EXAMPLE OF THE APPLICATION OF CLINICAL EXOME SEQUENCING TO THE EVALUATION OF A NEUROLOGIC DISEASE: CEREBELLAR ATAXIA

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The utilization of Clinical Exome Sequencing in neurologic practice requires specific adaptation to the condition under study (e.g., movement disorders, intellectual disability/neurodevelopmental disorders, autism, neuromuscular disease, epilepsy, dementia, etc.) including considerations regarding the diversity of genetic disease which contributes to the etiology, the respective modes of inheritance, and the clinical overlap between one genetic condition and another. While a comprehensive review of such an approach for every neurologic disease is beyond the scope of this course and this document, a detailed illustration of how these concepts can be applied to a single neurologic condition is presented to provide an example of how this technology can be used in routine clinical practice.

Cerebellar Ataxia

The cerebellar ataxias represent a diverse and heterogeneous group of disorders that are primarily characterized by abnormal function of the cerebellum and its pathways, typically those involving the brainstem and the spinal cord.[1-9] A major diagnostic challenge for clinicians involves the significant phenotypic overlap between acquired, idiopathic, and genetic etiologies.[1-9] This distinction is non-trivial, as many of the acquired causes can be modified.[2,3,5-9] and this can significantly impact the resulting level of disability and potential for successful rehabilitation for a given patient.

A thorough discussion of the clinical evaluation of such patients for acquired causes is beyond the scope of this course, but interested readers are referred to published articles discussing this topic in detail.[2,3,5-9]. Within this framework of disease we will now focus on the clinical approach to the genetic evaluation of a patient with cerebellar ataxia. It is assumed that all patients for which genetic etiologies are considered will have been previously evaluated and deemed negative for the common (and, if appropriate, more rare) acquired etiologies.

Clinical Evaluation of a Patient with Cerebellar Ataxia

The cerebellum is thought to functionally integrate sensory data (e.g., visual, tactile, proprioceptive, etc.) and modulate gross motor output predictions to obtain smooth coordinated movement.[10] In the absence of this, balance and coordination become impaired. Similar symptoms can arise from disorders involving the vestibular and proprioceptive systems and, in the case of the genetic cerebellar ataxias, these often occur in combination with cerebellar dysfunction,[1-9] resulting in a more pronounced clinical picture.

In general, patients with cerebellar ataxia demonstrate a primary cerebellar dysfunction, evidenced by either limb, trunk, or gait ataxia, alone or in combination. Beyond these symptoms, associated findings depend on the specific disorder and can include extrapyramidal and/or pyramidal features, peripheral neuropathy, cognitive impairment, seizures, and others. The autosomal dominant disorders (collectively designated as the “SCAs” or spinocerebellar ataxias) tend to present predominantly with cerebellar-specific features reflecting discoordinated motor actions such as dysarthria, dysphagia, eye movement disturbances (i.e., ocular dysmetria, saccadic visual pursuit, gaze-evoked nystagmus), limb dysmetria, and gait imbalance with subsequent falls.[1,2,4,6,9] The autosomal recessive disorders also feature predominant cerebellar signs but also often have an associated peripheral neuropathy and are frequently associated with additional disease-specific non-neurologic clinical symptoms as well.[3,6-8]

Genetic Cerebellar Ataxia

Essentially all modalities of genetic inheritance are represented among the hereditary cerebellar ataxias,[2,6] however, autosomal dominant and recessive conditions comprise the majority (see Tables 1 and 2). The dominant disorders (the SCAs) utilize a common sequential numbering scheme, with the most common disorders being SCA1, SCA2, SCA3, SCA6 and SCA7.[1,2,4,6,9] These five conditions, as well as several other SCAs, are members of a class of genetic diseases termed repeat expansion disorders. Repeat expansion disorders result

from expansion of nucleotide elements (either coding, noncoding, or intronic) within the specified gene.[11,12] In the case of these SCAs, the repeat element that undergoes expansion is CAG and it occurs within the coding sequences of each gene. Since the codon CAG represents the amino acid glutamine, this results in expansion of a polyglutamine tract within each protein.[1,2,4,6,9] These (and related rarer disorders) are sometimes called the polyglutamine (or CAG-repeat) ataxias.[1,2,4,6,9] The most common recessive ataxia is also a repeat expansion, in this case, an intronic GAA occurring within the frataxin gene, resulting in the disease known as Friedreich ataxia.[3,6-8] Together, these 6 disorders represent up to 50% of all hereditary ataxias worldwide.[1-4,6,8,9,13]

Unfortunately, beyond these most common forms there is a vast array of additional genetic etiologies. Primary cerebellar ataxias number over 30 dominant disorders[9] and over 25 recessive.[8] Overall, more than 300 genetic diseases exist with ataxia as either a primary or secondary symptom.[14] Mutation of a majority of these genes each represents <1% of hereditary ataxia patients worldwide.[1-4,6-9,13] Given the high degree of phenotypic overlap among the diseases, this presents a major diagnostic challenge to physicians as patients often do not present with clinical features specific to one (or even several) disorders to focus diagnostic testing.[1-4,6-9,13]

Genetic Testing for Cerebellar Ataxia

Current methods of diagnostic genetic testing (Table 3) for the hereditary cerebellar ataxias center initially on determining whether a patient requires repeat expansion testing. Such testing is typically performed using polymerase chain reaction (PCR) or Southern blotting to detect size differences associated with the expanded alleles.[12] This type of testing is essential for detection of the most common dominant and recessive ataxias described above. For disorders caused by point mutations, gene sequencing is the method most commonly employed and this can be performed for either the entire gene (whole gene sequencing) or for a specific mutation (targeted mutation analysis).[12] The sequencing methodology typically used is the Sanger method, which can be quite costly for sequencing genes when they are either large in size and or number.[12] Sanger (or Dye-Terminator) sequencing reads, on average, 500-800 nucleotides per reaction, a scale suitable for most

DISEASE	SYMBOL	LOCUS	GENE
Autosomal recessive cerebellar ataxia, type 2	ARCA2	1q42.13	*ADCK3*
AMACR deficiency	AMACR	5p13	*AMACR*
Spinocerebellar ataxia autosomal recessive, type 10	SCAR10	3p22.1	*ANO10*
Ataxia with oculomotor apraxia, type 1	AOA1	9p13.3	*APTX*
Cayman ataxia	CA	19p13.3	*ATCAY*
Ataxia telangiectasia	AT	11q22.3	*ATM*
Infantile-onset spinocerebellar ataxia	IOSCA	10q24	*C10orf2*
Dysequilibrium syndrome	DES	13q12 8q12.1 9p24 17p13.3	*ATP8A2* *CA8* *VLDLR* *WDR81*
Cerebrotendinous xanthomatosis	CTX	2q35	*CYP27A1*
Friedreich ataxia	FRDA	9q21.11	*FXN*
Late-onset Tay-Sachs	LOTS	15q23	*HEXA*
Rundataxin ataxia	RDTX	3q29	*KIAA0226*
Autosomal recessive spastic ataxia with leukoencephalopathy	ARSAL	2q33.1	*MARS2*
Ataxia telangiectasia-like disorder	ATLD	11q21	*MRE11A*
Abetalipoproteinemia	ABL	4q24	*MTTP*
Peroxin-associated ataxias	PEX	8q21.1 1p36.32 11p11.2	*PEX2* *PEX10* *PEX16*
Refsum disease	RD	6q23.3 10p13	*PEX7* *PHYH*
DNA polymerase gamma disorders	POLG	15q25	*POLG*
Autosomal recessive ataxia of Charlevoix-Saguenay	ARSACS	13q12	*SACS*
Ataxia with oculomotor apraxia, type 2	AOA2	9q34.13	*SETX*
Marinesco-Sjögren syndrome	MSS	5q31	*SIL1*
Spinocerebellar ataxia, autosomal recessive, type 14	SCAR14	11q13.2	*SPTBN2*
Autosomal recessive cerebellar ataxia, type1	ARCA1	6q25	*SYNE1*
Spinocerebellar ataxia autosomal recessive, type 11	SCAR11	1q32.2	*SYT14*
Spinocerebellar ataxia with axonal neuropathy	SCAN1	14q32.11	*TDP1*
Ataxia with vitamin E deficiency	AVED	8q12.3	*TTPA*

Table 1. Selected Autosomal Recessive Cerebellar Ataxias.
Disorders caused by repeat expansion are highlighted.

single gene analysis but with improved next-generation sequencing technologies available, no longer is practical for large scale multi-gene analysis.[12] Next-generation sequencing utilizes a massively parallel approach which can effectively and efficiently sequence entire genomes in weeks.[12] For clinical purposes, sequencing the 1-2% of the genome which encodes protein, termed the exome, where up to roughly 85% of disease-causing mutations are estimated to reside,[12] is the current method of choice for large scale gene analysis. Several recently published strategies for genomic assessment of ataxia genes incorporate this method into their algorithm.[8,9,12,15-17]

DISEASE	LOCUS	GENE
SCA1	6p23	*ATXN1*
SCA2	12q24	*ATXN2*
SCA3	14q24.3-q31	*ATXN3*
SCA5	11q13	*SPTBN2*
SCA6	19p13.2	*CACNA1A*
SCA7	3p21.1-p12	*ATXN7*
SCA8	13q21.33	*ATXN8OS, ATXN8*
SCA10	22q13.31	*ATXN10*
SCA11	15q15.2	*TTBK2*
SCA12	5q32	*PPP2R2B*
SCA13	19q13.3-13.4	*KCNC3*
SCA14	19q13.4	*PRKCG*
SCA15/SCA16/SCA29	3p26.1	*ITPR1*
SCA17	6q27	*TBP*
SCA19/SCA22	1p13.3	*KCND3*
SCA23	20p13	*PDYN*
SCA27	13q34	*FGF14*
SCA28	18p11.22-q11.2	*AFG3L2*
SCA31	16q22	*BEAN1, TK2*
SCA35	20p13	*TGM6*
SCA36	20p13	*NOP56*
Dentatorubral- Pallidoluysian Atrophy (DRPLA)	12p13.31	*ATN1*

Table 2. Selected Autosomal Dominant Cerebellar Ataxias.
Disorders caused by repeat expansion are highlighted.
SCA, spinocerebellar ataxia.

The Role of Clinical Exome Sequencing

A systematic approach is required when evaluating a patient with cerebellar ataxia (Figure 1), including a detailed medical and family history, a thorough clinical examination, and an MRI of the brain.[2,5,6,8,9] Every patient presenting with cerebellar ataxia also requires a complete evaluation for acquired causes as these are potentially treatable or modifiable and early recognition can significantly improve recovery outcomes.[2,5,6,8,9] Once acquired etiologies have been ruled out, idiopathic and genetic conditions are brought to the forefront. When considering genetic causes, phenotypic evaluation and family history are key features to next consider. A positive family history is certainly suggestive of an underlying genetic etiology and the pattern of inheritance within the family would suggest whether an emphasis should be placed on autosomal dominant, autosomal recessive, or other modalities (e.g., X-linked, mitochondrial, etc.). Unless there are key phenotypic features to focus genetic testing toward a specific disorder, the most common dominant or recessive disorders should be evaluated initially. This is also true for sporadic cases if a genetic etiology is to be investigated. If this initial evaluation is negative then more advanced testing is required. At this stage, clinical exome sequencing becomes highly valuable as a means of rapidly assessing the multitude of genes which represent 1% or less of the genetic causes of cerebellar ataxia worldwide.[8,9,12,15-17] This is also a cost effective strategy as multi-single gene and large gene panel testing using the Sanger method can be quite costly, particularly when not performed systematically based on phenotype.[18,19]

Several studies have examined the use of exome sequencing in early-onset ataxia. For example, Ohba et al. examined patients with childhood-onset ataxia with exome sequencing and diagnosed 9 of 23 cases (39%).[20] In a similar study, Sawyer et al. diagnosed 13 of 28 cases (46%).[21] Together these two studies suggest exome sequencing to be a valuable tool for diagnosing ataxic disorders in children.

Type of Test	Type of Mutation Identified
Whole Gene Sequencing	Point Mutations Small Frameshifts Splice Site Mutations
Targeted Mutation Analysis	Known Mutations
Repeat Expansion Testing (PCR or Southern Blot)	Repeat Expansion
Gene Copy Number Variation (Deletion/Duplication Testing)	Copy Number Variation
Chromosomal Microarray Analysis (Comparative Genomic Hybridization)	Genome-Wide Copy Number Variations
Clinical Exome Sequencing	Point Mutations Small Frameshifts Splice Site Mutations

Table 3. Diagnostic Genetic Testing Methodologies

Additionally other studies have examined the use of exome sequencing in adult-onset and sporadic ataxia cases. For example, Pyle et al. examined a cohort of 22 families (36% sporadic- and 50% adult-onset) and confirmed genetic diagnosis in 9 families (41%.).[22] Fogel et al. studied a cohort of 76 patients (74% sporadic- and 72% adult-onset) and identified pathogenic variants in 16 of these (21%),[23] demonstrating the effectiveness of this diagnostic test regardless of age of onset or family history, assuming a negative (and appropriately detailed) workup for acquired causes.[24]

In regard to the clinical use of exome sequencing, appropriate bioinformatic analysis and interpretation are a critical component to effective utilization,[12,15,23] and the strategy selected must be appropriate to evaluate ataxia genes. It is important to note that other repeat expansion disorders (e.g., some of the rarer SCAs), diseases caused by copy number variation (i.e., large deletions or duplications involving disease genes), or diseases affecting non-coding portions of a gene would not be detected by this method.[12] It is also important to recognize that, at present, only genes currently known to cause ataxia are likely be identified in this way. It is possible that genes known to cause other diseases (but not cerebellar ataxia) may be detected, depending on the method by which the results are analyzed, but this test is not designed to identify and validate genes never before shown to cause disease. However, because exome results will not change over time, the data can be re-examined periodically to incorporate new genetic discoveries and an initially negative test result could become positive later, a distinct advantage over other, more static, diagnostic methods.

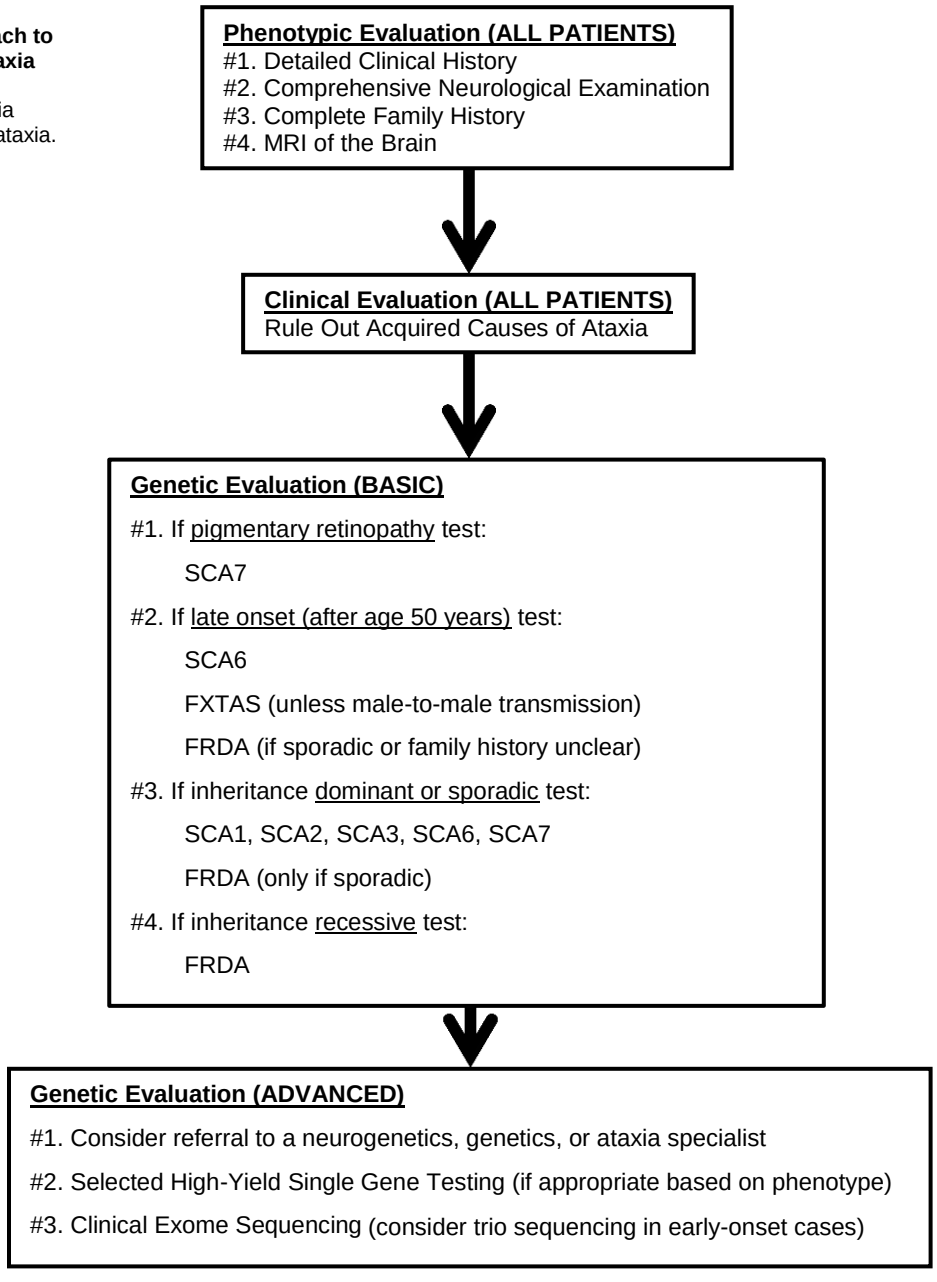

Figure 1. Diagnostic Approach to a Patient with Cerebellar Ataxia
SCA, spinocerebellar ataxia; FXTAS, Fragile X tremor ataxia syndrome; FRDA, Friedreich ataxia.

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