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A diagnostic ceiling for exome sequencing in cerebellar ataxia and related neurological disorders

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Abstract

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Genetic ataxias are associated with mutations in hundreds of genes with high phenotypic overlap complicating the clinical diagnosis. Whole-exome sequencing (WES) has increased the overall diagnostic rate considerably. However, the upper limit of this method remains ill-defined, hindering efforts to address the remaining diagnostic gap. To further assess the role of rare coding variation in ataxic disorders, we reanalyzed our previously published exome cohort of 76 predominantly adult and sporadic-onset patients, expanded the total number of cases to 260, and introduced analyses for copy number variation and repeat expansion in a representative subset. For new cases ($n = 184$), our resulting clinically relevant detection rate remained stable at 47% with 24% classified as pathogenic. Reanalysis of the previously sequenced 76 patients modestly improved the pathogenic rate by 7%. For the combined cohort ($n = 260$), the total observed clinical detection rate was 52% with 25% classified as pathogenic. Published studies of similar neurological phenotypes report comparable rates. This consistency across multiple cohorts suggests that, despite continued technical and analytical advancements, an approximately 50% diagnostic rate marks a relative ceiling for current WES-based methods and a more comprehensive genome-wide assessment is needed to identify the missing causative genetic etiologies for cerebellar ataxia and related neurodegenerative diseases.

1 | INTRODUCTION

Hereditary spinocerebellar ataxia (dominant SCA and recessive SCAR) and spastic paraplegia (HSP) are neurodegenerative disorders affecting the cerebellum, its pathways, and the corticospinal tracts that can result from mutations in one of the hundreds of genes (Online Mendelian Inheritance in Man [OMIM], https://www.omim.org/; OMIM, 2019). Both disorders manifest high degrees of phenotypic heterogeneity even with common specific causal mutations, necessitating genomic testing strategies to identify the relatively rare causal mutations that are pervasive in both disorders and heterogeneous in presentation (Anheim, Tranchant, & Koenig, 2012; Benini, Ben Amor, & Shevell, 2012; Brusse, Maat-Kievit, & van Swieten, 2007; Fogel & Perlman, 2006, 2007, 2011; Fogel, Satya-Murti, & Cohen, 2016; Klockgether, 2010; Manto & Marmolino, 2009). Both disorders include seemingly sporadic confirmed genetic causes at relatively high rates (~25%) in otherwise undiagnosed cases, commonly due to recessive inheritance, de novo mutations, or anticipation (Fogel et al., 2014; Nibbeling et al., 2017; Ohba et al., 2013; Pyle et al., 2015; Sawyer et al., 2013). In the last 5 years, whole-exome sequencing (WES) has come to the forefront of testing cases with suspected genetic causes of spinocerebellar ataxia or spastic paraplegia once the more common repeat expansion mutations have been ruled out (Fogel et al., 2014). WES offers cost-effective broad-coverage testing of almost all known coding variants due to single-nucleotide changes, small insertions/deletions, or proximal splice site variants (Fogel et al., 2016; Rexach, Lee, Martinez-Agosto, Nemeth, & Fogel, 2019). It also offers opportunities for the simultaneous analysis of comparator DNA (i.e., from parents or siblings) to facilitate identification of de novo or compound heterozygous mutations and rule

out rare familial benign polymorphisms based on segregation with disease status. Our initial studies identified pathogenic/likely pathogenic variants in 21% (16/76) of properly selected ataxia/spasticity patients with an additional 40% (30/76) with variants of uncertain significance (VUS) requiring clinical follow-up (Fogel et al., 2014; Richards et al., 2015). Other more recent studies examining similar cohorts have increased this overall detection rate in hereditary ataxias to approximately 40–50% (Farwell et al., 2015; Nibbeling et al., 2017; Sawyer et al., 2013; Sun et al., 2018). Diagnostic rates are frequently higher in patients with positive family histories, when the probability for identifying a monogenic disorder is highest, and when multiple family members are available for testing, where interpretation of variants is improved by segregation relationships (Farwell et al., 2015; Fogel et al., 2014; Sawyer et al., 2013). Here we present a more extensive follow-up study with a larger cohort consisting primarily of adult-onset sporadic ataxia and spastic paraplegia cases with suspicion for a possible genetic etiology. Our study was intended to maximize diagnostic potential using genomic technologies including an assessment of copy number variation (CNV) and repeat expansion in a representative subset of the cohort. Additionally, we reanalyzed our previously reported cases to assess the effect of interval advances in variant annotation and gene discovery. Finally, we compare our results with other published studies to assess the overall diagnostic capability of current next-generation sequencing and WES pipelines and discuss approaches to further improvement of patient diagnosis.

2 | METHODS

2.1 | Patient enrollment and clinical assessment

This study comprises 184 index patients with a phenotypic range of either pure cerebellar ataxia, spasticity, or complex neurologic disorders linked to either condition. All patients had an extensive clinical evaluation to rule out the acquired causes of ataxia (Fogel et al., 2014). To qualify for this study, patients were required to have negative test results for the most common repeat expansion disorders (SCA1, SCA2, SCA3, SCA6, SCA7, and Friedreich ataxia) causing hereditary cerebellar ataxia (Fogel & Perlman, 2006, 2011; Fogel, Vickrey, Walton-Wetzel, Lieber, & Browner, 2013; Shakkottai & Fogel, 2013). Genetic counseling was provided for all patients both before and following the completion of the study. All patients enrolled in this study provided written informed consent. All methods in this study were approved by the Institutional Review Board of the University of California at Los Angeles.

2.2 | Exome sequencing and data analysis

DNA samples were collected from the index patient and their family members based on family history and individual availability for exome sequencing. Exome capture was performed with commercially-available kits and sequencing was performed on the Illumina HiSeq platform with paired-end reads (Table S1). WES data analysis was conducted based on the Broad Institute's Genome Analysis Toolkit (GATK3) version 3 best practices guidelines (DePristo et al., 2011; McKenna et al., 2010; Van der Auwera et al., 2013). Sequencing reads were mapped to the human genome (hs37d5) using the Burrows–Wheeler Aligner (Li & Durbin, 2009) and postprocessed with SAMtools (Li et al., 2009). Picard

Tools (https://broadinstitute.github.io/picard/) was used to compute sequence alignment metrics and mark duplicate reads. The Qualimap tool was used to evaluate sequence alignment quality (Garcia-Alcalde et al., 2012; Okonechnikov, Conesa, & Garcia-Alcalde, 2015). The mean coverage of the protein-coding RefSeq genes was 94.3× with standard deviation 22.3× (range 50.3× to 204.5×). GATK was used for indel realignment, base quality score recalibration, joint genotyping, variant quality score recalibration, variant evaluation, and variant selection. Variant evaluation and selection were based off NCBI Reference Sequence Database RefSeq (https://www.ncbi.nlm.nih.gov/refseq; O'Leary et al., 2016) exon intervals. Variants were annotated with either the SNP & Variation Suite v8 or VarSeq v1 (both from Golden Helix, Inc., Bozeman, MT, www.goldenhelix.com). The Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org/) and the Genome Aggregation Database (gnomAD; http://gnomad.broadinstitute.org/) public databases were used to filter for variants with a minor allele frequency 2% (Lek et al., 2016). Variants that were found to be disproportionately common among internal controls (e.g., batch effects) were excluded from the analysis. Phenotypic keywords provided by clinicians were used to generate gene lists from the OMIM (https://www.omim.org/; OMIM, 2019) and Human Gene Mutation Database Professional Version (HGMD; https://www.qiagenbioinformatics.com/products/human-gene-mutation-database/; Stenson et al., 2014) databases. Variants found within exons and splice regions of genes from these gene lists were first assessed for their clinical significance as previously described (Fogel et al., 2014; Richards et al., 2015). Variants were designated as pathogenic or likely pathogenic based on information from clinical databases such as HGMD, ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), or reports from published studies. Novel variants have been submitted to ClinVar as clinically appropriate. Comparator WES data were incorporated into the analysis when available (Table S2). If available, variants within linkage peaks (see below) were also prioritized for initial analysis. Subsequently, variants outside of the gene lists and linkage peaks were then analyzed. Variant classification and interpretation were based on the American College of Medical Genetics and Genomics guidelines (Richards et al., 2015). Sanger sequencing was used to confirm variant segregation with disease status if family members were available. Identified variants that had <Q500 were confirmed by Sanger sequencing (Strom et al., 2014). Statistical analysis of comparative diagnostic efficacy was performed using data from multiple previously published studies (Table S3).

2.3 | Array genotyping and pedigree verification

Data from Illumina Infinium Human Exome v1–2 array and Human CytoSNP-12v1–0_D BeadChips (both Illumina, San Diego, CA) were generated for linkage analysis for selected index patients and their family members. The quality assessment included confirmation of sample identity and purity using the Error Rate In Sequencing (ERIS) pipeline. An "e-GenoTyping" approach was used to screen all sequence reads for exact matches to probe sequences defined by the variant and position of interest. Samples that passed quality control metrics of ERIS single nucleotide polymorphism (SNP) array concordance (>90%) and ERIS average contamination rate (<5%) were carried forward into quality filtering, pedigree validation, and linkage analysis. Insertions/deletions and nonautosomal polymorphisms and variants without an rs identifier were all removed. SNPs that had high missingness or mapped to identical locations (duplicates) were also removed. The prePRIMUS QC pipeline

was used to estimate pairwise kinship and PRIMUS (Staples et al., 2014; Staples et al., 2016) was used to reconstruct and validate pedigrees. In the absence of array data, pedigree verification was approximated off WES variant calls by calculating the unadjusted Ajk statistic (Yang et al., 2010) with the relatedness algorithm from VCFtools (Danecek et al., 2011). Patient-reported sex was verified by exome data coverage of the sex chromosomes. Two sample swaps were confirmed and the corresponding pedigrees were updated for analysis.

2.4 | Linkage analysis

Multifamily parametric linkage analysis was conducted with ALLEGRO (Gudbjartsson, Jonasson, Frigge, & Kong, 2000) using either a fully penetrant dominant model with no phenocopies (f_0, f_1, $f_2 = 0,1,1$) or a recessive model (f_0, f_1, $f_2 = 0,0,1$) corresponding to patterns of affection in the pedigree to identify overlapping haplotypes shared identically by descent (IBD). Identified candidate regions where IBD sharing was consistent with the model of inheritance for the disease were then used to prioritize variants for subsequent WES analyses. Because of power, the logarithm of the odds scores generated from these analyses did not meet genome-wide significance thresholds and thus was not considered.

2.5 | CNV analysis

CNV analysis was conducted off WES data with read depth approaches using both Copy Number Inference From Exome Reads (CoNIFER; Krumm et al., 2012; O'Roak et al., 2012) and HMZDelFinder (Gambin et al., 2016) as well as exome hidden Markov Model (XHMM; Fromer & Purcell, 2014; Fromer et al., 2012; Poultney et al., 2013). A total of 53 families from the expanded cohort that had WES on one the following captured kits: Nextera Rapid Capture Exome, NimbleGen Seqcap EZ GSC VCRome, or Agilent SureSelect Human All Exon V4 Capture (Table S1) were selected for CNV analysis. In addition, 15 families from the original cohort (Fogel et al., 2014) that had WES on either Nextera Rapid Capture Exome or NimbleGen Seqcap EZ GSC VCRome capture kits were also included for CNV analysis. Cases were grouped by their capture kit platform for analysis. CNV calls in low complexity regions were removed. Intersecting CNV calls between CoNIFER and XHMM as well as CNV calls from HMZDelFinder were analyzed. All CNV calls were compared to the following CNV databases: ExAC CNV (Ruderfer et al., 2016), Database of Genomic Variant (DGV; MacDonald, Ziman, Yuen, Feuk, & Scherer, 2014) and DECIPHER (Firth et al., 2009). CNV calls that were present in multiple studies in DGV were classified as likely benign. CNV calls that were found in ExAC CNV were evaluated based on population and frequency information. If appropriate, biallelic inheritance was considered. CNV calls that were found in DECIPHER were evaluated based on reported phenotypes, overlapping gene annotation, and pathogenicity/contribution.

2.6 | Short tandem repeat (STR) expansion detection analysis

STR expansion screening was conducted with STRetch (Dashnow et al., 2018) on the same families as the CNV analysis described above. BAM files were grouped by corresponding capture kit platforms and processed through the STRetch_exome_bam_pipeline.groovy pipeline. The EXOME_TARGET parameter was configured to the corresponding capture kit target coordinates for each run. A bed file with all STRs defined in the human genome was

provided for the input_regions parameter. STRetch calls that had locus coverage lower than 3 and p_adj value >.05 were filtered. Only calls within genic regions were analyzed. Calls in known STR disease-causing genes were further evaluated for pathogenicity based on segregation of disease status (if applicable) and phenotype.

3 | RESULTS

Overall, 49% of the cohort was female (90/184) with an average age of 50 years (standard deviation 20 years, range 2–88 years) and primarily of European (73%) descent (Tables S4–7). The majority of the cases were sporadic (124/184, 67%) and adult-onset (127/184, 69%; Table 1). Pathogenic or likely pathogenic variants were identified in 24% (44/184, Tables 1 and 2; Table S5) and VUS were identified in 23% (43/184, Tables 1 and 3; Table S6). Because VUS require clinical follow-up (e.g., additional confirmatory diagnostic testing, if available, or subsequent bioinformatic revaluation) we combined these categories to obtain an overall rate of 47% for identification of clinically relevant variation (87/184, Tables 1–3; Tables S5–7). There was no notable difference in overall observation of clinically relevant variants between familial (43%, 26/60) or sporadic cases (49%, 61/124) or in early-onset (58%, 33/57) versus adult-onset (43%, 54/127) cases (Tables 1–3; Tables S5 and S6). Generally, as expected, more pathogenic/likely pathogenic variants were found in familial (30%, 18/60) and early-onset cases (35%, 20/57) relative to sporadic and adult-onset cases (21%, 26/124 and 19%, 24/127, respectively; Tables 1–3; Tables S5 and S6). Pathogenic/ likely pathogenic mutations were predominantly identified in genes associated with recessive disorders with compound heterozygous variants being the most common (41%), followed by heterozygous variants in genes associated with dominant disorders (25%) and homozygous recessive variants (23%, Table 1). De novo variation made up 7% of the pathogenic/likely pathogenic variants (Table 1). The most frequently encountered genes identified with pathogenic/likely pathogenic variants were *SPG7* (20%, 9/44), *CACNA1G* (Ngo et al., 2018), and *SYNE1* (7%, 3/44 each), and *ITPR1, KCNA2*, and *SPG11* (5%, 2/44 each, Table 2) similar to the original cohort (Fogel et al., 2014).

Following exome analysis, CNV and repeat expansion analysis was performed on a representative subset of the overall cohort (26%, 68/260) consisting of 53 undiagnosed families from the expanded cohort (29%, 53/184) and 15 undiagnosed families from the initial cohort (20%, 15/76) where WES data were available from multiple members to confirm disease segregation. A pathogenic CNV deletion was identified in two families (2.9%, Tables 1, 2, and 4; Table S5). In one family with six members affected by progressive adult-onset ataxia across two generations, a CNV deletion (exons 3–33) in the *ITPR1* gene was identified. Similar deletions have previously been reported to cause SCA15/16 (van de Leemput et al., 2007). The CNV deletion was detected by CoNIFER and XHMM in both the patient and affected son's WES data. Actual breakpoints were clinically confirmed through NGS and quantitative PCR. In the other family, a pathogenic deletion in the FARS2 gene was identified during the reanalysis of the original cohort, described in detail below. A pathogenic repeat expansion was also identified in one family (1.5%) by STRetch (Tables 1 and 2; Table S5). This expansion is associated with Spinocerebellar ataxia type 8 (Mundwiler & Shakkottai, 2018; Paulson, 2018) and was subsequently confirmed by clinical testing in the proband.

Reanalysis of variants from the original cohort at 5 years resulted in an unchanged diagnostic interpretation in 58 of 76 cases (76%), and reclassification in the remaining 18 cases (24%, Table 4). In six subjects, variants previously classified as VUS were reclassified as benign based on either being too common in the human genome using updated ExAC/ gnomAD frequency data or because they did not segregate with the disease when additional family members were tested. Conversely, six nondiagnostic cases were reclassified as having a reportable VUS based on interval evidence supporting disease. Two nondiagnostic cases and two with a previously reported VUS were reclassified as now having pathogenic/likely pathogenic variants based on interval updates. One patient had an originally reported VUS in the *PNPLA6* gene reclassified as benign and a previously unreported VUS reported in the *TBK1* gene due to a more consistent phenotype. Last, one patient had originally reported VUS in the *ZFYVE26* gene reclassified as benign and a previously unreported pathogenic variant reported in the *FARS2* gene (Sahai et al., 2018) due to a more consistent phenotype along with the identification of the second pathogenic variant through our CNV analysis (Table 4). As described above, the CNV analysis of 15 families from this original cohort (20%, 15/76) identified one (7%, 1/15) pathogenic/likely pathogenic variant. Together, these reclassifications resulted in a modest increase in pathogenic/likely pathogenic variant calls (28%, 21/76 vs. 21%, 16/76) and overall clinically relevant variants (63%, 48/76 vs. 61%, 46/76, Table 4). Combining these data with our expanded cohort, our collective observation of pathogenic/likely pathogenic variation is 25% (65/260) and clinically relevant variants were seen in 52% (135/260, Tables 1–4; Tables S5 and S6). For the combined cohort the most commonly identified genes with pathogenic/likely pathogenic variants were *SPG7* (17%, 11/65), *SYNE1* (9%, 6/65), *CACNA1G, ITPR1*, and *SPG11* (5%, 3/65 each), and *GBE1, KCNA2, SPAST*, and *WFS1* (3%, 2/65 each, Tables 2 and 4; Table S5; Fogel et al., 2014).

An important diagnostic question concerns how effective exome detection truly is for all known genetic ataxias. To estimate this, we examined the diagnostic rates for families with multiple affected members (either parents or siblings) where a Mendelian genetic etiology can most strongly be presumed. Next we combined our data with the clinically relevant exome diagnostic rates from five recent independent WES studies (Montaut et al., 2018; Nibbeling et al., 2017; Ohba et al., 2013; Pyle et al., 2015; Sawyer et al., 2013) of similar undiagnosed families with ataxia obtaining a mean diagnostic rate of 42% from 139 total families (58/139, Table 1 and Table S3). To assess the efficiency of exome sequencing as a diagnostic test we assumed all these families had a Mendelian genetic cause detectable by exome sequencing, thus setting the maximum diagnostic rate at 100% (adjusted to 96% for average exome coverage). Using a one proportion test, the observed diagnostic rate of 42% differs significantly from the maximum expected rate of 96% (z score = 32.5; $p < .0001$; 95% confidence interval = 34–51%). These data are sufficiently powered at this level of significance to show a significant discrepancy between the observed diagnostic rate and an expected rate as low as 51%. Therefore, even in a population with the strongest likelihood of having a Mendelian genetic cause identifiable by WES, half of the patients remain undiagnosed. Incorporating our data with that from a total of six additional studies that examined ataxia patients using NGS panel testing (Coutelier et al., 2018; Farwell et al., 2015; Marelli et al., 2016; Nemeth et al., 2013; Sun et al., 2018; van de Warrenburg et al.,

2016) did not improve the maximum observed diagnostic confidence interval above 51% (Table 1 and Table S3). Incorporating sporadic cases from the above studies further weakens the maximum potential diagnostic rate (Table S3).

4 | DISCUSSION

In this report, we performed exome sequencing on 184 patients with undiagnosed familial and sporadic ataxia and/or spastic paraplegia with suspicion for a genetic cause based on either familial inheritance patterns or negative screening for alternative acquired causes of ataxia (Fogel et al., 2014). CNV and repeat expansion analysis were also performed in a representative subset of the cohort, representing approximately one-third of undiagnosed cases. We identified pathogenic/likely pathogenic variants in 24% (44/184), and VUS in 23% (43/184) of cases for an overall clinically relevant detection rate of 47% (87/184). This included identification of a pathogenic CNV in one family and a pathogenic repeat expansion in another. Of note, 11 cases classified as nondiagnostic in this study were ultimately clinically diagnosed with multiple system atrophy, cerebellar type, and another case was identified with a VUS associated with increased risk for this condition (Gilman et al., 2008; Zhao et al., 2016). We also reclassified variants from our previously reported 76 cases (Fogel et al., 2014) based on current annotation, which has been shown to improve diagnosis over time (Alfares et al., 2018; Ewans et al., 2018; Fogel, 2018b; Fogel, Lee, Strom, Deignan, & Nelson, 2016; Fogel et al., 2016; Nambot et al., 2018; Rexach et al., 2019; Wright et al., 2018). This resulted in four cases previously classified as nondiagnostic or having a reportable VUS being reclassified with a pathogenic/likely pathogenic genetic variant. Six previous nondiagnostic cases were reclassified as having a reportable VUS, and six cases with variants previously designated as VUS or likely pathogenic reclassified as benign. Two cases had a previous VUS reclassified as benign but also had either a new VUS or pathogenic variant reported in a different gene. Despite these adjustments, our overall rate of detecting clinically relevant variants remained similar to our previous study (63%, 48/76 vs. 61%, 46/76) although pathogenic/likely pathogenic numbers improved (28%, 21/76 vs. 21%, 16/76). Combining these datasets, the overall detection of clinically relevant variants was 52% (135/260) with 25% (65/260) classified as pathogenic or likely pathogenic.

Collectively, numerous studies evaluating WES and NGS-based ataxia gene panels have achieved diagnostic rates of 32% across all patients (278/873) and 47% for familial cases (43/92), which improves slightly if focused on WES in familial cases (53%, 30/57, Table S3; Coutelier et al., 2018; Farwell et al., 2015; Keogh et al., 2015; Lee et al., 2014; Marelli et al., 2016; Montaut et al., 2018; Nemeth et al., 2013; Nibbeling et al., 2017; Ohba et al., 2013; Pyle et al., 2015; Sawyer et al., 2013; Sun et al., 2018; van de Warrenburg et al., 2016). By combining our current data with these studies we evaluated the capability of current exome sequencing and analysis pipelines to achieve a diagnosis of the remaining unsolved ataxia and spasticity cases. Even when combining the data from these multiple independent studies (Table S3) and assessing diagnostic rates in familial ataxia, that most likely to have an identifiable Mendelian cause, the maximum overall rate of detecting clinically significant findings consistently hovers at approximately 50%, well below even a conservative estimate of the expected rate.

While WES efficiently identifies certain types of disease-causing variants including small indels, single base changes disrupting protein function, and canonical splice acceptor/donor variants, the fact that approximately 50% of cases still remain unresolved highlights gaps in sensitivity of detecting of other classes of mutations (Rexach et al., 2019). In particular, many structural variants and repeat expansions present in the coding sequence are not detected well or reliably genome-wide by WES. For example, exome sequencing can be limited in the comprehensive detection of mutations within the mitochondrial genome and is incapable of detecting the majority of repeat expansions and noncoding genomic variation which can contribute to the modulation of gene expression (Rexach et al., 2019). With recent advances in analyses methods, WES can be used to detect CNV affecting multiple exons, as illustrated here, but continues to be limited for detecting small CNV (i.e., involving single-exons), which may be better identified with whole-genome sequencing (WGS) methods (Rexach et al., 2019). We assessed 26% (68/260) of our cohort for CNV and repeat expansions using the depth of read coverage data from WES, which resulted in the diagnosis of only three additional cases (4%). This suggests that the addition of detection methods for CNV and known repeat expansions to current diagnostic pipelines may not contribute much overall to the missing heredity. However, repeat expansions and single exon heterozygous deletions are challenging to reliably call from exome data so this may also indicate that current methods need further refinement. Extension to WGS using short read (to observe CNV) and long read (to observe CNV and repeat expansion) technologies in the evaluation of ataxia promises to improve detection of these genetic causes (Rexach et al., 2019). Noncoding variation, such as point mutations in promoter regions, splice sites, or other RNA-processing regulatory regions, all potentially detectable by WGS, must also account for some disease-causing mutations as well, but their categorization has remained difficult (Rexach et al., 2019).

The results of this study further emphasize the need for continued investigation into methods to complement and extend the diagnostic value of current next-generation sequencing datasets. It is certainly possible, and likely probable, that additional undiscovered genes responsible for these phenotypes exist and are causative in a percentage of our population. Even with the use of linkage analysis and the addition of multiple family members when available, such genes are challenging to detect if they cause extremely rare or private disorders. The development of collaborative resources to merge data and analysis from large cohorts of patients with ataxic phenotypes may aid in the discovery of such genes (Fogel, 2018a), as will a focus on mutation types not typically detected by exome sequencing. For example, novel disease-causing repeat expansion disorders continue to be described and subsequently identified in undiagnosed patients (Cortese et al., 2019; Ishikawa et al., 2011; Kobayashi et al., 2011; Rafehi et al., 2019; Seixas et al., 2017; Valera et al., 2017). Furthermore, variants whose effect is determined in combination with additional genes (digenic, polygenic), epigenetic, or environmental factors, or causal mutations in the noncoding genome that affect gene regulation would be difficult to detect by current DNA-only methods. The application of "multi-omic" strategies is increasingly being applied to facilitate variant interpretation by assessing their effects on the transcriptome, including alteration in messenger RNA splicing (Cummings et al., 2017; Elsaid et al., 2017; Kremer et al., 2017). Coupling next-generation sequencing methods with transcriptome analysis has

already shown diagnostic utility in other rare diseases (Lee et al., 2019). In addition to WGS, new sequencing platforms and analysis strategies are in various stages of development that could facilitate the identification of rare or novel repeat expansions (Gymrek, Golan, Rosset, & Erlich, 2012; Rafehi et al., 2019), or smaller or more complex CNV or CNV mediated by difficult to map repeat elements (SINEs, LINEs, etc.; Turner et al., 2016). Additionally, pathway or network-based analytic methods have been utilized to identify rare or polygenic candidate disease genes based on their convergence upon common disease-associated biological pathways (Nibbeling et al., 2017). Finally, epidemiological and computational medicine approaches, which are increasingly enabled through large-scale precision health initiatives and availability of electronic medical records, have the potential to identify gene-environment interactions that have long eluded detection (Rexach et al., 2019).

Much of the above discussion still represents areas of advancing research investigation but, given the dynamic nature of the field, can be rapidly translated to clinical practice. For the clinician evaluating patients with ataxic and related disorders suspected to have a genetic etiology, standard of care would still include the use of exome sequencing (Fogel, 2018b; Fogel et al., 2014; Rexach et al., 2019) or a comprehensive next-generation sequencing panel targeting currently known ataxia genes (Sun et al., 2018) if exome sequencing is unavailable. However, once performed and if nondiagnostic, for the remaining undiagnosed familial cases with apparent monogenic inheritance, a key focus should be on repeating bioinformatic analysis at regular intervals, as well as the implementation of more comprehensive genomic tools and more complete methods to identify mutation types currently not observed in WES as they become clinically available.

5 | CONCLUSION

Exome sequencing performed in a predominantly adult- and sporadic-onset cohort of 260 patients with cerebellar ataxia and/or spastic paraplegia observed clinically relevant genetic variation in 52% and pathogenic or likely pathogenic variants in 25% of cases, emphasizing the importance of coding variation to these disorders. However, improved annotation methods and the inclusion of CNV and repeat expansion analysis in a representative subset of this cohort did not dramatically improve overall diagnostic rates from prior studies, even among familial cases with the highest evidence for monogenic disorders. The discrepancy between observed and expected diagnostic rates in familial cases from this and other published studies supports a current diagnostic ceiling for exome sequencing of approximately 50%, suggesting that a critical limitation to genetic diagnosis in these patients rests on the high likelihood that missing pathogenic mutations lie outside the exome and must be identified by other methods and more comprehensive genome-wide strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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TABLE 1

Distribution of variant types detected by WES for expanded ataxia cohort

A.

	Total		Familial		Sporadic		EO		AO	
	N	%	N	%	N	%	N	%	N	%
Path/LP	44	24	18	30	26	21	20	35	24	19
VUS	43	23	8	13	35	28	13	23	30	24
NSV	97	53	34	57	63	51	24	42	73	57
Total	184	100	60	33	124	67	57	31	127	69

B.

	Pathogenic/likely pathogenic variants (N = 44)	
	No. of cases	%
De novo	3	7
Homozygous	10	23
Compound heterozygous	18	41
Inherited heterozygous[a]	11	25
Copy number variant	1	2
Repeat expansion	1	2

Abbreviations: AO, adult-onset; EO, early-onset (age 20 years); LP, likely pathogenic; N, number of index patients; NSV, no significant variants identified; Path, pathogenic; WES, Whole-exome sequencing.

[a]Variants were considered inherited heterozygous unless parents or other family members were available for testing for de novo confirmation or variant was previously reported as de novo through another clinical test.

TABLE 2

Patients with pathogenic/likely pathogenic variants identified

Patient no./sex/age/onset	Family history	Primary symptom	Gene	Inheritance	cDNA	Protein	OMIM disease phenotype
ATX77/F/39/EO	Affected sister	Ataxia	SH3TC2	AR	c.1546A>T c.1586G>A	p.Lys516Ter p.Arg529His	601596
ATX78/F/59/AO	Multiple affected	Ataxia	ITPR1	AD	c.(4494513_4735417)del	p.?	606658
ATX79/F/46/EO	Affected brother and sister	Ataxia	COQ8A	AR	c.74delC c.1555delA	p.His26Thrfs*11 p.Thr519Profs*24	612016
ATX80/F/50/EO	2 Affected sisters	Ataxia	SPG11	AR	c.6091C>T c.6526T>C	p.Arg2031Ter p.Phe2176Leu	604360
ATX81/F/42/AO	Sporadic	Ataxia	ERCC4	AR	c.2395C>T c.2117T>C c.1765C>G	p.Arg799Trp p.Ile706Thr p.Arg589Gly	278760
ATX82/M/17/EO	Affected brother	Ataxia	AARS2	AR	c.706delC c.984C>G	p.Gln236Serfs*3 p.Ile328Met	615889
ATX83/F/41/EO	Affected sister	Ataxia	EXOSC3	AR	c.395A>C c.572G>A	p.Asp132Ala p.Gly191Asp	614678
ATX84/M/58/AO	Affected brother	Ataxia	ANO10	AR	c.289delA homozygous	p.Met97Ter	613728
ATX85/M/46/EO	Affected brother	Ataxia	KIF1C	AR	c.927_928insAAGGA homozygous	p.Asn312Argfs*12	611302
ATX86/M/8/EO	Sporadic	Ataxia	GOSR2	AR	c.430G>T homozygous	p.Gly144Trp	614018
ATX87/M/27/EO	Affected sister	Ataxia	DOCK6	AR	c.2294G>A homozygous	p.Arg765His	614219
ATX88/M/41/AO[a]	Multiple affected	Ataxia	CACNA1G	AD	c.5144G>A	p.Arg1715His	616795
ATX89/M/10/EO	Sporadic	Ataxia	KCNA2	AD	c.890G>A	p.Arg297Gln	616366
ATX90/M/60/AO	Sporadic	Ataxia	SPG7	AR	c.(1_?)del c.1529C>T	p.? p.Ala510Val	607259
ATX91/M/28/AO[a]	Sporadic	Ataxia	CACNA1G	AD	c.5144G>A	p.Arg1715His	616795
ATX92/M/74/AO[a]	Multiple affected	Ataxia	CACNA1G	AD	c.5144G>A	p.Arg1715His	616795
ATX93/F/63/AO	Affected brother	Ataxia	SPG7	AR	c.1454_1462delGGCGGGAGA c.1529C>T	p.Arg485_Glu487del p.Ala510Val	607259
ATX94/M/77/AO	Affected sister	Ataxia	SPG7	AR	c.1454_1462delGGCGGGAGA c.1529C>T	p.Arg485_Glu487del p.Ala510Val	607259
ATX95/F/67/AO	Sporadic	Ataxia	SYNE1	AR	c.16927C>T c.20236C>T	p.Arg5643Trp p.Arg6746Trp	610743

Patient no./sex/age/onset	Family history	Primary symptom	Gene	Inheritance	cDNA	Protein	OMIM disease phenotype
ATX96/M/61/AO	Sporadic	Ataxia	SYNE1	AR	c.2380_2381delinsAA c.23102G>A	p.Ala794Lys p.Arg7701Gln	610743
ATX97/F/65/AO	Sporadic	Ataxia	SPG7	AR	c.1529C>T homozygous	p.Ala510Val	607259
ATX98/F/22/EO	Sporadic	Ataxia	WFS1	AR	c.1082C>T homozygous	p.Thr361Ile	222300
ATX99/M/61/EO	Sporadic	Ataxia	CACNA1A	AD	c.1997C>T	p.Thr666Met	108500
ATX100/M/57/AO	Sporadic	Ataxia	SPG7	AR	c.1529C>T homozygous	p.Ala510Val	607259
ATX101/F/43/EO	Sporadic	Ataxia	SYNE1	AR	c.22958G>A c.13210A>T	p.Trp7653Ter p.Lys4404Ter	610743
ATX102/M/70/AO	Sporadic	Ataxia	TMEM240	AD	c.509C>T	p.Pro170Leu	607454
ATX103/F/71/AO	Sporadic	Ataxia	ALDH18A1	AD	c.755G>A	p.Arg252Gln	601162
ATX104/F/48/AO	Multiple affected	Spasticity	SPAST	AD	c.1103T>C	p.Phe368Ser	182601
ATX105/F/35/EO	Sporadic	Spasticity	CYP7B1	AR	c.392dupA c.825T>A c.889A>G	p.Asn131Lysfs*3 p.Tyr275Ter p.Thr297Ala	270800
ATX106/M/51/AO	Affected mother (not examined)	Ataxia	SPG7	AR	c.1454_1462delGGCGGGAGA c.1529C>T	p.Arg485_Glu487del p.Ala510Val	607259
ATX107/F/27/EO	Sporadic	Spasticity	ATL1	AD	c.742G>A	p.Glu248Lys	182600
ATX108/F/58/AO	Affected brother	Ataxia	SPG7	AR	c.233T>A c.291_294delTACT	p.Leu78Ter p.Thr98Serfs*8	607259
ATX109/M/12/EO	Sporadic	Ataxia	ITPR1	AD	c.7471G>A	p.Gly2491Arg	206700
ATX110/M/48/AO	Sporadic	Ataxia	SPG7	AR	c.1529C>T c.1552+1G>T	p.Ala510Val p.?	607259
ATX116/M/52/AO	Sporadic	Ataxia	ATXN8OS/ATXN8	AD	Repeat expansion	CTG/CAG expansion	608768
ATX222/F/13/EO	Sporadic	Ataxia	SCN8A	AD	c.4850G>A	p.Arg1617Gln	614306
ATX223/M/30/EO	Sporadic	Spasticity	SPG11	AR	c.6266dupT c.1013G>A	p.Cys2090Valfs*14 p.Trp338Ter	604360
ATX224/M/38/AO	Sporadic	Ataxia	ABHD12	AR	c.527G>A homozygous	p.Gly176Glu	612674
ATX233/F/39/AO [b]	Multiple affected	Ataxia, spasticity	ELOVL4	AD	c.512T>C	p.Ile171Thr	133190
ATX234/F/44/EO	Sporadic	Ataxia	SACS	AR	c.3484G>T c.4880_4881dupGT	p.Glu1162Ter p.Gln1628Valfs*5	270550
ATX237/F/69/AO	Sporadic	Spasticity	GBE1	AR	c.986A>C homozygous	p.Tyr329Ser	263570

Patient no./sex/age/onset	Family history	Primary symptom	Gene	Inheritance	cDNA	Protein	OMIM disease phenotype
ATX238/M/26/EO	Sporadic	Ataxia	*SLC2A1*	AD	c.458G>A	p.Arg153His	606777 612126
ATX242/M/14/EO	Affected mother	Ataxia	*KCNA2*	AD	c.890G>A	p.Arg297Gln	616366
ATX250/M/48/AO	Sporadic	Ataxia	*SPG7*	AR	c.1529C>T homozygous	p.Ala510Val	607259

Note: Age reported in years.

Abbreviations: AD, autosomal dominant; AO, adult-onset; AR, autosomal recessive; cDNA, complementary DNA; EO, early-onset (age 20 years); F, female; M, male; OMIM, Online Mendelian Inheritance in Man.

[a]Family originally reported in Ngo et al. (2018).

[b]Family originally reported in Xiao et al. (2019).

TABLE 3

Patients with variants of uncertain significance identified

Patient no./sex/age/onset	Family history	Primary symptom	Gene	Inheritance	cDNA	Protein	OMIM disease phenotype
ATXI113/M/30/AO	Sporadic	Ataxia	FOXRED1	AR	c.7C>T	p.Arg3Trp	618241
ATXI115/F/ND/AO	Sporadic	Ataxia	CLCN2	AR	c.1153C>T	p.Gln385Ter	615651
ATXI117/F/48/AO	Sporadic	Ataxia	FUS	AD	c.253C>T	p.Gln85Ter	608030
ATXI118/F/66/AO	Sporadic	Ataxia	ELOVL5	AD	c.412C>T	p.Arg138Cys	615957
ATXI119/F/49/AO	Sporadic	Ataxia	SACS / SPART	AR / AR	c.2329T>C / c.1939G>A	p.Ser777Pro / p.Val647Met	270550 / 275900
ATXI120/F/53/EO	Affected sister	Ataxia	SPG11	AR	c.2561C>T	p.Ala854Val	604360
ATXI121/M/57/AO	Sporadic	Ataxia	PEX6	AR	c.853C>G homozygous	p.Pro285Ala	614863
ATXI122/M/35/EO	Sporadic	Ataxia	PSAP	AR	c.1085A>T	p.Glu362Val	249900
ATXI123/F/48/AO	Multiple affected	Ataxia	COQ2	AD/AR	c.1178T>C	p.Val393Ala[a]	146500
ATXI124/F/65/AO	Sporadic	Ataxia	KCNC3	AD	c.691G>A	p.Gly231Ser	605259
ATXI125/M/61/AO	Sporadic	Ataxia	PAX6	AD	c.179A>G	p.Gln60Arg	106210
ATXI128/M/50/AO	Sporadic	Ataxia	SYNE1	AR	c.4843G>A / c.17614T>C / c.23102G>A	p.Ala1615Thr / p.Ser5872Pro / p.Arg7701Gln	610743
ATXI129/F/71/AO	Affected mother	Ataxia	EIF4G1	AD	c.3470G>T	p.Arg1157Leu	614251
ATXI130/M/42/AO	Sporadic	Ataxia	SYNE1	AR	c.13696G>A / c.14050C>T / c.23102G>A	p.Asp4566Asn / p.Leu4684Phe / p.Arg7701Gln	610743
ATXI131/F/23/EO	Affected sister	Ataxia	ADAR	AR	c.577C>G	p.Pro193Ala	615010
ATXI132/M/53/AO	Sporadic	Spasticity	SYNE1	AR	c.19321G>T	p.Ala6441Ser	610743
ATXI133/M/12/EO	Multiple affected	Ataxia	MYH14	AD	c.1864G>A	p.Val622Ile	614369
ATXI135/M/53/AO	Sporadic	Ataxia	USP9X	XLR	c.2515A>G	p.Ser839Gly	300919
ATXI136/F/80/AO	Sporadic	Ataxia	CLCN1	AD/AR	c.689G>A	p.Gly230Glu	160800 / 255700
ATXI137/F/8/EO	Sporadic	Ataxia	SYNE1	AR	c.2198A>G / c.11440A>G	p.Glu733Gly / p.Thr3814Ala	610743
ATXI138/M/72/AO	Sporadic	Spasticity	LAMA1	AR	c.3724G>A / c.5302G>A	p.Ala1242Thr / p.Val1768Met	615960
ATXI139/M/56/AO	Sporadic	Ataxia	PNPLA6	AR	c.2922_2923insAA	p.Thr975Lysfs*10	612020
ATXI140/M/60/AO	Sporadic	Spasticity	SPTLC2 / ZFYVE26	AD / AR	c.994G>T / c.5518C>T	p.Ala332Ser / p.Arg1840Trp	613640 / 270700

Patient no./sex/age/onset	Family history	Primary symptom	Gene	Inheritance	cDNA	Protein	OMIM disease phenotype
ATX141/M/63/AO	Sporadic	Ataxia	HEXA	AR	c.739C>T	p.Arg247Trp	272800
ATX142/M /18/EO	Sporadic	Ataxia	BICD2	AD	c.684T>A	p.Asp228Glu	615290
ATX143/M/56/AO	Sporadic	Spasticity	PNPLA6	AR	c.2783G>C	p.Cys928Ser	612020
ATX144/M/56/AO	Sporadic	Ataxia	TDP1	AR	c.346A>G	p.Ile116Val	607250
ATX145/M/54/AO	Sporadic	Ataxia	SYT14	AR	c.573T>G	p.Asp191Glu	614229
ATX146/M/24/EO	Sporadic	Ataxia	TUBB4A	AD	c.439G>A	p.Gly147Arg	612438
ATX147/M/56/AO	Sporadic	Ataxia	ZFYVE26 CACNA1G	AR AD	c.5584C>T c.6170G>C	p.Arg1862Cys p.Gly2057Ala	270700 616795
ATX148/M/74/AO	Sporadic	Spasticity	GBA2	AR	c.1196G>C	p.Arg399Pro	614409
ATX150/F/64/EO	Sporadic	Ataxia	GRID2 SETX	AR AR	c.845C>T c.806C>T	p.Thr282Met p.Ser269Leu	616204 606002
ATX151/M/62/AO	Affected father (not examined)	Ataxia	SACS	AR	c.8245A>G	p.Ile2749Val	270550
ATX226/M/55/AO	Multiple affected	Spasticity	EIF2B3	AR	c.464G>A	p.Arg155His	603896
ATX227/M/20/EO	Sporadic	Ataxia	SPG7	AR	c.488G>A	p.Gly163Asp	607259
ATX228/F/8/EO	Sporadic	Ataxia	TPP1	AR	c.1496C>G	p.Pro499Arg	609270
ATX229/M/16/EO	Sporadic	Ataxia	SPTBN2	AR	c.6577G>A	p.Ala2193Thr	615386
ATX230/F/16/EO	Sporadic	Ataxia	HTRA2	AR	c. 346dupG c.1085T>C	p.Ala116Glyfs*22 p.Ile362Thr	617248
ATX235/M/62/AO	Sporadic	Ataxia	SYNE1 SACS	AR AR	c.12142G>A c.22432C>T c.9305T>A	p.Glu4048Lys p.Arg7478Cys p.Leu3102Ter	610743 270550
ATX239/F/66/AO	Affected father (not examined)	Spasticity	SAMD9L	AD	c.3892C>G	p.Arg1298Gly	159550
ATX251/M/77/AO	Sporadic	Ataxia, Spasticity	ATP13A2	AR	c.3057delC	p.Tyr1020Thrfs*3	617225
ATX252/M/18/EO	Sporadic	Ataxia	MFSD8 CTSF	AR AR	c.929G>A c.1046−2A>C	p.Gly310Asp p.?	610951 615362
ATX260/F/56/AO	Sporadic	Ataxia	GRM1	AD	c.2155A>C	p.Thr719Pro	617691

Note: Age reported in years.

Abbreviations: AD, autosomal dominant; AO, adult-onset; AR, autosomal recessive; cDNA, complementary DNA; EO, early-onset (age 20 years); F, female; M, male; ND, not disclosed; OMIM, Online Mendelian Inheritance in Man; XLR, X-linked recessive.

[a] Previously reported as a risk variant for multiple system atrophy (Zhao et al., 2016).

TABLE 4

Reclassification of previously analyzed cases

Patient index	Pub. status	Reclassified status	Reason	OMIM disease phenotype	Variant summary			
					Gene	Genomic	cDNA	Protein
ATX12	VUS	N	Variants too common in population	270700	ZFYVE26	14:68236320C>T 14:68265135G>A	c.5612G>A c.1844C>T	p.Cys1871Tyr p.Ser615Phe
	N	Path	Known pathogenic variant, consistent phenotype	617046	FARS2	6:5369210C>A Conifer: 6:5109590–5613554del XHMM: 6:5404729–5431977del	c.407C>A c.(613_46)del	p.Pro136His p.?
ATX15[a]	N	Path	Known pathogenic variant, consistent phenotype, segregates with disease in family	545000	MT-TK	m.8344A>G	-	-
ATX19	N	VUS	Rare variant, consistent phenotype	617931	PUM1	1:31532131C>T	c.283G>A	p.Gly95Arg
ATX23[a]	N	Path	Rare variants, consistent phenotype, segregates with disease in family	616907	CAPN1	11:64953668_64953669delAG 11:64974125_64974129delAGAGA	c.618_619delAG c.1545_1549delAGAGA	p.Gly208Glnfs*7 p.Lys517Cysfs*8
ATX27	VUS	N	Variant too common in population	600224	SPTBN2	11:66472288G>A	c.2459C>T	p.Thr820Met
ATX31	N	VUS	Rare variants, consistent phenotype	610743	SYNE1	6:152470724C>T 6:152737976C>T	c.24317G>A c.5617G>A	p.Ser8106Asn p.Ala1873Thr
ATX36	VUS	N	Variant does not segregate with disease in family	222300	WFS1	4:6302816C>G	c.1294C>G	p.Leu432Val
ATX38	VUS	Path[b]	Known pathogenic variant, consistent phenotype[b]	604360	SPG11	15:44876420delCT	c.5456_5457delAG	p.Glu1819Alafs*10
ATX39	VUS	LP	Known pathogenic variant, consistent phenotype	614436	LRSAM1	9:130265074T>C	c.2068T>C	p.Cys690Arg
ATX40	N	VUS	Rare variant, consistent phenotype	615889	AARS2	6:44269870C>T	c.2525G>A	p.Arg842Gln
ATX42	N	VUS	Rare variant, consistent phenotype	607259	SPG7	16:89598384G>A	c.1060G>A	p.Gly354Arg
ATX45	N	VUS	Rare variant, consistent phenotype	615889	AARS2	chr6:44269119_44269120delGA	c.2680_2681delTC	p.Val895Alafs*10
ATX50	N	VUS	Rare variant, consistent phenotype	213600	SLC20A2	8:42297115C>A	c.787G>T	p.Val263Phe
ATX52	VUS N	N VUS	Variant is too common in population	612020 616439	PNPLA6 TBK1	19:7607741C>T 12:64879243C>T	c.1340C>T c.1198C>T	p.Pro447Leu p.Pro400Ser

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Patient index	Pub. status	Reclassified status	Reason	OMIM disease phenotype	Gene	Variant summary		
						Genomic	cDNA	Protein
			Rare variant, consistent phenotype					
ATX64	VUS	N	Variant is too common in population	614251	EIF4G1	3:184039843A>T	c.1492A>T	p.Ile498Phe
ATX66	VUS	N	Variants too common in population	263570	GBE1	3:81640290A>C 3:81810551G>T	c.1134T>G c.118C>A	p.Ser378Arg p.Pro40Thr
ATX68	VUS	N	Variants do not segregate with disease in family	614895 271245	PRX TWNK	19:40900066G>T 10:102749087C>T	c.4193C>A c.1120C>T	p.Ala1398Asp p.Arg374Trp
ATX74	VUS	N	Variant is too common in population, does not segregate with disease in family	613908	TGM6	20:2375121T>G	c.31T>G	p.Trp11Gly

Note: A Total of 76 cases from Fogel et al. (2014) were reanalyzed using current methods (see text). Population frequency determined by use of the ExAC (http://exac.broadinstitute.org/) and gnomAD (https://gnomad.broadinstitute.org/) databases.

Abbreviations: cDNA, complementary DNA; LP, likely pathogenic; N, nondiagnostic; OMIM, Online Mendelian Inheritance in Man; Path, pathogenic; VUS, variant of uncertain clinical significance.

[a] These patients were originally reported with sporadic disorders but subsequently reclassified as familial when additional family members were clinically evaluated.

[b] This recessive disorder was diagnosed clinically due to its distinctive phenotype, a second pathogenic variant is presumed to be noncoding.