently been identified in the majority of patients with CANVAS (Cerebellar ataxia, neuropathy, and vestibular areflexia syndrome) and in a proportion of late onset ataxia patients[4]. Worldwide SCA1, 2, 3, 6, and 7 explain 50-60% of all cases of autosomal dominant ataxia, while other known SCA subtypes are rare (<1%) [5]. In general, recessive ataxias often present as multisystem disorders, while autosomal dominant ataxias are typically restricted to the central nervous system[1].

Being able to provide a genetic diagnosis allows for the opportunity for genetic counseling, long-term investigations and development of therapeutic strategies, and has implications for patient management and prognosis [2].

Genetic Testing Options for Ataxia

- Repeat expansion testing
- Exome-based sequencing

Our Ataxia Repeat Expansion Panel includes repeat expansion testing for 13 genes associated with ataxia.

Ataxia Repeat Expansion Panel					
Disorder	**Gene**	**Normal repeat number**	**Uncertain/reduced penetrance repeat number**	**Full/high penetrance repeat number**	**CPT code**
SCA1	ATXN1	≤44 (CAT present), ≤35 (CAT absent	36-38 (CAT absent)	≥45 (CAT present), ≥39 (CAT absent)	81178
SCA2	ATXN2	≤31	32	≥33	81179
SCA3 (Machado-Joseph disease)	ATXN3	≤44	45-59	≥60	81180
SCA6	CACNA1A	≤18	19	≥20	81184
SCA7	ATXN7	≤18	19-36	≥37	81181
SCA8	ATXN8OS	≤50	51-79	≥80	81182
SCA10	ATXN10	≤32	33-799	≥800	81183
SCA12	PPP2R2B	≤32	33-50	≥51	81343
SCA17	TBP	≤40	41-48	≥49	81344
DRPLA (Dentatorubral-pallidoluysian atrophy)	ATN1	≤35	36-47	≥48	81177
FXTAS (Fragile X-related tremor/ataxia syndrome	FMR1	5-44	45-54	55-200 (premutation)	81243
FRDA (Friedreich ataxia)	FXN	≤33	34-65	≥66	81284
CANVAS (Cerebellar ataxia, neuropathy, and vestibular areflexia syndrome)	RFC1	AAAAG normal	NA	AAGGG expansion	81479

Our Ataxia Exome includes analysis of 565 genes associated with ataxia.

Ataxia Exome		
The Ataxia Exome includes exome sequencing and analysis of a predefined set of 565 genes associated with ataxia and assembled by research and clinical experts in the field. These include genes known to be associated with ataxia as the predominant feature, genes associated with ataxia as part of the phenotype, and genes speculated to be involved in an ataxia phenotype based on expert opinion.		
Autosomal Recessive Genes Over 230 ataxia genes including: Spastic paraplegia Joubert syndrome Ceroid lipofuscinosis Mitochondrial disorders Inborn errors of metabolism Muscular dystrophy-dystroglycanopathies Mendelian Genetic syndromes And Many More	**Autosomal Dominant Genes** Over 70 genes including: Spinocerebellar ataxias Mendelian genetic syndromes And Many More	**X-linked Genes** Including: Epileptic encephalopathy Intellectual disability Mendelian genetic syndromes And Many More

***For a list of the 565 genes analyzed, please visit our website.*

Analysis and Methods

Repeat Expansion Panel

Repeat sizing is performed by standard flanking-PCR (F-PCR) and repeat primed PCR (RP-PCR) followed by capillary electrophoresis. F-PCR amplifies across the repeat region while RP-PCR amplifies within the repeat region. RP-PCR will detect large expansions that may not be detected by F-PCR and provides more accurate repeat sizing information. RP-PCR is performed using a fluorescently labeled primer specific to the target of interest, a "repeat primer" consisting of multiple repeats in tandem, and an anchor primer specific to a tail attached to the repeat primer. A 'ladder' of repeat size products is generated and sizing determined by counting the number of peaks of the ladder (Warner *et al.*, 1996. J Med Genet. 33(12):1022-1026). Expansions larger than 100 repeats for all the repeats tested can be detected but may not be sized by this test. SCA10 fully penetrant alleles ($\geq$800) will not be differentiated from reduced penetrant alleles that are >100 repeats. For repeat sizes in the normal range the accuracy of the assay is +/- 1 repeat. For repeat sizes in the uncertain significance/reduced penetrance range and full mutation expansions that can be sized the accuracy of the assay is +/- 3 repeats. For *RFC1* several different repeat conformations have been observed, of which the main ones include: a wild type sequence AAAAG (11 repeats or more), and longer expansions of AAAAG, AAAGG and AAGGG sequences. However, in reported ataxia cases only the AAGGG expansion has been shown to be pathogenic (PMIDs: 31028356, 31230722). This assay only detects the reference AAAAG and pathogenic AAGGG repeat conformations.

Ataxia Exome

Of the thousands of variants identified by exome sequencing, a list of variants that are located within a predefined set of 565 genes that have been associated with ataxia is generated. Variants within this gene list are analyzed. In some cases, exome sequencing data may also be used to detect larger copy number variations (CNVs) such as whole or partial gene deletions/duplications. The sensitivity of exome sequencing to detect intragenic deletions/duplications >20bp in size is not currently known. For cases without a clearly pathogenic variant identified in the predefined list of 565 genes, an additional analysis of previously reported pathogenic variants and truncating variants in known disease genes (present in the HGMD database) will be performed. For variants outside of the predefined list of 565 genes, only those considered to be the likely cause of the patient's phenotype will be reported. Mutations in genes unrelated to the individual's reported phenotype are considered secondary or incidental findings. Secondary or incidental findings will not be reported in the Ataxia Exome, unless they occur within the predefined list of 565 genes. Most variants identified as part of exome sequencing will NOT undergo interpretation by a laboratory staff member. Only those variants considered to be potentially relevant to the patient's condition are reviewed by a team of Board-Certified PhD geneticists, MD geneticists, and genetic counselors who will determine the likelihood of the variant being related to the patient's disorder based on the phenotypic information provided by the ordered clinician.

Exome sequencing is performed using the Agilent SureSelect Clinical Research Exome kit that is designed to target the exome with greater coverage of known disease-associated genes. Sequencing is performed using the Illumina technology and reads are aligned to the reference sequence. Approximately 97-98% of exons in the genes of interest are targeted at a minimum depth of 10X in the diagnostic Ataxia Exome. Our analytical pipeline presents variants on only the preselected 565 genes implicated in ataxia. Variants are identified and evaluated using a custom collection of bioinformatic tools and comprehensively interpreted by our team of directors and genetic counselors. All pathogenic and likely pathogenic variants are confirmed by Sanger sequencing.

Re-analysis of the Ataxia Exome

As new gene discoveries and associations are reported in the literature, we can review past cases for findings in these genes. Re-analysis of exome sequencing data is available upon request. Reanalysis can be performed once at no additional charge; additional charges may apply for further reanalysis requests.

Pricing and Turnaround Times

Ataxia Repeat Expansion Panel

Sample specifications:	3 to 10 cc of blood in a purple top (EDTA) tube
Cost:	$1250
Turn-around time:	2-4 weeks

Repeat Expansion Testing for a Single Ataxia Disorder

Sample specifications:	3 to 10 cc of blood in a purple top (EDTA) tube
Cost:	$750
Turn-around time:	2-4 weeks

Note: We do not bill insurance directly for RFC1 single gene repeat expansion testing

Ataxia Exome

Sample specifications:	3 to 10 cc of blood in a purple top (EDTA) tube
Cost:	$4000
Turn-around time:	6 weeks

Note: We do not bill insurance directly for this specific test

Comprehensive Ataxia Testing **CONCURRENT** (Ataxia Repeat Expansion Panel & Ataxia Exome)

Sample specifications:	3 to 10 cc of blood in a purple top (EDTA) tube
Cost:	$5000
Turn-around time:	6 weeks

Note: We do not bill insurance directly for this specific test

Comprehensive Ataxia Testing **REFLEX** (Ataxia Repeat Expansion Panel **with reflex to** Ataxia Exome)

Sample specifications:	3 to 10 cc of blood in a purple top (EDTA) tube
Cost:	$1250 for Ataxia Repeat Expansion Panel $3750 for Ataxia Exome
Turn-around time:	6-8 weeks

Note: We do not bill insurance directly for this specific test

For more information about our testing options, please visit our website at dnatesting.uchicago.edu or contact us at 773-834-0555.

Additional Resources for Hereditary Ataxias

Research studies on the genetics of ataxia are available in the laboratory of Dr. Margit Burmeister at the University of Michigan. For more information please visit http://www.hg.med.umich.edu/faculty/margit-burmeister-phd.

References:

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3. Bird T. Hereditary Ataxia Overview. In: MP A, HH A, RA P, eds. *GeneReviews [Internet].* Seattle, WA: University of Washington; 1998 (last update 2019).
4. Cortese A, Simone R, Sullivan R, et al. Biallelic expansion of an intronic repeat in RFC1 is a common cause of late-onset ataxia. *Nat Genet.* 2019;51(4):649-658.
5. Brusse E, Maat-Kievit JA, van Swieten JC. Diagnosis and management of early- and late-onset cerebellar ataxia. *Clin Genet.* 2007;71(1):12-24.

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