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Exome Sequencing in the Clinical Diagnosis of Sporadic or Familial Cerebellar Ataxia

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Abstract

IMPORTANCE—Cerebellar ataxias are a diverse collection of neurologic disorders with causes ranging from common acquired etiologies to rare genetic conditions. Numerous genetic disorders have been associated with chronic progressive ataxia and this consequently presents a diagnostic challenge for the clinician regarding how to approach and prioritize genetic testing in patients with such clinically heterogeneous phenotypes. Additionally, while the value of genetic testing in early-

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onset and/or familial cases seems clear, many patients with ataxia present sporadically with adult onset of symptoms and the contribution of genetic variation to the phenotype of these patients has not yet been established.

OBJECTIVE—To investigate the contribution of genetic disease in a population of patients with predominantly adult- and sporadic-onset cerebellar ataxia.

DESIGN, SETTING, AND PARTICIPANTS—We examined a consecutive series of 76 patients presenting to a tertiary referral center for evaluation of chronic progressive cerebellar ataxia.

MAIN OUTCOMES AND MEASURES—Next-generation exome sequencing coupled with comprehensive bioinformatic analysis, phenotypic analysis, and clinical correlation.

RESULTS—We identified clinically relevant genetic information in more than 60% of patients studied (n = 46), including diagnostic pathogenic gene variants in 21% (n = 16), a notable yield given the diverse genetics and clinical heterogeneity of the cerebellar ataxias.

CONCLUSIONS AND RELEVANCE—This study demonstrated that clinical exome sequencing in patients with adult-onset and sporadic presentations of ataxia is a high-yield test, providing a definitive diagnosis in more than one-fifth of patients and suggesting a potential diagnosis in more than one-third to guide additional phenotyping and diagnostic evaluation. Therefore, clinical exome sequencing is an appropriate consideration in the routine genetic evaluation of all patients presenting with chronic progressive cerebellar ataxia.

The diagnostic evaluation of a patient with chronic progressive cerebellar ataxia is clinically challenging. Cerebellar ataxia is associated with a heterogeneous array of neurologic conditions spanning common acquired etiologies to rare genetic disorders present only in single families.[1–5] Currently, more than 60 distinct neurogenetic conditions are known to cause primary cerebellar ataxia. In most cases, it is difficult to differentiate these disorders owing to phenotype variability within a disorder and overlap between disorders.[1–5] Further complicating matters, there are nearly 300 additional genetic conditions that can include cerebellar ataxia as a clinical finding.[6] Many patients present to clinicians with late-onset symptoms and no reported family history[7] and, in the absence of other identifying etiologies, the role of genetic disease in this population is not well understood.[1,3,5]

The availability of next-generation clinical exome sequencing (CES) has made it possible to perform genome-wide genetic evaluations for patients as part of a detailed clinical workup.[8,9] This is a potentially useful addition to the physician's armamentarium because, given the number of rare genes related to ataxia, overuse of low-yield single-gene and genetic panel testing represents a significant cost to patient health care.[7,10] The anticipated widespread benefits of CES have already prompted recommendations for its inclusion as part of routine clinical algorithms.[4,5,9,11,12] Although CES is much less expensive than sequentially examining multiple single genes, there are limited data to support the widespread use of this testing as part of the standard evaluation of patients with cerebellar ataxia especially those without a family history.

To date, several studies have examined the use of exome sequencing in ataxia of early onset (at or prior to age 20 years). Ohba et al[13] examined patients with childhood-onset ataxia and cerebellar atrophy and identified molecular etiologies in 39% (9 of 23 families). Sawyer et

al[14] also evaluated childhood-onset ataxia cases and reported a 46% success rate (13 of 28 families). Most positive cases identified by Ohba et al[13] had sporadic onset, while Sawyer et al[14] found a higher success rate among familial cases or those with consanguineous parentage (69%, 9 of 13). Overall, the few studies published thus far support the use of CES in patients with early-onset/childhood ataxia particularly in those with a positive family history.

However, childhood-onset and familial cases are relatively rare and most patients presenting to ataxia clinics have adult onset of symptoms and lack a family history.[7] In such sporadic cases, the contribution of genetic mutations is at best uncertain, potentially diminishing enthusiasm for the regular use of exome sequencing in these patients. Furthermore, as the most common familial adult-onset ataxias are repeat-expansion disorders,[1,3,5] the usefulness of exome sequencing (which is not reliable for determining the length of tandem repeats) has not been established for this population.

To assess the value of next-generation sequencing in the clinical diagnosis of patients with cerebellar ataxia, we performed CES in 76 consecutive cases, predominantly adult- and sporadic-onset (72% and 74%, respectively), seen in our tertiary ataxia referral center. Pathogenic diagnoses were established for 16 of the 76 patients (21%), of which 38% (6 of 16) were adult-onset cases and 69% (11 of 16) presented sporadically. Furthermore, using a detailed bioinformatic approach, we identified variants of potential pathogenicity in an additional 30 patients (40%), of which 77% (23 of 30) were adult-onset cases and 73% (22 of 30) presented sporadically. In only 30 of the 76 cases (40%) was CES unable to provide any additional genetic information to assist in establishing a clinical or molecular diagnosis or in directing further workup. This study supports the use of CES as an important tool in the evaluation of patients with both early- and adult-onset ataxia, with or without a family history, in the presence of an otherwise non-diagnostic clinical workup.

Methods

Participants

Patients were seen in the UCLA Ataxia Center, a tertiary referral site for disorders of gait and balance serving primarily the population of southern California and southern Nevada. Our sample was obtained from a consecutive series of qualifying patients seen by our neurogenetics specialists (S.P., B.L.F., or D.H.G.). To qualify, all patients were initially required to have a chronic and progressive gait and/or limb ataxia on clinical examination. However, because severity varied, patients were classified based on their most significant of 3 common symptoms: ataxia, spasticity, or parkinsonism. Presentation of symptoms was categorized as (1) either early onset (at or before age 20 years) or adult onset and as (2) either familial or sporadic onset based on history. Prior to enrollment, all patients were required to have a complete negative evaluation for acquired causes[1,3,5,7] (eAppendix in the Supplement) and, as appropriate, screening for the most common repeat expansion disorders causing hereditary cerebellar ataxia (spinocerebellar ataxia type 1 [SCA1], SCA2, SCA3, SCA6, SCA7, and Friedreich ataxia). Specific patients received additional single-gene testing based on phenotype. All patients received genetic counseling both before and after exome sequencing. All study methods were approved by the institutional review board of

the University of California at Los Angeles. Written informed consent was obtained from all patients enrolled in the study.

Exome Sequencing and Bioinformatic Analysis

Clinical exome sequencing and data analysis were performed using a standard protocol[15] that has been fully validated and conducted under stringent quality control (eTables 1–4 in the Supplement). Once the variants causing nonsynonymous amino acid changes, stop codons, stop loss changes, inframe insertions/deletions, frameshifts, or changes to splice site sequences were identified, the following strategy was used to prioritize the variant list.[16] Population allele frequency compiled from public databases of normal human variation (National Center for Biotechnology Information Database of Single Nucleotide Polymorphisms[17]; National Heart, Lung and Blood Institute Exome Variant Server[18]; and the 1000 Genomes Project[19]) was used to filter the data set to exclude all variants present in the population at greater than 1% frequency based on the low probability that these directly cause Mendelian disease,[20] the observation that no previously reported ataxia gene mutations are seen in the population above this frequency (eTable 5 in the Supplement), and because this study was not designed to assess the contribution of common alleles to the development of ataxia. We next identified a list of keywords best defining the phenotype of each patient, which was used to prioritize genes based on clinical information in the Online Mendelian Inheritance in Man database (http://www.omim.org/) and the Human Gene Mutation Database Professional Version (http://www.hgmd.org/). An example gene list for the keyword *cerebellar ataxia* is shown in eTable 6 in the Supplement and would be further expanded with genes derived from additional keywords and additional contributions to these databases over time. This initial prioritization of genetic variants included the previous classification of patients based on their most prominent neurologic symptom (80% ataxia, 61 of 76; 17% spasticity, 13 of 76; and 3% parkinsonism, 2 of 76) and additional phenotypic features (eTables 1–3 in the Supplement). All data sets were annotated for previously reported disease-causing variants using the Human Gene Mutation Database Professional Version. Variants were also analyzed using the following predictive software: SIFT,[21] Condel,[22] and PolyPhen-2,[23] although these data were not used to exclude any variants. Conservation at the base position was analyzed using GERP[24] and conservation at the amino acid level was checked in orthologs. In some cases, CES was also performed on 1 or more family members and used to verify allelic segregation or whether a variant was inherited or de novo (eTables 1–3 in the Supplement). For each case, all this collective information was reviewed by at least 1 bioinformatics specialist (H.L. or S.P.S.) and 1 neurogenetics specialist (B.L.F.) to correlate genomic data to clinical phenotype. Genotype/phenotype correlation was based on the most current diagnostic criteria available in the medical literature for each given gene and included an allowance for presentations with unanticipated phenotypic variability. Because of potential inaccuracies in reporting, except for cases in which additional family members were clinically examined, a family history of symptoms was not used to exclude variants with potentially inconsistent modes of inheritance. These highlighted variants were further reviewed by members of the multidisciplinary UCLA Genomics Data Board to obtain a consensus opinion for each case. Single nucleotide variants with suboptimal quality[16] and all insertion/deletions were confirmed by Sanger sequencing (primers available on request).

Statistical Analysis

Statistical comparisons were performed using standard tests of the normal distribution with a 5% level of significance.

Results

To assess the value of CES in patients with cerebellar ataxia, we systematically examined a consecutive series of patients referred to our tertiary ataxia center. On clinical examination, all patients had a chronic progressive gait disorder and a gait and/or limb ataxia. To be eligible for CES, a full diagnostic workup for acquired etiologies was required, along with basic screening for common repeat expansion disorders (SCA1, SCA2, SCA3, SCA6, SCA7, and Friedreich ataxia), representing an estimated 40% to 50% of genetic ataxias worldwide.[2–5] Those patients whose illness remained undiagnosed underwent CES (n = 76). To obtain as broad a perspective as possible for the use of CES, family history and age at onset were not considered as criteria for performing genomic analysis. Overall, 55% of the cohort was female (42 of 76) with an average (SD) age of 49 (21) years (range, 2–81 years) and primarily of European (59%, 45 of 76) and/or Hispanic (18%, 14 of 76) descent. Most of the patients had sporadic onset of symptoms (74%, 56 of 76), primarily as adults (53%, 40 of 76). Of the familial presentations (26%, 20 of 76), most also had onset in adulthood (20%, 15 of 76) (Tables 1, 2, and 3; eTables 1–3 in the Supplement).

Following CES, all sample data were examined using our bioinformatic pipeline (see the Methods section). We used a stringent clinically stratified approach to identify disease-causing variants. Our most stringent level was termed *pathogenic* (Table 1; eTable 1 in the Supplement) and equated to a confirmed molecular diagnosis of the patient's clinical phenotype. To directly qualify as pathogenic, variants had to either be previously reported as a disease mutation or cause protein truncation via a frameshift or generation of a stop codon in a previously established clinical ataxia gene. Nonsynonymous variants were further required to (1) be present at or below a defined minor allele frequency threshold (1% for a recessive model and 0.1% for dominant), (2) involve a conserved nucleotide position based on a positive GERP score,[24] (3) be classified as damaging using at least 2 of 3 bioinformatic prediction models (see the Methods section), and (4) be de novo or inherited from an affected parent (dominant model), segregate independently (recessive model, compound heterozygote), or be homozygous (recessive model, homozygous). Nonsynonymous variants that met 2 or more of these criteria, but not all, were designated as *potential pathogenic*, the equivalent of recommending further clinical, diagnostic, or, in some cases, research evaluation to confirm. We also correlated genetic variation with clinical presentation to determine whether the patient's phenotype matched all key features of the associated disease as a confirmatory measure (eTables 1 and 2 in the Supplement). To allow for clinical variability, we did not exclude any variants based on this criteria but, if there was a unique phenotypic feature(s) present, this was used to advance a potential variant to full pathogenicity status (explained further on; Tables 1 and 2; eTables 1 and 2 in the Supplement). Using this method, we obtained clinically relevant variants for 61% of the cases (46 of 76) (Tables 1 and 2; eTables 1 and 2 in the Supplement). Of these, 16 cases

were designated as pathogenic (21%, 16 of 76) (Table 1; eTable 1 in the Supplement) and another 30 as potential pathogenic (40%, 30 of 76) (Table 2; eTable 2 in the Supplement).

Of the 16 cases with pathogenic variants, most presented sporadically (69%, 11 of 16) and there were more early-onset cases observed (63%, 10 of 16). Fourteen of the cases had autosomal recessive inheritance and 2 were autosomal dominant. Two recessive genes were found in more than 1 individual, *SYNE1* (3 cases) and *SPG7* (2 cases) (Table 1). We observed 8 variants that had previously been reported in patients and another 16 that were novel across 13 disease genes, providing a clear illustration of the advantage of CES in patients with heterogeneous phenotypes encompassed by a large number of genes.

Pathogenic variants were identified in 5 genes that would not have been initially considered clinically in any of these patients based on age at onset and phenotype. Case ATX58 was found to have a novel unreported homozygous variant in *NDUFS7*, a gene previously only associated with severe mitochondrial complex I deficiency and early death.[25] Our patient, now age 14 years, had mitochondrial dysfunction confirmed on muscle biopsy (Figure 1) but has a much milder early-onset phenotype characterized primarily by spasticity, optic atrophy, white matter hyperintensities, and autism spectrum disorder (Table 1; eTable 1 in the Supplement). Case ATX26 was found to be homozygous for a known variant in *SLC52A2* associated with the severe Brown-Vialetto-Van Laere syndrome.[26,27] Our patient, now age 10 years, presented with cerebellar ataxia and sensory neuropathy, lacking most of the key features of this disorder (Table 1; eTable 1 in the Supplement) such as optic atrophy, sensorineural hearing loss, respiratory insufficiency, or motor neuropathy. This mild phenotype has not previously been reported in patients with this or other *SLC52A2* mutations.[26,27] Diagnosis was confirmed biochemically (data not shown).

Cases ATX1 and ATX48 were notable for both having mutations in different subunits of the DNA-directed RNA polymerase III (*POLR3A* and *POLR3B*, respectively), typically associated with leukodystrophy presentations.[28] Both patients had variants that were previously unreported (Table 1; eTable 1 in the Supplement). Phenotypically, both patients possessed unusual clinical features associated with mutations in these genes but not typical of other ataxic disorders[28] (hypomyelination on brain magnetic resonance imaging and oligodontia for ATX1 and hypogonadotropic hypogonadism for ATX48) (Figure 1; Table 1; eTable 1 in the Supplement). The variant found in patient ATX1 was designated as pathogenic, despite being heterozygous, based on the presence of a unique characteristic phenotype associated with this disorder (cerebellar ataxia, hypomyelination on brain magnetic resonance imaging, oligodontia, and polyneuropathy)[28] (Figure 1; Table 1; eTable 1 in the Supplement). The entire gene and its intron-exon junctions were resequenced in this patient and no additional variants were identified; therefore, we hypothesized that a second yet-to-be-identified pathogenic variant may be present in noncoding sequence.

The final unexpected pathogenic case, ATX29, had 2 previously reported disease-causing variants in *WFS1* present on the same allele in conjunction with a third novel variant on the other allele (Table 1; eTable 1 in the Supplement). Although the patient lacked early diabetes mellitus, seen in classic Wolfram syndrome or other multisystem *WFS1*-related disorders, the clinical features present, which included cerebellar ataxia, optic atrophy,

sensorineural hearing loss, dementia, and respiratory insufficiency, are common features of Wolfram syndrome,[29,30] suggestive of a variant presentation.

For the 30 cases found to have variants of potential pathogenicity, most were sporadic (73%, 22 of 30) and adult onset (77%, 23 of 30) (Table 2; eTable 2 in the Supplement). We identified 49 variants, 13 of which had been previously reported as pathogenic, in 25 disease-associated genes, predominantly of autosomal recessive inheritance (64%, 16 of 25) (Table 2; eTable 2 in the Supplement). All of the previously reported variants were found in autosomal recessive genes.

Potential compound heterozygous variants were found in 7 cases (*ZFYVE26* in ATX12; *SETX* in ATX17; *WFS1* and *DYSF* in case ATX32; *PNPLA6* in ATX52; *SYNE1* in cases ATX63 and ATX69; and *GBE1* in case ATX66) (Table 2; eTable 2 in the Supplement). In keeping with our stringently defined criteria, these cases were not considered fully pathogenic because allelic segregation could not be confirmed owing to limited availability of additional family members. Additionally, for case ATX12, public genomic data suggest the observed variants (previously reported as disease causing) may commonly co-occur in certain racial/ethnic backgrounds (Table 2; eTable 2 in the Supplement).

We identified 11 cases with novel variants in autosomal dominant genes and 2 cases with previously reported variants in recessive genes that had been associated with dominant disease (*SETX* in ATX8 and *C10ORF2* in ATX68); however, in maintaining with our stringent diagnostic criteria, we did not designate any as fully pathogenic because we could not verify inheritance from an affected parent or confirm they arose de novo (Table 2; eTable 2 in the Supplement).

Lastly, in one-half of the potential pathogenic cases (50%, 15 of 30), we identified a single heterozygous variant in a recessive gene whose phenotype could explain the clinical presentation of the case (Table 2; eTable 2 in the Supplement). While these patients might simply be carriers of the variants in question, for several patients, we held strong clinical suspicion that they may harbor a second undetected pathogenic variant on the opposite allele and, therefore, from the standpoint of a clinician, would warrant further investigation. For example, case ATX38 was found to have an unreported heterozygous frameshift variant in the *SPG11* gene and presented with cerebellar ataxia, spastic paraplegia, progressive dementia involving frontal/executive function, and pseudobulbar involvement (Table 2; eTable 2 in the Supplement)—all typical features of disease associated with that gene.[31] In this and the other cases, the finding of a presumed pathogenic variant in a highly suspicious clinical gene supports a possible diagnosis and warrants further testing for noncoding or other mutations not detected by CES (eg, copy number variation). These cases illustrate how genomic data can provide clinically useful information despite not clearly identifying the molecular etiology.

Cases ultimately having nondiagnostic results showed no difference in the proportion of patients with familial histories (23%, 7 of 30 vs 28%, 13 of 46; $P = .63$) (Table 3; eTable 3 in the Supplement), potentially reflecting the presence of novel disease genes in these individuals. We did observe a significant trend toward more adult-onset cases in this group

(87%, 26 of 30 vs 63%, 29 of 46; $P = .02$); however, we did not observe any other demographic or phenotypic features consistently associated with nondiagnostic testing results.

Discussion

In this report, we performed CES on 76 consecutive patients presenting with cerebellar ataxia to a tertiary referral center. Using a clinically stratified bioinformatic approach to facilitate identification of disease-causing sequence variation, we identified 16 cases (21%, 16 of 76) considered to have pathogenic variants that explained their disease (Table 1; eTable 1 in the Supplement) and an additional 30 cases (40%, 30 of 76) with variants of potential pathogenicity warranting further investigation (Table 2; eTable 2 in the Supplement). Overall, 61% of cases (46 of 76) yielded genetic information useful for evaluating clinicians to either establish a molecular diagnosis or to direct additional confirmatory testing. Furthermore, this work extends previous studies that successfully used CES in children with ataxia[13,14] by demonstrating success in adult-onset cases (38%, 6 of 16 pathogenic and 77%, 23 of 30 potential pathogenic; 63% of total cases, 29 of 46). Because most cases with identified genetic variants were sporadic (69%, 11 of 16 pathogenic and 73%, 22 of 30 potential pathogenic; 72% of total cases, 33 of 46), these results indicate that CES should be considered part of the routine genetic evaluation of patients with cerebellar ataxia (Figure 2).

Broadly speaking, to our knowledge, there are few clinical tests with yields this high, especially in such a diverse patient population where the underlying causes are rare, most of the diagnostic test results are negative, and the clinical work-ups can go on for years. Case ATX26 is an example of the clinical benefits of genomic testing. This patient presented with a phenotype most reminiscent of Friedreich ataxia, and the ultimate causative gene, *SLC52A2* (Table 1; eTable 1 in the Supplement), would not have been foremost on any clinical algorithm.[2–4] Rapid identification of this mutation was critical for this patient because this is a potentially treatable disorder.[26,27] Immediately on diagnosis, our patient started treatment with daily oral riboflavin, with normalization of all biochemical abnormalities (data not shown) and has been clinically stable for more than 12 months.

The most common rare ataxia gene detected in this study was *SYNE1*. We identified 3 pathogenic cases (cases ATX6, ATX18, and ATX35) (Table 1; eTable 1 in the Supplement) and 2 additional potential pathogenic cases (ATX63 and ATX69) (Table 2; eTable 2 in the Supplement). The classic phenotype associated with this gene is an autosomal recessive adult-onset pure cerebellar ataxia,[3,4,32] a presentation commonly seen in sporadic cases presenting to an ataxia clinic.[10] Because of the large size of this gene with 146 exons in the canonical transcript (ENST00000423061; Ensembl database; http://www.ensembl.org/), next-generation sequencing is the only cost-effective means for routine clinical screening.[10] Previously only reported in the French-Canadian population, more widespread sequencing efforts have identified additional cases from France (1 case), Brazil (1 case), and Japan (3 cases),[32–34] consistent with the observation of multiple cases in this cohort and suggesting this to be a worldwide disorder with higher prevalence than previously known.

Targeted next-generation sequencing approaches have been suggested as a more cost-effective means of evaluating patients with ataxia by limiting the amount of bioinformatic analysis required.[35] However, as this study demonstrated, it is possible to effectively evaluate this degree of variation using a comprehensive bioinformatic approach correlating exome sequencing findings with clinical presentation. Furthermore, this method of diagnostic testing is cost-effective as the identical exome sequence pipeline reported here costs approximately US $4500 (including bioinformatic analysis), whereas some of the larger Sanger sequencing gene panels currently offered commercially can cost up to approximately US $30 000. Targeted sequencing panels could cost less at approximately US $2000 but would examine 2 orders of magnitude fewer genes (about 200 vs 21 000), reducing the cost advantage.[35] Furthermore, targeted sequencing approaches depend on previously reported clinical findings to select key genes to target. As more and more exome sequences are performed, variability in presentation and expressivity associated with genes having previously defined phenotypes will likely become even more apparent. As we showed, several very rare and unexpected diagnoses were made based on CES in this initial cohort of only 76 patients, emphasizing clearly the power of this approach relative to any other. Previous next-generation sequencing studies in patients with ataxia have already noted such variant phenotypes as well.[14,35] In this study, it was unlikely that 5 pathogenic cases (31%, 5 of 16) would have been identified were analysis focused solely on disorders known to cause primary cerebellar ataxia (cases ATX1, ATX26, ATX29, ATX48, and ATX58) (Table 1; eTable 1 in the Supplement). Targeted approaches lack the power to identify unexpected novel or extremely rare presentations in genes associated with more common phenotypes that do not include a primary ataxia. Vetting of identified genetic variants by clinicians with experience diagnosing the relevant phenotypes is important in establishing genotype-phenotype correlations and is a recommended step in the exome diagnostic pipeline, particularly when considering previously unreported variants of uncertain pathogenicity.

The analytic strategy used here has been demonstrated as effective in the identification of pathogenic variants in genes associated with cerebellar ataxia in patients exhibiting typical and related phenotypes. The disadvantage to this method is that the full spectrum of phenotypic variability and variable expressivity for many genetic disorders is not yet known. Therefore, it is possible that pathogenic variants may be missed because the observed phenotype is quite different than expected. We have attempted to mitigate this possibility by using data review by multiple bioinformatic, neurogenetic, and ataxia specialists. Furthermore, although the most common repeat expansion disorders were ruled out in these patients, it is possible that cases with a rarer expansion disorder or a DNA structural variation (eg, copy number variation) could be missed as these would not be detected by the next-generation sequencing methods used.[8] Some variants might also be missed owing to technical limitations in the CES method, which prevents sequencing of 100% of some exons in certain genes. Additional cases may also be missed because of mutation of genes not yet associated with a clinical phenotype or, in some cases, a human disease (eg, case ATX63; Table 2; eTable 2 in the Supplement). For cases of uncertain pathogenicity or non-diagnostic findings, reexamination of the exome results at regular intervals may yield new diagnostic clues over time as new genetic information is added to clinical databases and bioinformatic

prediction methods improve. The integration of CES with research programs at academic institutions may facilitate the discovery of novel disease genes in families with non-diagnostic results. Lastly, the clinical use of whole-genome sequencing will likely also add benefit by potentially improving exon capture statistics and enabling the detection of noncoding pathogenic variation.[35] Given the already high diagnostic rate observed here in this predominantly adult- and sporadic-onset cohort, this is particularly encouraging for the future of genetic diagnostic evaluation.

Conclusions

The clinical use of next-generation exome sequencing is becoming more widespread; however, there is limited information available to direct clinicians in identifying which patients would most benefit from such testing. Our findings suggested that patients with chronic progressive cerebellar ataxia would benefit diagnostically from exome sequencing irrespective of a positive family history or early age at onset. We further suggest strategies for the integration of genomic testing into the clinical evaluation and effective bioinformatic methods of data analysis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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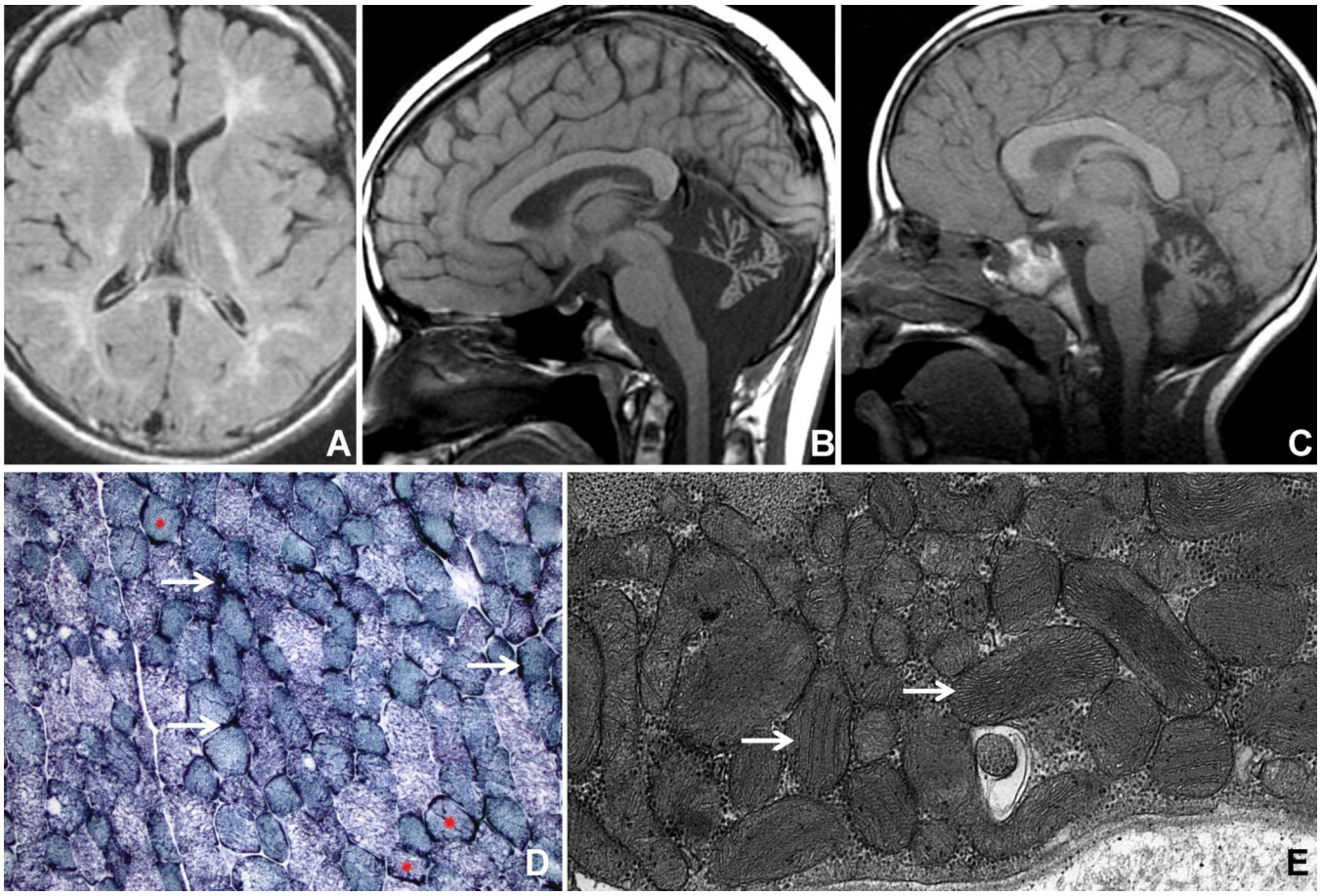

Figure 1. Neuroimaging and Pathology From Selected Cases With Pathogenic Variants

A, Case ATX1. T2 fluid-attenuated inversion recovery axial imaging shows diffuse white matter hyperintensities in a patient with disease due to *POLR3A* mutation. B, Case ATX48. T1 sagittal imaging shows severe cerebellar atrophy in a patient with disease due to *POLR3B* mutation. C, Case ATX67. T1 sagittal imaging shows cerebellar atrophy in a patient with disease due to a de novo mutation in *ITPR1*. Mitochondrial disease due to *NDUFS7* mutation in case ATX58 (D and E). D, Subsarcolemmal linear aggregates are seen with the oxidative enzyme SDH (original magnification ×20, arrowheads). Occasional small ragged blue fibers are also seen (asterisks). E, Electron microscopy demonstrates aggregates of subsarcolemmal mitochondrial hyperplasia with pleioconia, occasional megaconial forms, abnormal cristae architecture, and crystalline inclusions (original magnification ×25 000, arrowheads).

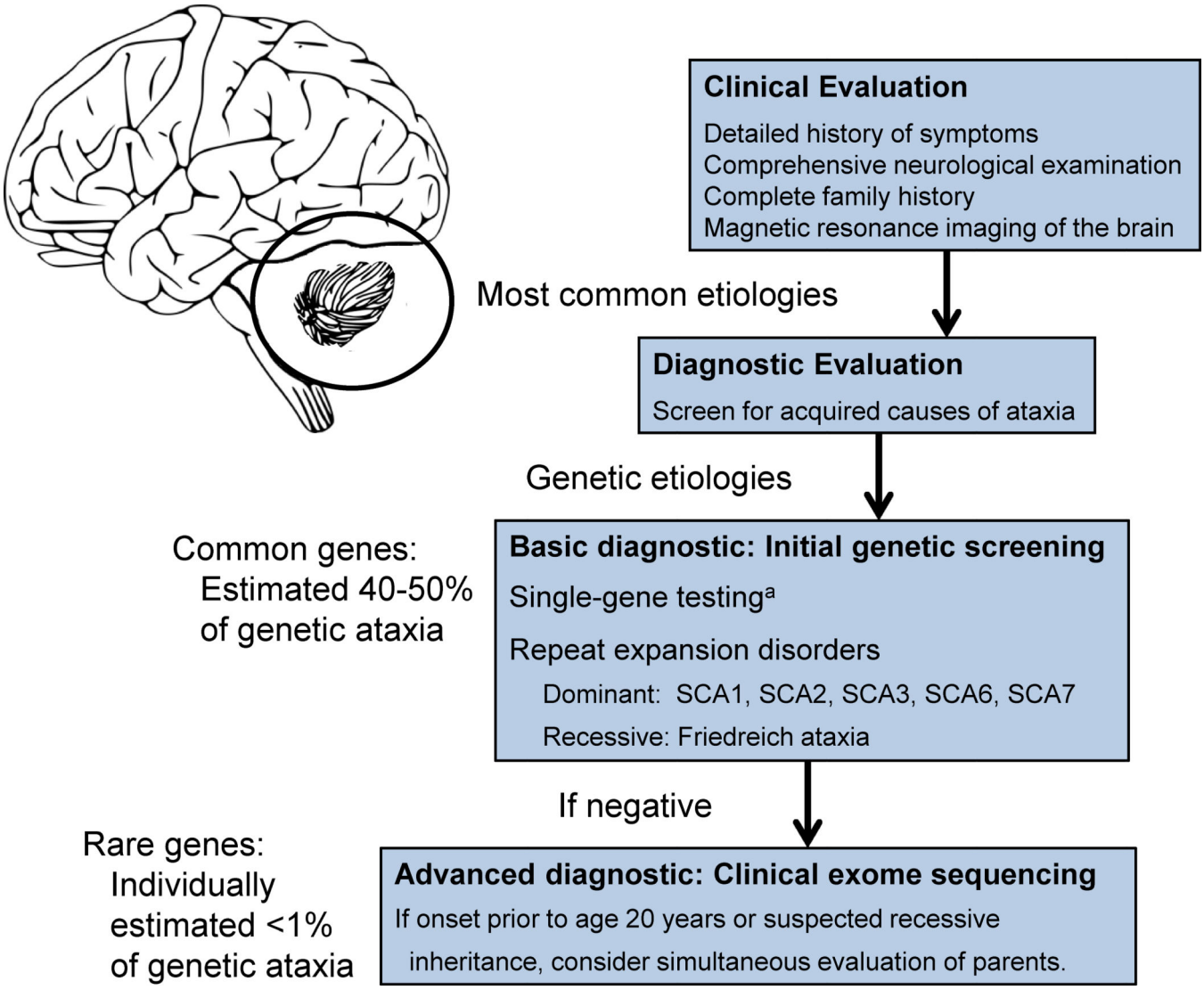

Figure 2. Diagnostic Evaluation for Patients Presenting With Chronic Progressive Cerebellar Ataxia

A general flowchart for the clinical evaluation of a patient presenting with chronic progressive cerebellar ataxia. Initial diagnostic testing should address acquired etiologies because these are more common than genetic causes and often treatable or modifiable. Once acquired conditions are ruled out, a more formal genetic evaluation would include basic testing for either high-yield single genes or, if presentation is sporadic, the most common genetic causes worldwide. If nondiagnostic, a more detailed genetic evaluation is recommended using clinical exome sequencing for rare genetic causes or variant presentations of other disorders. Trio testing of parents and probands may be useful in cases of early-onset (at or before age 20 years) or suspected recessive inheritance to evaluate allelic segregation or de novo mutation. [a]Single-gene testing for high-yield disorders based on clinical phenotype or family history. If initial differential includes strong consideration of multiple single genes, exome sequencing is likely preferable because of cost.

SCA indicates spinocerebellar ataxia.

Table 1

Cases With Pathogenic Variants Identified by Clinical Exome Sequencing

Patient No./Sex/Age, y	Family History	Primary Symptom	Phenotype	Gene	Inheritance	mRNA	Protein	OMIM Disease Phenotype
ATX1/M/22	Sporadic	Ataxia	EO, MHA, OLG, PN, UMN, WMA	*POLR3A*	Recessive[a]	c.2521G>A	p.Gly841Ser	607694
ATX6/M/45	Sporadic	Ataxia	PCA	*SYNE1*	Recessive	c.9646A>T c.4482+1G>T	p.Lys3216* p.?[b]	610743
ATX9/M/51	Affected sister	Ataxia	PCA	*ANO10*	Recessive	c.123_124insA homozygous	p.Asp45Argfs*9	613728
ATX18/F/49	Sporadic	Ataxia	PCA	*SYNE1*	Recessive	c.20050C>T c.7938G>A	p.Arg6684* p.Trp2646*	610743
ATX26/F/9	Sporadic	Ataxia	EO, PN	*SLC52A2*	Recessive	c.916G>A[c] homozygous	p.Gly306Arg[c]	607882
ATX29/M/16	Affected sister	Ataxia	DEM, EO, OA, SHL	*WFS1*	Recessive	c.683G>A c.1495C>T[c] c.2335G>A[c]	p.Arg228His p.Leu499Phe[c] p.Val779Met[c]	222300
ATX35/F/57	Sporadic	Ataxia	PCA	*SYNE1*	Recessive	c.3417-10_3417delinsC homozygous	p.?[b]	610743
ATX43/M/50	Affected brother	Spasticity	PN, WMA	*SPG7*	Recessive	c.1529C>T[c] c.2120_2121delTins CCAAGTCTGTA	p.Ala510Val[c] p.Val707Alafs*38	607259
ATX48/F/16	Sporadic	Ataxia	EO, HGH	*POLR3B*	Recessive	c.1244T>C c.3052G>A	p.Met415Thr p.Asp1018Asn	614381
ATX58/M/13	Sporadic	Spasticity	EO, OA, PSY, WMA	*NDUFS7*	Recessive	c.313C>T homozygous	p.Arg105Cys	256000
ATX59/M/33	Sporadic	Ataxia	EO, PN	*SETX*	Recessive	c.6106G>A[c] c.7149_7151delinsAT	p.Gly2036Arg[b,c] p.Asp2383Glufs*26	606002
ATX60/M/26	Sporadic	Ataxia	EO, UMN	*SPG7*	Recessive	c.1729G>A[c] homozygous	p.Gly577Ser[c]	607259
ATX67/F/20	Sporadic	Ataxia	EO, ID	*ITPR1*	Dominant	c.830G>T	p.Ser277Ile	117360
ATX70/F/37	Affected brother	Ataxia	EO, EP, OMA	*MRE11A*	Recessive	c.497C>T c.168G>T	p.Pro166Leu p.Leu56Phe	604391
ATX72/M/16	Affected mother	Spasticity	EO, PN	*SPAST*	Dominant	c.1409A>C[c]	p.Asp470Ala[c]	182601
ATX76/M/62	Sporadic	Ataxia	PN, WMA	*GBE1*	Recessive	c.986A>C[c] homozygous	p.Tyr329Ser[c]	263570

Abbreviations: DEM, dementia; EO, early onset (≤age 20 years); EP, extrapyramidal features; F, female; HGH, hypogonadotropic hypogonadism; ID, intellectual disability; M, male; MHAs, migraine headaches; mRNA, messenger RNA; OA, optic atrophy; OLG, oligodontia; OMA, oculomotor apraxia; OMIM, Online Mendelian Inheritance in Man; PCA, pure cerebellar ataxia; PN, polyneuropathy; PSY, psychiatric symptoms; SHL, sensorineural hearing loss; UMN, upper motor neuron features; WMAs, white matter hyperintensities on brain magnetic resonance imaging.

[a] Only single heterozygous coding variant identified for a recessive disorder, second mutation presumed to be noncoding.

[b] Variant predicted to disrupt normal RNA splicing.

[c] Variant previously reported as pathogenic (Human Gene Mutation Database Professional Version, http://www.hgmd.org/).

Table 2

Cases With Variants of Uncertain Significance and/or Potential Pathogenicity Identified by Clinical Exome Sequencing

Patient No./Sex/Age, y	Family History	Primary Symptom	Phenotype	Gene	Inheritance	mRNA	Protein	OMIM Disease Phenotype
ATX5/F/62	Sporadic	Ataxia	PN	*WFS1*	Recessive[a]	c.1672C>T[b]	p.Arg558Cys[b]	222300
ATX8/F/20	Sporadic	Ataxia	EO, PCA	*SETX*	Recessive[c]	c.1957C>A[b] c.1807A>G[b]	p.Gln653Lys[b] p.Asn603Asp[b]	606002
ATX10/F/22	Sporadic	Ataxia	DEM, EO, WMA	*GRID2*	Recessive[a]	c.989C>T	p.Thr330Met	No entry
ATX12/F/61	Affected twin	Spasticity	GA	*ZFYVE26*	Recessive[d]	c.5612G>A[b] c.1844C>T[b]	p.Cys1871Tyr[b] p.Ser615Phe[b]	270700
ATX13/M/32	Sporadic	Ataxia	ID	*AFG3L2*	Dominant	c.292G>C	p.Glu98Gln	610246
ATX17/F/29	Sporadic	Ataxia	EO, PN	*SETX*	Recessive	c.6536T>G c.7366G>A	p.Ile2179Ser p.Ala2456Thr	606002
ATX21/F/47	Sporadic	Ataxia	PN, UMN	*TGM6*	Dominant	c.516A>G	p.Ile172Met	613908
ATX24/F/61	Sporadic	Ataxia	PCA	*GRID2*	Recessive[a]	c.52T>C	p.Trp18Arg	No entry
				SPTBN2	Dominant	c.2161C>A	p.Arg721Ser	600224
				WFS1	Recessive[a]	c.1058C>T	p.Ser353Phe	222300
ATX27/F/29	Sporadic	Ataxia	UMN	*SPTBN2*	Dominant	c.2459C>T	p.Thr820Met	600224
ATX30/F/65	Sporadic	Ataxia	PCA	*CACNA1A*	Dominant	c.903C>G	p.Phe301Leu	601011
				WFS1	Recessive[a]	c.1705-1706GC>AG	p.Ala569Ser	222300
ATX32/F/81	Sporadic	Ataxia	PCA	*DYSF*	Recessive	c.383G>A[b] c.3967C>G[b]	p.Gly128Glu[b] p.Gln1323Glu[b]	253601
				WFS1	Recessive	c.728C>T c.1366C>T	p.Ala243Val p.Arg456Cys	222300
ATX34/M/54	Sporadic	Ataxia	DEM, UMN, WMA	*LRRK2*	Dominant	c.5417C>T	p.Thr1806Ile	607060
ATX36/M/54	2 Affected brothers	Ataxia	EP, PSY, UMN	*WFS1*	Recessive[a]	c.1294C>G[b]	p.Leu432Val[b]	222300
ATX37/F/10	Sporadic	Ataxia	EO, PN	*WFS1*	Recessive[a]	c.694C>T	p.Arg232Cys	222300
ATX38/M/74	Affected son	Ataxia	DEM, SZ	*SPG11*	Recessive[a]	c.5456_5457delAG[b]	p.Glu1819Alafs*10[b]	604360
ATX39/M/72	Affected sibling, father	Parkinsonism	EP, PN	*LRSAM1*	Dominant	c.2068T>C	p.Cys690Arg	614436
				DYSF	Recessive[a]	c.3067C>T	p.Arg1023Trp	253601

Patient No./Sex/Age, y	Family History	Primary Symptom	Phenotype	Gene	Inheritance	mRNA	Protein	OMIM Disease Phenotype
ATX46/M/44	Affected brothers	Spasticity	EO, EP, GA	*KIF1A*	Recessive[a]	c.4672G>A	p.Glu1558Lys	610357
ATX52/M/58	Sporadic	Spasticity	UMN	*PNPLA6*	Recessive	c.1340C>T c.3598C>G	p.Pro447Leu p.Gln1200Glu	612020
ATX54/F/52	Sporadic	Ataxia	DEM	*TGM6*	Dominant	c.1090G>A	p.Glu364Lys	613908
				PSEN1	Dominant	c.1085A>C	p.Gln362Pro	600274
ATX55/F/51	Sporadic	Ataxia	MHA	*PDYN*	Dominant	c.79T>G	p.Ser27Ala	610245
ATX61/M/36	Affected father	Spasticity	EO, WMA	*B4GALNT1*	Recessive[a]	c.1577G>A	p.Arg526Gln	609195
				WFS1	Recessive[a]	c.1675G>A[b]	p.Ala559Thr[b]	222300
ATX62/M/70	Sporadic	Ataxia	PN	*AMACR*	Recessive[a]	c.554T>C[b]	p.Val185Ala[b]	614307
ATX63/M/2	Sporadic	Ataxia	EO, episodic	*KLHL1*	Recessive[a,e]	c.241T>G	p.Ser81Ala	No entry
				SYNE1	Recessive	c.24617G>C c.11181G>T	p.Ser8206Thr p.Met3727Ile	610743
ATX64/M/71	Sporadic	Ataxia	EP, PN	*EIF4G1*	Dominant	c.1471A>T	p.Ile491Phe	614251
ATX66/F/60	Multiple members with diverse symptoms	Ataxia	DEM, PN, SZ	*GBE1*	Recessive	c.1134T>G c.118C>A	p.Ser378Arg p.Pro40Thr	263570
ATX68/F/81	Sporadic	Ataxia	PN	*PRX*	Recessive[a]	c.4193C>A	p.Ala1398Asp	605725
				C10ORF2	Recessive[a,f]	c.1120C>T[b]	p.Arg374Trp[b]	271245
ATX69/M/68	Sporadic	Ataxia	PN	*SYNE1*	Recessive	c.17016T>G c.3209T>C	p.Tyr5672* p.Val1070Ala	610743
ATX73/F/61	Sporadic	Ataxia	UMN	*SPG7*	Recessive[a]	c.1948G>A	p.Asp650Asn	607259
ATX74/M/72	Sporadic	Ataxia	PN	*TGM6*	Dominant	c.31T>G	p.Trp11Gly	613908
ATX75/M/55	Affected mother, 3 siblings	Ataxia	PN	*SPG7*	Recessive[a]	c.1045G>A[b]	p.Gly349Ser[b]	607259
				WFS1	Recessive[a]	c.1364C>T	p.Thr455Met	222300

Abbreviations: DEM, dementia; EO, early onset (≤ age 20 years); EP, extrapyramidal features; F, female; GA, gait ataxia; ID, intellectual disability; M, male; MHA, migraine headaches; mRNA, messenger RNA; OMIM, Online Mendelian Inheritance in Man; PCA, pure cerebellar ataxia; PN, polyneuropathy; PSY, psychiatric symptoms; SZ, epilepsy; UMN, upper motor neuron features; WMAs, white matter hyperintensities on brain magnetic resonance imaging.

[a] Single heterozygous coding variant identified for a recessive disorder, second mutation (if present) presumed to be noncoding. We cannot rule out the possibility these patients are carriers of a single recessive allele.

[b] Variant previously reported as pathogenic (Human Gene Mutation Database Professional Version, http://www.hgmd.org/).

[c] These compound *SETX* variants are in *cis* and previously reported to act dominantly.

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[d] These *ZFYVE26* variants have been observed together in control samples of the same genetic background.

[e] *KLHL1* causes ataxia in an animal model; no known human disease yet identified.

[f] This *C10ORF2* variant is reported to cause dominant disease.

Table 3

Cases With Nondiagnostic Results Following Exome Sequencing

Patient No./Sex/Age, y	Family History	Primary Symptom	Phenotype
ATX2/M/54	Sporadic	Ataxia	PCA
ATX3/F/77	Sporadic	Ataxia	PN
ATX4/M/59	Sporadic	Ataxia	UMN
ATX7/F/24	Sporadic	Ataxia	ID, PN, PSY, WMA
ATX11/F/41	Affected brother	Ataxia	UMN
ATX14/F/60	Sporadic	Ataxia	WMA
ATX15/M/40	Sporadic	Ataxia	EP, PN
ATX16/F/62	Sporadic	Ataxia	UMN
ATX19/F/35	Sporadic	Ataxia	EO
ATX20/F/46	Sporadic	Ataxia	DEM, EP, UMN, WMA
ATX22/M/69	Sporadic	Ataxia	PN
ATX23/F/39	Sporadic	Spasticity	UMN
ATX25/M/52	Sporadic	Spasticity	UMN
ATX28/F/61	Sporadic	Ataxia	PSY
ATX31/M/72	Sporadic	Ataxia	PN
ATX33/F/23	Sporadic	Ataxia	EO
ATX40/F/73	Multiple members with diverse symptoms	Ataxia	EP, WMA
ATX41/F/69	Sporadic	Ataxia	WMA
ATX42/M/78	Sporadic	Ataxia	PCA
ATX44/M/66	Affected sister	Ataxia	PN
ATX45/F/78	Affected brother	Ataxia	DEM, EP, WMA
ATX47/F/55	Sporadic	Spasticity	PN
ATX49/F/51	Sporadic	Ataxia	PCA
ATX50/F/65	2 Affected sisters	Ataxia	MHA
ATX51/F/70	Sporadic	Parkinsonism	PCA
ATX53/F/73	Affected mother	Ataxia	PN
ATX56/M/14	Sporadic	Spasticity	EO
ATX57/F/23	Sporadic	Ataxia	EO, ID, PSY
ATX65/F/57	Affected mother, daughter	Spasticity	PN
ATX71/M/60	Sporadic	Spasticity	PN, WMA

Abbreviations: DEM, dementia; EO, early onset (≤ age 20 years); EP, extrapyramidal features; F, female; ID, intellectual disability; M, male; MHA, migraine headaches; PCA, pure cerebellar ataxia; PN, polyneuropathy; PSY, psychiatric symptoms; UMN, upper motor neuron features; WMA, white matter hyperintensities on brain magnetic resonance imaging.