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Cross-Sectional Analysis of Exome Sequencing Diagnosis in Patients With Neurologic Phenotypes Facing Barriers to Clinical Testing

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Abstract

Background and Objectives

Exome sequencing (ES) demonstrates a 20–50 percent diagnostic yield for patients with a suspected monogenic neurologic disease. Despite the proven efficacy in achieving a diagnosis for such patients, multiple barriers for obtaining exome sequencing remain. This study set out to assess the efficacy of ES in patients with primary neurologic phenotypes who were appropriate candidates for testing but had been unable to pursue clinical testing.

Methods

A total of 297 patients were identified from the UCLA Clinical Neurogenomics Research Center Biobank, and ES was performed, including bioinformatic assessment of copy number variation and repeat expansions. Information regarding demographics, clinical indication for ES, and reason for not pursuing ES clinically were recorded. To assess diagnostic efficacy, variants were interpreted by a multidisciplinary team of clinicians, bioinformaticians, and genetic counselors in accordance with the American College of Medical Genetics and Genomics variant classification guidelines. We next examined the specific barriers to testing for these patients, including how frequently insurance-related barriers such as coverage denials and inadequate coverage of cost were obstacles to pursuing exome sequencing.

Results

The cohort primarily consisted of patients with sporadic conditions (n = 126, 42.4%) of adult-onset (n = 239, 80.5%). Cerebellar ataxia (n = 225, 75.8%) was the most common presenting neurologic phenotype. Our study found that in this population of mostly adult patients with primary neurologic phenotypes that were unable to pursue exome sequencing clinically, 47 (15.8%) had diagnostic results while an additional 24 patients (8.1%) had uncertain results. Of the 297 patients, 206 were initially recommended for clinical exome but 88 (42.7%) could not pursue ES because of insurance barriers, of whom 14 (15.9%) had diagnostic findings, representing 29.8% of all patients with diagnostic findings. In addition, the incorporation of bioinformatic repeat expansion testing was valuable, identifying a total of 8 pathogenic repeat expansions (17.0% of all diagnostic findings) including 3 of the common spinocerebellar ataxias and 2 patients with Huntington disease.

Discussion

These findings underscore the importance and value of clinical ES as a diagnostic tool for neurogenetic disease and highlight key barriers that prevent patients from receiving important clinical information with potential treatment and psychosocial implications for patients and family members.

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Glossary

DOC = depth-of-coverage; **EH** = ExpansionHunter; **EMR** = electronic medical record; **ES** = exome sequencing; **LP** = likely pathogenic; **P** = pathogenic; **SCA** = spinocerebellar ataxia; **VUS** = variants of uncertain significance.

Introduction

Monogenic etiologies have been identified as the cause of numerous neurologic diseases that affect hundreds of millions of people worldwide.[1] Recent meta-analyses reveal the prevalence of these monogenic neurologic disorders to be greater than 1 in every 1,100 people.[2] Patients with phenotypes spanning all fields of neurology and all ages of onset receive valuable molecular insight into symptomology, pathogenesis, and prognosis through genetic testing. While a variety of genetic testing methods and strategies exist, exome sequencing (ES) analyzes the coding region of almost all ~20,000 human genes, targeting more than 7,000 genetic conditions with a single test. Patients with neurologic presentations including neurodevelopmental delay, intellectual disability, epilepsy, neuromuscular disease, and cerebellar ataxia are typically diagnosed at a rate of 20–50 percent by ES.[3-11] Recent publications demonstrate that ES provides valuable molecular insight that results in direct treatment changes in both adult-onset and childhood-onset neurologic conditions.[11-14] Furthermore, medical management changes such as early therapeutic interventions, supportive options, and surveillance decisions are routinely made based on genetic test results.[11] As we begin to witness an unprecedented increase in the availability of gene-specific treatments and clinical trials,[15] achieving a molecular diagnosis quickly has become vital to patient care, and ES is at the forefront of clinical utility. In addition, to its clinical utility, studies demonstrate that ES allows for a quicker and less costly route to diagnosis,[7,14] benefiting both the patient and health insurance companies. Furthermore, the benefits of molecular diagnoses extend beyond the matter of clinical utility when familial and psychosocial implications are considered.[11,16]

Despite its high yield and increasing clinical utility, historically, patients with genetic disease face barriers to pursuing recommended genetic testing.[17] Previous studies assessing these barriers to ES demonstrate that between 20% and 25% of patients offered the genetic test are denied authorization by their insurer.[17,18] A previous study examining patients with a diverse range of indications for genetic testing demonstrated that ES can be effective in reaching diagnoses for patients who faced insurance barriers, with diagnostic rates comparable to those of previous reports.[18] Our study sought to evaluate the reasons exome sequencing had not been pursued clinically in a cohort of primarily adult patients with neurologic diseases seen at a tertiary referral center. Furthermore, this research aimed to assess the efficacy of ES in patients with adult-onset neurologic conditions who faced such barriers to clinical testing.

In addition, we sought to evaluate the usefulness of ES for bioinformatically detecting coding repeat expansions related to neurologic disease using ExpansionHunter, a tool that computationally predicts the number of repeats across known genomic loci.[19] A recent publication assessing the use of ExpansionHunter found high sensitivity and specificity of this tool for genome sequencing data.[20] ES poses more bioinformatic limitations on such an analysis but has broad clinical utility, so we sought to further our analysis by assessing its efficacy in this setting.

Methods

Participants

The cohort comprised 297 participants with primary neurologic presentations who consented to donate DNA to the UCLA Clinical Neurogenomics Research Center Biobank between 1999 and 2022. Retrospective electronic medical record review was conducted to determine whether participants met inclusion criteria. Participants were included if they had a documented neurologic phenotype with a suspected genetic etiology and were unable to pursue exome or genome sequencing clinically by the time of review. Patients who previously received single gene or gene panel testing were enrolled if such testing was nondiagnostic. Participants were excluded if a genetic diagnosis had been reached previously by any testing method, exome or genome sequencing had been previously performed elsewhere, or if their phenotype was determined to be inconsistent with a genetic etiology.

Standard Protocol Approvals, Registrations, and Patient Consents

All study methods were approved by the Institutional Review Board at UCLA. Written informed consent was obtained from all patients or legal guardians of patients participating in the study.

Barriers to Clinical Genetic Testing

Retrospective electronic medical record (EMR) review was conducted to gather demographic information including age, race, sex, and ethnicity (Table 1). Clinical indication for exome sequencing and family history of symptoms were recorded as documented by a neurologist (Table 2). Reasons for not pursuing ES clinically were organized into 5 categories: *insurance coverage denied* includes patients with documented insurance prior authorization denials for ES; *Medicare* includes patients with Medicare at the time of neurogenetics workup, which does not prior authorize genetic testing and therefore does not guarantee payment for clinical ES; *inadequate insurance coverage* includes patients with

Table 1 Demographics

	Count (%)
Race	
White	240 (80.8)
Asian	19 (6.4)
Alaskan/Native American	1 (0.3)
Black	9 (3.0)
Other	2 (0.7)
Not disclosed	18 (6.0)
Unknown	8 (2.7)
Ethnicity	
Not Hispanic or Latino	202 (68.0)
Hispanic or Latino	26 (8.8)
Unknown	36 (12.1)
Not disclosed	33 (11.1)
Sex	
Male	133 (44.8)
Female	164 (55.2)
Age at consent, y	
Average	58.5 ± 16.8
Range	0–89

documented partial coverage which was reported unaffordable to the patient or patients with commercial plans that do not prior authorize genetic testing resulting in the patient's decision to decline pursuing ES as clearly documented in their EMR; *patient seen before exome clinically available* includes patients seen before 2011 when ES was not commercially available and then subsequently lost to follow-up; and *did not pursue ES (other)* includes patients with no clearly documented reason noted in the patient chart, provider did not offer sequencing at the time of visit, or patient chose not to pursue testing for another reason.

Exome Sequencing

DNA from whole blood or saliva was isolated by the UCLA Clinical Neurogenomics Research Center following standard protocols. ES was performed using IDT xGen Exome Research Panel v2 (Integrated DNA Technologies, Coralville, IA) for exome capture and NovaSeq 6000 system (Illumina, San Diego, CA) for sequencing. 150bp paired-end sequencing was performed. The base call (BCL) sequence files were converted and demultiplexed to FASTQ files using bcl2fastq v2.20.0.422 (Illumina). Sequence reads were aligned to the Genome Reference Consortium Human Build 37 (GRCh37) and Revised Cambridge Reference Sequence of the mitochondrial genome using BWA-mem 0.7.17 to generate BAM files.[21] BAM files were processed following the GATK best practices (GATK v.3.8) for single-nucleotide variants and small insertions/deletions (indel) variant calling to generate VCF files.[22,23] AutoMap v1.2[24] was used for detecting regions of homozygosity.

The mean depth of coverage (DOC) was 200× per exome with a minimum 98% of the targeted regions covered at 20×. Quality control metrics were within the acceptable ranges. Variants were annotated, filtered, and classified using EVIDENCE, an internally developed tool.[24] Once the variants were annotated, common (allele frequency >5% in gnomAD[26]) variants were removed. Variants were then classified based on the American College of Medical Genetics and Genomics and the American Molecular Pathology guidelines[27,28] as pathogenic (P), likely pathogenic (LP), variants of uncertain significance (VUS), likely benign (LB), or benign (B). For repeat expansions, the variant was considered pathogenic if the repeat number was equal to or above

Table 2 Primary Neurologic Presentation

Phenotype, n (%)	
Ataxia, cerebellar	225 (75.8)
Autonomic dysfunction	1 (0.3)
Brain calcifications	6 (2.0)
Chorea	1 (0.3)
Dementia	6 (2.0)
Developmental delay	4 (1.4)
Dystonia	2 (0.7)
Epilepsy	1 (0.3)
Leukodystrophy	4 (1.4)
Migraine	2 (0.7)
Muscular atrophy	2 (0.7)
Neuropathy	5 (1.7)
Parkinsonism	12 (4.0)
Spastic paraplegia	19 (6.4)
Tremor	6 (2.0)
Vestibular dysfunction	1 (0.3)
Familial presentation, n (%)	
Sporadic	126 (42.4)
Familial	76 (25.6)
Unknown	95 (32.0)
Onset, n (%)	
Childhood (<18 y)	28 (9.4)
Adult (≥18 y)	239 (80.5)
Unknown	30 (10.1)

Table 3 Results by Barrier to Clinical Exome Testing

Exome sequencing barrier (patients [percent total, percent eligible])	Exome sequencing result (patients [percent total, percent eligible])			
	Diagnostic (n = 47, 15.8%)	Uncertain (n = 24, 8.1%)	Secondary (n = 11, 3.7%)	Negative (n = 220, 74.1%)
Medicare (n = 30, 10.1%, 14.6%)	3 (6.4%, 8.8%)	3 (12.5%, 17.7%)	0 (0.0%, 0.0%)	24 (10.9%, 15.7%)
Inadequate insurance coverage (n = 10, 3.4%, 4.9%)	4 (8.5%, 11.8%)	1 (4.2%, 5.9%)	2 (18.2%, 22.2%)	3 (1.4%, 2.0%)
Insurance coverage denied (n = 48, 16.2%, 23.3%)	7 (14.9%, 20.6%)	6 (25.0%, 35.3%)	1 (9.0%, 11.1%)	35 (15.9%, 22.9%)
Patient seen before exome clinically available (n = 91, 30.6%, n/a)	13 (27.7%, n/a)	7 (29.2%, n/a)	2 (18.2%, n/a)	67 (30.5%, n/a)
Did not pursue exome clinically (n = 118, 39.7%, 57.3%)	20 (42.6%, 58.8%)	7 (29.2%, 41.2%)	6 (54.5%, 66.7%)	91 (41.4%, 59.5%)

the minimum number for pathogenicity provided in the literature and/or OMIM database.[29] The final list of rare variants was manually reviewed, and the results were clinically interpreted as diagnostic, uncertain, secondary, or negative by a multidisciplinary team including a medical geneticist, a clinical neurologist, and a genetic counselor.

Copy number and repeat expansion analyses were performed independently in 2 separate laboratories. Copy number variants were called based on the DOC information using either CoNIFER[30] and 3bCNV (manuscript in preparation) or the eXome-Hidden Markov Model (XHMM) tool for CNV analysis.[31] Repeat expansions were called using ExpansionHunter using RepeatCatalogs-v1.0.0[32] with loci downloaded from the UCSC genome browser's Simple Repeats Track that were located by Tandem Repeats Finder (TRF).[33]

Data Availability

The corresponding author has full access to the data used in the analyses and takes full responsibility for the data, the analyses and interpretation, and the conduct of the research. The corresponding author has the right to publish any and all data separate and apart from any sponsor.

Results

Using the UCLA Clinical Neurogenomics Research Center Biobank, a biorepository of DNA and other biospecimens collected broadly across all patients presenting to the UCLA Department of Neurology, we identified a total of 297 patients with suspected neurogenetic disease who were unable to receive exome sequencing clinically because of various barriers. This cohort primarily consisted of adults (n = 290, 97.6%) with an average age of 58.5 years (Table 1). The most common primary indication was ataxia (n = 225, 75.8%), followed by spastic paraplegia (n = 19, 6.4%) and parkinsonism (n = 12, 4.0%) (Table 2). Symptom onset was older than age 18 years (n = 239, 80.5%) in most patients, with only a few of childhood-onset (n = 28, 9.4%) and the remainder without a documented age at onset (n = 30, 10.1%). Most patients in this cohort had no family history of disease and were considered sporadic cases (n = 126, 42.4%), fewer had relevant family history of disease (n = 76, 25.6%), and the rest of the patients did not have this information clearly recorded in their electronic medical record (n = 95, 32.0%) (Table 2).

Access Barriers to Clinical Exome Sequencing

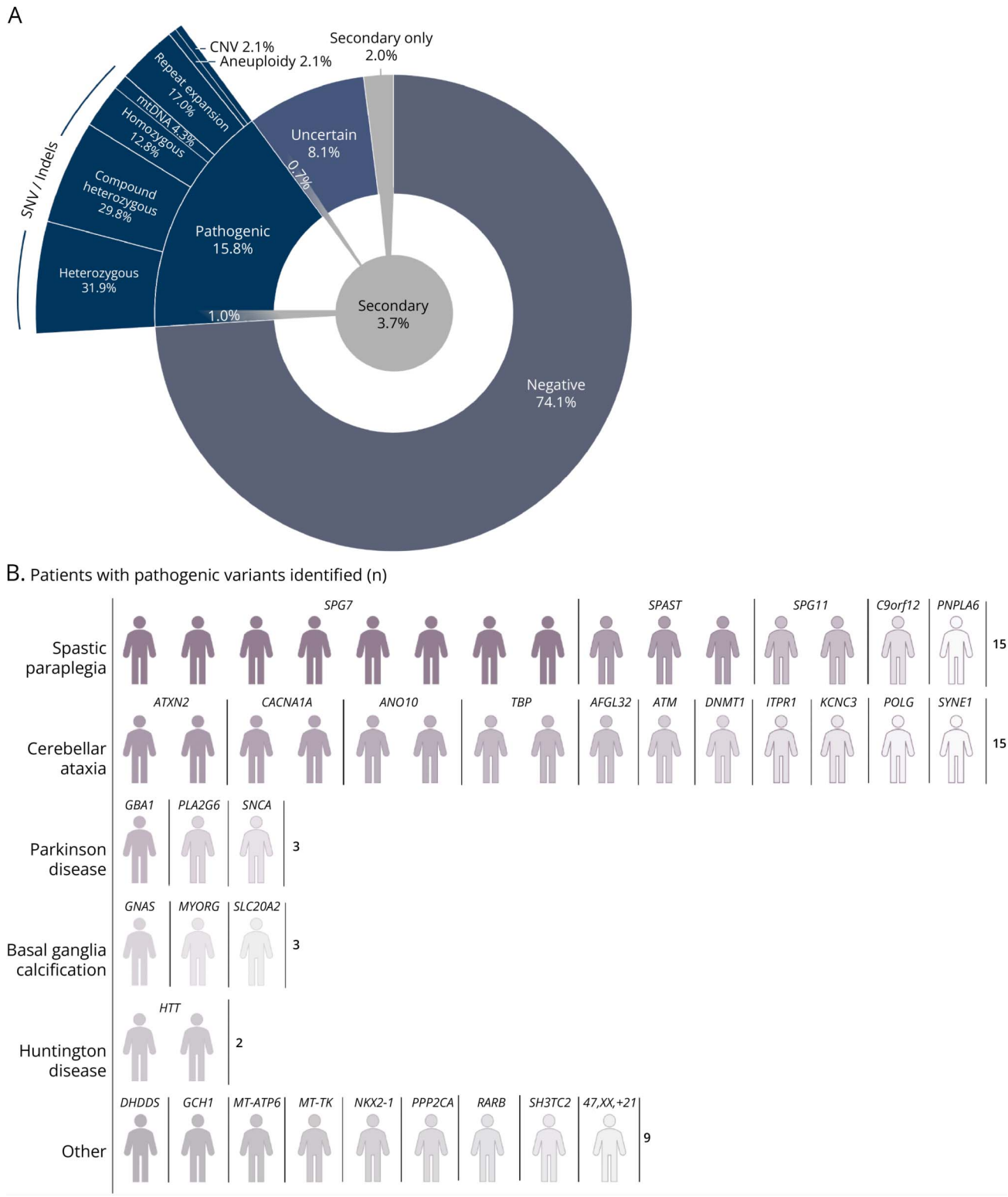
The barriers to accessing clinical exome sequencing in our cohort are depicted in Table 3. Ninety-one (30.6%) of the patients were evaluated before the clinical availability of exome sequencing and subsequently lost to follow-up, leaving 206 patients (69.4%) clinically eligible for exome sequencing. There were a total of 88 patients who faced insurance barriers when pursuing exome clinically (29.6% of total, 42.7% of eligible patients). Insurance barriers can be broken down into denial of coverage for 48 patients (16.2% total, 23.3% eligible) and inadequate insurance coverage for 10 patients (3.4% total, 4.9% eligible). Another 30 patients (10.1% total, 14.6% eligible) had Medicare which does not preauthorize genetic testing so coverage could not be assured. The remaining 118 patients (39.7% total, 57.3% eligible) were categorized as not pursuing clinical exome sequencing for other reasons unrelated to insurance.

Exome Sequencing Diagnostic Outcomes

In our cohort of primarily adult patients presenting with neurologic phenotypes who were unable to pursue clinical ES, 47 patients (15.8%) had a diagnostic finding with pathogenic/likely pathogenic (P/LP) variants identified (Table 3). An additional 24 patients (8.1%) had variants of uncertain significance, resulting in a total of 71 patients (23.9%) with clinically relevant results (Table 3). In addition, 11 patients (3.7%) received a medically actionable secondary finding (Table 3).

For the 225 participants whose primary clinical indication was ataxia, 49 (21.7%) had clinically relevant findings (eTables 1 and 2). For the 19 participants who presented with spasticity, 7 (36.8%) had clinically relevant findings (eTables 1 and 2). Of the 12 participants with parkinsonism as their primary neurologic phenotype, 6 (50.0%) had clinically relevant findings (eTables 1 and 2). All other primary neurologic indications and the rate at which exome sequencing identified a

Figure Exome Sequencing Results and Most Common Gene-Disease Associations Identified



(A) Exome sequencing results for all 297 patients are depicted. For patients with diagnostic findings (n = 47), the results are broken down into zygosity and shown as percentage of pathogenic results; heterozygous, compound heterozygous, homozygous, repeat expansion, copy number variant, aneuploidy, and mitochondrial DNA. A total of 24 patients had uncertain findings. Eleven patients (3.7%) had secondary findings, 3 had both a P/LP and a secondary finding, 2 had both a VUS and secondary finding. (B) Depicted are the gene-disease associations identified via exome sequencing. Genes are grouped on the basis of their typical phenotypic classification which, because of clinical variability of these disorders, may not reflect the presenting phenotype of all the patients diagnosed in this study (see eTables 1 and 2). Of the 47 patients with diagnostic findings, 15 patients had pathogenic variants identified in genes associated with spastic paraplegia, with *SPG7*, being the most common seen in 8 patients. The next most common disease was spinocerebellar ataxia, identified in 15 patients. Parkinson disease, basal ganglia calcifications, and Huntington disease were the other diseases identified in more than one patient (n = 3, n = 3, and n = 3, respectively). A total of 9 additional gene-disease associations were identified in only one patient in the cohort.

Table 4 Pathogenic Repeat Expansions Identified in Cohort

Phenotype	Gene	Disease association	Predicted expansion (ExpansionHunter)	Calculated expansion (PCR)	CLIA, calculated (PCR)
Ataxia	*HTT*	Huntington disease	42–45	NP	42
Ataxia	*HTT*	Huntington disease	35–39	NP	36
Ataxia	*ATXN2*	SCA 2	39–40	38	NP
Ataxia	*ATXN2*	SCA 2	41–42	38	NP
Parkinsonism	*CACNA1A*	SCA 6	21–23	21	21
Ataxia	*CACNA1A*	SCA 6	22–23	21	NP
Parkinsonism	*TBP*	SCA 17	44–45	44	NP
Parkinsonism	*TBP*	SCA 17	41–42	43	43

Abbreviation: NP = not performed.

pathogenic, likely pathogenic, or uncertain finding is reported in (eTables 1 and 2).

For patients who received a diagnostic result, the P/LP variant types observed are depicted in Figure, A. The most common type of result was a heterozygous sequence variant (n = 15, 31.9%), followed by compound heterozygous (n = 14, 29.8%). Using ExpansionHunter, 8 patients were diagnosed with 4 repeat expansion conditions (17.0% of diagnostic results). A comparison of the repeat size estimated by ExpansionHunter across 2 research laboratories is shown in Table 4 along with subsequent clinical confirmation of the findings if available.

P/LP/VUS variants were identified in a total of 56 unique genes with disease associations (eTable 1). Of the 47 patients who received diagnostic results, the most common diagnosis was hereditary spastic paraplegia type 7 (HSP7, HSP-*SPG7*) with a total of 8 patients receiving this diagnosis (17.0%) (Figure, B). The next most common condition identified was hereditary spastic paraplegia type 4 (HSP4, HSP-*SPAST*) observed in 3 (6.4%) patients (Figure, B). Of the 15 total patients who received a diagnostic finding in a gene associated with hereditary spastic paraplegia, 13 (86.7%) presented with ataxia while only 2 (13.3%) presented with spastic paraplegia (eTable 1). Spinocerebellar ataxia type 2 (SCA2, SCA-*ATXN2*), SCA5 (SCA-*SPTBN2*), SCA6 (SCA-*CACNA1A*), SCAR10 (SCA-*ANO10*), HSP 11 (HSP-*SPG11*), and Huntington disease (*HTT*) were all seen in 2 (4.3%) patients each (Figure, B). When assessing the clinical presentation of the 15 patients identified to have a diagnostic finding in a gene associated with cerebellar ataxia, 11 (73.3%) presented with ataxia, 3 (20%) presented with parkinsonism, and 1 (6.7%) presented with spasticity (eTable 1). All other pathogenic disease gene associations identified were only observed in a single patient in our cohort. Two patients were identified with variants in *GBA1*, a known genetic risk factor of Parkinson disease; however, only one of those patients was considered to have a diagnostic finding because they had both a known risk allele variant and a phenotype consistent with classic Parkinson disease. In addition, there was one patient in our cohort who presented with Down syndrome complicated by cognitive decline and behavioral changes with iron deposition on brain imaging. ES confirmed that Down syndrome/trisomy 21 and no other potential causative variants were identified. Given the recent evidence of abnormal brain iron accumulation as a rare finding in Down syndrome regression disorder,[34] consistent with the patient's phenotype, they were considered as diagnosed.

Insurance Access Barriers in Patients Who Received Clinically Relevant ES Results

Of the 47 patients in our cohort who received diagnostic results, 14 of them (29.7% total, 41.2% of eligible patients) had faced insurance barriers. Of the 24 patients who received clinically relevant results of uncertain significance, 10 had faced insurance barriers (41.7% total, 58.8% of eligible patients). The remainder were patients seen before exome sequencing was clinically available or patients who did not pursue ES for other reasons (Table 3).

For patients with diagnostic or secondary findings who were denied insurance coverage or reported inadequate insurance coverage (n = 14), unique barriers faced as documented in the EMR are listed in Table 5. The most common reasons cited included failure to provide preauthorization or ES not being a covered benefit (8, 57.1%) and lack of medical necessity or proof of improved health outcomes (3, 21.4%).

Discussion

This study set out to identify barriers that prevent patients with primary neurologic phenotypes from pursuing exome sequencing on a clinical basis. In our cohort of patients who were appropriate candidates for ES but had been unable to pursue it clinically, 2 in 5 eligible patients faced insurance

Table 5 Specific Insurance Barriers in Patients Who Received Diagnostic and/or Actionable Secondary Results

ES barrier	Unique ES barrier	ES result
Denied coverage	Not enough proof ES improves health outcomes	Diagnostic
Denied coverage	ES is not a covered benefit	Diagnostic
Denied coverage	Not enough proof ES improves health outcomes	Diagnostic
Denied coverage	No identifiable reason for denial in chart	Diagnostic
Denied coverage	ES is considered experimental, lack of medical necessity	Diagnostic
Denied coverage	ES is not a covered benefit	Diagnostic
Denied coverage	ES is not a covered benefit	Diagnostic
Denied coverage	ES is not a covered benefit	Secondary
Inadequate coverage	Payer does not provide prior authorization or guarantee coverage	Diagnostic
Inadequate coverage	Patient reported high deductible and/or copay	Diagnostic
Inadequate coverage	Payer does not provide prior authorization or guarantee coverage	Diagnostic
Inadequate coverage	Payer does not provide prior authorization or guarantee coverage	Diagnostic
Inadequate coverage	Patient unable to obtain testing at the location set by the insurance provider	Secondary
Inadequate coverage	Payer does not provide prior authorization or guarantee coverage	Secondary

barriers. This is higher than the averages previously reported in the literature for pediatric patients or those seen in general genetics practices.[17,18] A more detailed examination of why commercial insurers denied coverage for patients in this cohort revealed the use of outdated ideologies such as ES having no proof of improved health outcomes or lacking medical necessity in over one-fifth of denials. Given that 41.2% of eligible patients found to have diagnostic findings and 58.8% of those with variants of uncertain significant faced insurance barriers, payer-related issues appear to be a significant hurdle for patients who would otherwise receive benefit from ES. Of note, the total number of patients that reported inadequate coverage was low, 4.9%, but this is likely an underestimate as patients do not always document this information with their providers and therefore would not have been captured by our retrospective chart review. Finally, nearly one-seventh of our cohort were Medicare patients at the time of their clinical evaluation. Medicare does not grant prior authorization for genetic testing; therefore, patients have no assurance the test will be covered nor are they provided an estimate of their potential out-of-pocket costs. Considering clinical ES can cost well into the thousands of dollars, this practice can be a barrier for patients toward following through with clinical ES. This may disproportionately affect persons of lower socioeconomic status and has the potential to perpetuate health disparities already seen in the field of genetics.[35]

This study also set out to assess whether the diagnostic rate of ES for our cohort of patients unable to pursue such testing clinically was comparable with the reported diagnostic efficacy in patients with primary adult neurologic phenotypes. Of 297 patients determined to have suspected genetic disease, 47 patients received a genetic diagnosis, thereby ending their diagnostic odyssey. When compared with the reported ES diagnostic rate in similar populations,[3-11] the diagnostic rate of 15.8% (95% CI 11.9%–20.5%) was similar, but at the lower margin. Reasons for this lower diagnostic rate in our population are limited, but one potential reason could be that patients were included in the study based on review of providers' notes in the electronic medical record, which may have lacked sufficient detail, been incomplete, or lacked more recent clinical information which would have altered the diagnostic impression or the indication for genetic testing. This is supported by the fact that 91 patients (30.6%) were lost to follow-up before exome sequencing. Similarly, it is possible that some of the providers had not pursued exome sequencing on these patients because of clinical reasoning which they did not enter into the medical records. This limitation may be especially pertinent when comparing our diagnostic rate with previous reports of exome sequencing efficacy in patients with adult-onset ataxia, the most common presenting phenotype in the cohort. While the majority of the literature indicates diagnostic rates for such cohorts ranging from 25% to 50%,[36] these prospective studies involve detailed phenotyping of participants and diagnostic evaluations to ensure that acquired etiologies of ataxia and related phenotypes are ruled out before inclusion. By contrast, our cross-sectional study included patients for whom a provider designated a phenotype with a suspected a genetic etiology, a process which has been observed to result in lower diagnostic rates for adult-onset ataxia in some studies.[10,37] Finally, our study is limited in that additional downstream clinical actions, such as cascade testing in patients who received variants of uncertain

significance, were not pursued nor captured by this review which could have led to additional diagnoses on further follow-up.

Finally, we assessed the use of ExpansionHunter (EH) to investigate its potential to increase the diagnostic rate in patients with adult neurologic phenotypes. Diseases caused by pathogenic repeat expansions have long been recognized in the field of adult neurology; nearly 50 percent of inherited adult-onset cerebellar ataxias are caused by repeat expansions, as well as other conditions, including muscular dystrophies and Huntington disease.[38] Recent research has demonstrated the utility of EH for use in whole-genome and whole-exome sequencing for patients with specific neurologic phenotypes.[20,39,40] We sought to expand on this work in our population of primarily adult patients with neurologic phenotypes. Our analysis bioinformatically identified 8 pathogenic trinucleotide repeat expansions in this cohort which were orthogonally confirmed by PCR. Six (6) of these patients had a phenotypic diagnosis of spinocerebellar ataxia (SCA) and were found to have repeat expansions corresponding to SCA types 2, 6, and 17. This demonstrates that the diagnostic yield of exome sequencing can be increased through the incorporation of repeat expansion testing. This could reduce the need for multigene repeat expansion panels and the tiered genetic testing that is the current standard of care for patients with disorders such as ataxia, where coding repeat expansions are common.[41] Of interest, 2 patients were found to have pathogenic repeat expansions associated with Huntington disease which was unexpected as the patients did not present with typical clinical features of HD. Both patients had presented with ataxia, without evidence of chorea or other HD-specific findings, including a negative family history. This observation warrants further investigation and perhaps a closer look for the presence of *HTT* expansions in patients presenting with sporadic ataxia. Overall, this work demonstrates that ExpansionHunter is a viable bioinformatic tool for increasing the diagnostic yield in ES.

In conclusion, these data show that, despite high diagnostic yield, patients continue to face barriers to pursuing exome sequencing. Our detailed examination of insurance barriers to ES demonstrate that some insurers continue to deny coverage for ES while others are still selectively denying ES for adult patients based on outdated claims, such as lack of clinical utility for ES. More work is needed in this area to better assess how these practices affect patients with adult neurologic diseases to allow for future advocacy efforts to address these major health care issues. This work also highlights the additional value of new computational tools in detecting common pathogenic variants in adult neurology, such as repeat expansions, through ES. There is limited published data on the diagnostic use of ExpansionHunter in exome sequencing for patients with adult neurologic presentations and our data support its potential efficacy in this area. Further investigation of the sensitivity and specificity of this tool in these and other clinical populations is important before widespread use in clinical practice. However, should this and other similar tools prove useful, they may further streamline the genetic testing process, bringing us closer to achieving a "one-test-fits-all" model for genetic evaluation.

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Appendix *(continued)*

Name	Location	Contribution
Eugene Lee, PharmD, PhD	3billion, Inc.	Analysis or interpretation of data
Gohun Seo, MD, PhD	3billion, Inc.	Drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data
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