Molecular genetic testing for hereditary ataxia

What every neurologist should know

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Neurology: Clinical Practice February 2018 vol. 8 no. 1 27-32 doi:10.1212/CPJ.0000000000000421

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Abstract

Purpose of review

Because of extensive clinical overlap among many forms of hereditary ataxia, molecular genetic testing is often required to establish a diagnosis. Interrogation of multiple genes has become a popular diagnostic approach as the cost of sequence analysis has decreased and the number of genes associated with overlapping phenotypes has increased. We describe the benefits and limitations of molecular genetic tests commonly used to determine the etiology of hereditary ataxia.

Recent findings

There are more than 300 hereditary disorders associated with ataxia. The most common causes of hereditary ataxia are expansion of nucleotide repeats within 7 genes: *ATXN1*, *ATXN2*, *ATXN3*, *ATXN7*, *ATXN8*, *CACNA1A* (spinocerebellar ataxia type 6), and *FXN* (Friedreich ataxia). Recent reports describing the use of clinical exome sequencing to identify causes of hereditary ataxia may lead neurologists to start their clinical investigation with a less sensitive molecular test providing a misleading “negative” result.

Summary

The majority of individuals with hereditary ataxias have nucleotide repeat expansions, pathogenic variants that are not detectable with clinical exome sequencing. Multigene panels that include specific assays to determine nucleotide repeat lengths should be considered first in individuals with hereditary ataxia.

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Page 4

Multigene panels including a variety of genes associated with ataxia are clinically available. These panels may or may not include specific assays to determine nucleotide repeat lengths within the genes most commonly associated with hereditary ataxia. Therefore, pathogenic mutations in these common ataxia genes may be missed. Neurologists should be careful to choose the best set of tests most likely to identify the genetic cause of ataxia in any given patient.

Hereditary ataxias are a group of more than 100 genetic disorders primarily characterized by slowly progressive incoordination of gait.^{1,2} Additional features often include poor coordination of the upper extremities, abnormal eye movements, and dysarthria.

Ataxia also occurs in hundreds of additional genetic disorders not considered primary hereditary ataxias. Multiple inheritance patterns occur in this large group of disorders, including autosomal dominant, autosomal recessive, X-linked, and mitochondrial. Specific treatments are beneficial in individuals with a few of the known hereditary ataxias including ataxia with

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Funding information and disclosures are provided at the end of the article. Full disclosure form information provided by the authors is available with the full text of this article at Neurology.org/cp.

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vitamin E deficiency, Refsum disease, cerebrotendinous xanthomatosis, and CoQ10 deficiency. Establishing a diagnosis in an individual with ataxia can clarify recurrence risk and lead to specific treatments for some individuals.

Distinguishing clinical features or a positive family history of ataxia can suggest a specific diagnosis in some individuals. However, the number of genes associated with hereditary ataxia continues to increase. Clinicians are faced with the challenge of trying to identify a diagnosis in an individual presenting with ataxia as our understanding of the clinical spectrum of many hereditary ataxias is expanding. Results of molecular genetic testing have broadened the phenotypes to include mildly affected individuals and individuals with clinical findings that differ from previously established diagnostic criteria. Simultaneous interrogation of multiple genes using clinical exome sequencing or a large multigene panel is increasingly reported as an efficient method for identifying a diagnosis in individuals with ataxia.^{3–5} However, the most common causes of hereditary ataxia are due to nucleotide repeat expansions that would not be identified by these sequencing techniques.

A nucleotide repeat is a sequence of nucleotides repeated a number of times in tandem; nucleotide repeats can occur within or near a gene. The size of nucleotide repeats varies. Smaller numbers of repeats are common and not often associated with phenotypic abnormalities. Abnormally large numbers of repeats may be associated with phenotypic abnormalities and are classified as (in increasing order of size) mutable normal alleles, premutations, reduced-penetrance alleles, and full-penetrance alleles.

Nucleotide repeats increase the risk of DNA replication errors, which can lead to an expansion or contraction of the number of repeats. Larger nucleotide repeats are associated with increased severity of symptoms (full-penetrance alleles). When the size of a nucleotide repeat increases from one generation to the next, anticipation is observed. Anticipation is the tendency in certain genetic disorders for individuals in successive generations to present at an earlier age or with more severe manifestations. Larger nucleotide repeats can also expand during cell division, leading to variable nucleotide repeat sizes in neighboring cells.

Molecular genetic testing used to sequence a nucleotide repeat is more difficult than sequencing nonrepetitive regions of the exome because many of the known nucleotide repeats

contain a higher proportion of guanine and cytosine nucleotides compared to adenine and thymine nucleotides. Regions with high guanine and cytosine content are more difficult to amplify by PCR. In addition, repetitive regions do not align uniquely; thus, the length and therefore the pathogenicity of the repeated sequence cannot be determined.

Specific assays are required to analyze each nucleotide repeat of interest. DNA containing smaller nucleotide repeats can be amplified by PCR. The amplified segments of DNA are then separated by gel or capillary electrophoresis to determine repeat length. Highly expanded nucleotide repeats may not be detected by PCR-based assays due to difficulty in aligning the sequence to a unique genomic position. Additional testing (e.g., Southern blot analysis or triplet repeat primed PCR) may be required to determine the length of highly expanded nucleotide repeats.

Hereditary ataxias and nucleotide repeats

The most common hereditary ataxia in Caucasians is Friedreich ataxia, an autosomal recessive ataxia that is due to a repeat expansion in *FXN* in more than 90% of affected individuals. The 5 most common autosomal dominant hereditary ataxias include spinocerebellar ataxia (SCA) type 1, SCA 2, SCA 3, SCA 6, and SCA 7; all are due to nucleotide repeat expansions. The X-linked tremor/ataxia syndrome (FXTAS) associated with the fragile X gene (*FMR1*) is also caused by a repeat expansion. Several additional less common hereditary ataxias are also due to nucleotide repeat expansions (table 1). Establishing the diagnosis in an individual with any of these types of hereditary ataxia requires identification of an expanded nucleotide repeat and determination of nucleotide repeat size. Although a nucleotide repeat within a coding or noncoding region is expanded in all 16 of these types of hereditary ataxia, commercial exome sequencing will not identify these expansions.

Molecular genetic testing options for individuals with hereditary ataxia

Single gene testing

Distinguishing clinical features, a family history consistent with autosomal dominant or autosomal recessive inheritance, or ethnic background may sometimes be highly suggestive of a specific hereditary ataxia, in which case single gene testing should be considered first. Examples of these suggestive clinical features are presented in table 2. Single gene testing

Multigene panels that only include sequence analysis will not detect the majority of pathogenic variants associated with hereditary ataxia.

Table 1 Hereditary ataxias caused by nucleotide repeat expansions

Ataxia disorder	Gene	Percent of pathogenic variants ^a	Nucleotide repeat (amino acid)	Repeat location	Normal repeat number	Full-penetrance pathogenic repeat number
SCA type 1	<i>ATXN1</i>	100	CAG (Gln)	Exon 8	≤35 GR	≥39
SCA type 2	<i>ATXN2</i>	100	CAG (Gln)	Exon 1	≤31 GR	≥33
SCA type 3	<i>ATXN3</i>	100	CAG (Gln)	Exon 8	12–44	52–87
SCA type 6	<i>CACNA1A</i>	>99	CAG (Gln)	Exon 47	≤18	20–33
SCA type 7	<i>ATXN7</i>	100	CAG (Gln)	Exon 1	10–19	≥36
SCA type 8	<i>ATXN8OS</i>	100	CTG	3' UTR	15–50 CTA/CTG	≥71–1,300 CTA/CTG
	<i>ATXN8</i>	100	CAG (Gln)	Exon 1	~80	Unknown
SCA type 10	<i>ATXN10</i>	100	ATTCT	Intron 9	10–32	≥800
SCA type 12	<i>PPP2R2B</i>	100	CAG	5' UTR	8–23	51–78
SCA type 17	<i>TBP</i>	100	CAG or CAA (Gln)	Exon 3	25–44	47–63
SCA type 31	<i>BEAN1</i>	100	TGGAA	Intron 6	0	2.5–3.8 kb insertion
SCA type 36	<i>NOP56</i>	100	GGCCTG	Intron 1	3–14	≥650
DRPLA	<i>ATN1</i>	100	CAG (Gln)	Exon 5	6–35	≥48
Friedreich ataxia (autosomal recessive)	<i>FXN</i>	>90	GAA	Intron 1	5–33	≥66
Fragile X-associated tremor/ataxia syndrome	<i>FMR1</i>	>99	CGG	Exon 1 (UTR)	5–44	>200
Unverricht-Lundborg disease (autosomal recessive)	<i>CSTB</i>	>90	CCCCGCCCGCG	5' promoter	2–3	≥30

Abbreviations: DRPLA = dentatorubral-pallidoluysian atrophy; SCA = spinocerebellar ataxia; UTR = untranslated region.

Adapted from Bird TD. Hereditary ataxia overview. 1998 Oct 28 [Updated 2016 Nov 3]. In: Adam MP, Ardinger HH, Pagon RA, et al, eds. GeneReviews® [Internet]. Seattle: University of Washington; 1993–2017. Available at: ncbi.nlm.nih.gov/books/NBK1138/. © University of Washington 1993–2017. GeneReviews® is a registered trademark of the University of Washington, Seattle. The content is used with permission. All rights reserved.

^a Proportion of pathogenic variants in this gene that are caused by a nucleotide repeat expansion or contraction.

including nucleotide repeat analysis or sequence analysis can be the first approach when these features are present.

Multigene panels are molecular genetic tests designed by reference laboratories to simultaneously interrogate multiple

genes that are associated with similar or overlapping phenotypes. The diagnostic sensitivity of multigene panels varies because (1) different laboratories may include different genes and (2) laboratories frequently update panels to include analysis of noncoding regions (promoters) and additional

Table 2 Clinical features, mode of inheritance, and ancestry suggestive of a specific hereditary ataxia

Distinguishing clinical features	Mode of inheritance	Ancestry	Hereditary ataxia	Recommended single gene testing
Dystonia or distal muscle weakness and atrophy	AD	Portuguese	SCA 3	<i>ATXN3</i> nucleotide repeat analysis
Progressive visual loss	AD		SCA 7	<i>ATXN7</i> nucleotide repeat analysis
Slowly progressive ataxia, seizures	AD	Latin American	SCA 10	<i>ATXN10</i> nucleotide repeat analysis
Chorea or psychosis	AD		SCA 17	<i>TBP</i> nucleotide repeat analysis
Chorea	AD	Japanese	DRPLA	<i>ATN1</i> nucleotide repeat analysis
Childhood onset decreased DTR	AR		Friedreich ataxia	<i>FXN</i> nucleotide repeat analysis

Abbreviations: AD = autosomal dominant; AR = autosomal recessive; DRPLA = dentatorubral-pallidoluysian atrophy; DTR = deep tendon reflexes; SCA = spinocerebellar ataxia.

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Table 3 Other neurologic disorders caused by nucleotide repeat expansions and contractions

Disorder	Gene	% Of pathogenic variants ^a	Nucleotide repeat (amino acid)	Repeat location	Normal repeat number	Full-penetrance pathogenic repeat number
<i>C9orf72</i> -related ALS and FTD	<i>C9orf72</i>	5–30	GGGGCC (Gly-Ala)	Promotor or intron 1	2–9	700–1,600
Congenital central hypoventilation syndrome	<i>PHOX2B</i>	97	GCN (Ala)	Exon 3	≤20	25–33
Fragile X	<i>FMR1</i>	>99	CGG	Exon 1 (UTR)	5–44	>200
Fragile X, FRAXE type	<i>AFF2</i>	>99	GCC	5' UTR	6–25	>200
Early infantile epileptic encephalopathy	<i>ARX</i>	Most common	GCG (Ala)	Exon 2 aa 110-115	10–16	17–27
Early infantile epileptic encephalopathy and Partington syndrome	<i>ARX</i>	Most common	GCG (Ala)	Exon 2 aa 144-155	12	20
Holoprosencephaly type 5	<i>ZIC2</i>	~40	GCN (Ala)	Exon 3	15	25
Huntington disease	<i>HTT</i>	100	CAG (Gln)	Exon 1	≤26	>40
Huntington disease–like 2	<i>JPH3</i>	~100	CTG/CAG (Ala)	Exon 2A	6–28	≥41
Laing distal myopathy	<i>MYH7</i>	5 families	AAG (Lys)	Exon 36	3	2
Mental retardation type 12A	<i>DIP2B</i>	100	CGG	5' UTR	6–23	>350
Myotonic dystrophy type 1	<i>DMPK</i>	100	CTG	3' UTR	5–34	>50
Myotonic dystrophy type 2	<i>CNBP</i>	100	CCTG	Intron 1	≤26	>75
Oculopharyngeal muscular dystrophy	<i>PABPN1</i>	100	GCN (Ala)	Exon 1	10	12–17
Panhypopituitarism Mental retardation with growth hormone deficiency	<i>SOX3</i>	2 families	GCN (Ala)	Exon 1	15	22–26
Spinal and bulbar muscular atrophy	<i>AR</i>	100	CAG (Gln)	Exon 1	≤34	≥38

Abbreviations: ALS = amyotrophic lateral sclerosis; FTD = frontotemporal dementia; UTR = untranslated region.

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^a Proportion of pathogenic variants in this gene that are caused by a nucleotide repeat expansion or contraction.

genes as they are discovered. The methods used in a multi-gene panel may include sequence analysis, deletion/duplication analysis, specific assays for nucleotide repeats, or other nonsequencing-based tests.

Ten reference laboratories in the United States offer multi-gene panels designed to identify causes of hereditary ataxia.

1. One reference laboratory offers a multigene panel that can detect expanded nucleotide repeats in the 5 genes most often associated with hereditary ataxia.
2. One reference laboratory offers 4 different multigene panels for hereditary ataxia, including (1) a panel that can detect expanded nucleotide repeats in 8 genes, (2) a panel that includes nucleotide repeat analysis of 11 genes and simultaneous sequence analysis of 33 genes, (3) an autosomal dominant ataxia panel that includes nucleotide repeat analysis of 10 genes and simultaneous sequence analysis of 16 genes, and (4) a panel for autosomal recessive ataxias including nucleotide repeat analysis of *FXN*, sequence analysis of 18 genes, and *ATM*

deletion testing. The physician must decide which panel to order.

3. One reference laboratory offers 5 different multigene panels for hereditary ataxia. All of these panels include sequence analysis of 300 genes, deletion/duplication analysis of an unspecified number of genes, analysis of mitochondrial DNA, and 4 panels include nucleotide repeat analysis of *HTT* or *FXN* or an unspecified number of genes associated with SCA. The physician must decide which panel to order.
4. The remaining 7 laboratories offering molecular genetic testing for hereditary ataxia only offer sequence analysis of the associated genes. Repeat expansion sizes are not determined.

Multigene panels that only include sequence analysis will not detect the majority of pathogenic variants associated with hereditary ataxia. Several of the genes included on these large panels do not have any reported pathogenic variants detected by sequence analysis as all of the known pathogenic variants in the genes are nucleotide repeat expansions. The

limitations of molecular genetic tests that only include sequence analysis are often not obvious to the ordering provider and may or may not be included in detailed information on the reference laboratory website. When selecting a multigene panel, it is critical to review the genes included in the panel and review the methods used by the reference laboratory to investigate each gene for pathogenic variants.

Clinical exome sequencing

Whole exome sequencing (WES) is a laboratory test designed to identify and analyze the sequence of all protein-coding nuclear genes in the genome (about 20,000). Approximately 95% of the exome can be sequenced with currently available techniques. However, regions of the exome that contain highly repetitive DNA sequences (including nucleotide repeats) are resistant to sequence analysis. Research laboratories have used WES to identify new genes associated with ataxia in groups of patients with no specific genetic diagnosis.^{3,6} Note that these studies have already identified and excluded patients with the more common nucleotide repeat expansion diseases.

An individual will have approximately 200–500 variants identified on clinical exome sequencing that are non-synonymous (protein-altering) and not present in available databases.⁷ It requires additional filtering and expert curation of these variants to determine pathogenicity. Despite the best curation efforts, because of the large number of genes associated with hereditary ataxia, variants of uncertain significance are likely to be identified in one or more of these genes on exome sequencing. Identification of variants of uncertain significance can lead to additional costly testing of relatives that may or may not clarify the diagnosis. Focusing on a variant of uncertain significance can also lead to a missed opportunity to identify the correct diagnosis in an individual with an expanded nucleotide repeat. Finally, errors in analysis can lead to erroneous diagnoses such as the report of a gene newly associated with hereditary ataxia that was subsequently proven to be unrelated.⁶

Clinical exome sequencing may detect some pathogenic variants that are present in noncoding regions (e.g., intronic pathogenic variants near splice sites). However, deep intronic pathogenic variants that have been described in some individuals with hereditary ataxia are not reliably detected by clinical exome sequencing.⁸

How to compare molecular genetic testing options?

If the clinical features or family history suggest a specific hereditary ataxia, single gene testing can be considered. It is important that the provider select a nucleotide repeat assay or sequence analysis consistent with the known pathogenic variants in the gene. If the clinical features or family history are not suggestive of a specific hereditary ataxia, a multigene panel should be considered. Multigene panels that include specific assays for nucleotide repeats should be considered in

individuals with hereditary ataxia. In addition, multigene panels that include sequence analysis may have a higher sensitivity than WES and should identify less variants of uncertain significance. Providers should be aware that some multigene panels employ the same test methods used in exome sequencing, resulting in clinical sensitivity equal to WES for the genes included on the multigene panel. WES should be considered if the genes associated with the patient's phenotype are not well-covered by an available multigene panel. In individuals with hereditary ataxia, WES or panels using only WES should only be considered after specific assays for nucleotide repeats have not identified a pathogenic variant. A hereditary ataxia cannot be ruled out with currently available molecular genetic testing. Individuals without an etiology identified after nucleotide repeat assays and WES may benefit from reanalysis of the exome data after a year or two, as new genes are expected to be identified.

Further research

The human exome contains 1,030 trinucleotide repeats in exons of 878 genes.⁹ Only a small percentage of identified trinucleotide repeats have been associated with human disease. Nucleotide repeat expansions have primarily been associated with neurologic phenotypes (table 3). Investigation of known trinucleotide repeats that have not been associated with a described phenotype may further our understanding of known neurologic conditions, result in identification of new neurologic disorders, or allow diagnoses in individuals with previously negative molecular genetic testing.

Author contributions

S.E. Wallace: drafting/revising the manuscript, analysis or interpretation of data. T.D. Bird: drafting/revising the manuscript, acquisition of data.

Study funding

No targeted funding reported.

Disclosure

S.E. Wallace reports no disclosures. T.D. Bird serves on the editorial board of *GeneReviews.org*; holds patents re: Genetic testing technology for CMT1C and SCA14; and receives research support from Department of Veterans' Affairs. Full disclosure form information provided by the authors is available with the full text of this article at Neurology.org/cp.

Received June 16, 2017. Accepted in final form August 15, 2017.

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