Diagnostic yield of genome sequencing in children with progressive movement disorders

Luca Schierbaum,[1] Enrique Gonzalez Saez-Diez,[1,2] Amy Tam,[1] Joshua Rong,[1] Umar Zubair,[1] Katerina Bernardi,[1] Kathryn Yang,[1] Vicente Quiroz,[1] Zainab Zaman,[1] Afshin Saffari,[1] Siofra Carty,[1] Habibah A. P. Agianda,[1] Sanda Alexandrescu,[3] Florian Eichler,[4] Abigail Sveden,[5] Maya Chopra,[5] Daniel G. Calame,[6,7] Matt C. Danzi,[7,8] Stephan Zuchner[7,8] and Darius Ebrahimi-Fakhari[1,7]

Abstract

Childhood-onset movement disorders are clinically and genetically heterogeneous, with over 500 implicated genes. Standard clinical genetic testing, including exome sequencing, has limited sensitivity for certain variants, including repeat expansions, structural variants (SVs), copy number variants (CNVs), and deep intronic changes. We evaluated the diagnostic utility of short-read whole genome sequencing (srWGS) and, in selected cases, long-read genome sequencing (lrWGS) in a real-world cohort of children and young adults with early-onset progressive movement disorders and prior nondiagnostic genetic testing.

One hundred individuals (<30 years) with progressive movement disorders with a suspected genetic etiology were recruited from a tertiary pediatric movement disorders program. All had prior nondiagnostic testing. SrWGS (Illumina NovaSeq 6000) assessed single nucleotide variants (SNVs), CNVs, SVs, and repeat expansions; lrWGS (Pacific Biosciences) was applied to select unsolved trios. Variants were reviewed by a multidisciplinary team using standard variant interpretation guidelines and phenotype correlation.

A molecular diagnosis was achieved in 27% (27/100) of cases, and candidate variants were identified in an additional 33% (33/100). Among solved cases, 81.5% (22/27) were identified from exome-level data, while 18.5% (5/27) required genome-level analysis to detect variants such as repeat expansions in *HTT* and *FXN*, an intragenic duplication in *MECP2*, an *Alu* insertion in *ATM*, and a deletion in *FA2H*. Genome-level analysis contributed an additional diagnostic yield of 5% (5/100) only. Notably, in 33.3% (9/27) of solved cases, variants had been previously reported but not recognized as diagnostic. LrWGS of 14 unsolved trios did not yield additional diagnoses.

SrWGS provided a modest incremental yield over exome sequencing in early-onset movement disorders, with most diagnoses achieved through reanalysis of exome-level data. Findings highlight the importance of iterative variant interpretation and the need for improved analytic pipelines to fully realize the potential of genome sequencing.

Author affiliations:

1 Movement Disorders Program, Department of Neurology and F.M. Kirby Neurobiology Center, Boston Children's Hospital, Harvard Medical School, Boston, MA, 02215 USA

2 Medical Faculty, Heidelberg University, Heidelberg, 69120, Germany

3 Department of Pathology, Boston Children's Hospital, Harvard Medical School, Boston, MA, 02115, USA

4 Department of Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA, 02115, USA

5 Rosamund Stone Zander Translational Neuroscience Center, Boston Children's Hospital, Boston, MA, 02115, USA

6 Section of Pediatric Neurology and Developmental Neurosciences, Department of Pediatrics, Baylor College of Medicine, Houston, TX, 77030, USA

7 Spastic Paraplegia - Centers of Excellence Research Network (SP-CERN) – RDCRC, Bethesda, MD, 20824, USA

8 Dr. John T. Macdonald Foundation Department of Human Genetics and John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, FL, 33136, USA

Correspondence to: Prof Darius Ebrahimi-Fakhari

Department of Neurology

Boston Children's Hospital

300 Longwood Avenue, Boston, MA 02215, USA

E-mail: darius.ebrahimi-fakhari@childrens.harvard.edu

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Introduction

Childhood-onset movement disorders represent a growing area at the intersection of clinical neurology, neuroscience and genetics. The spectrum ranges from hyperkinetic disorders, such as dystonia, chorea, and myoclonus, to less common hypokinetic disorders. Mixed phenomenologies combining more than one movement disorder are common, and additional motor disorders such as spasticity and ataxia frequently coexist, adding diagnostic complexity. Accurate evaluation requires an integrated approach combining detailed clinical assessment, neuroimaging, and genetic testing. While acquired causes, such as hypoxic/ischemic injury, autoimmune conditions, and CNS infections remain relevant, a substantial proportion of pediatric movement disorders have a genetic basis. More than 500 disease-causing genes have been implicated to date, underscoring the central role of genetic testing in clinical care[1].

Whole exome sequencing (WES) has become the standard first-line test in many clinical settings. Despite this, a considerable proportion of patients with suspected genetic movement disorders remain undiagnosed. This persistent diagnostic gap reflects several challenges intrinsic to genomic medicine and particularly relevant in childhood-onset movement disorders, including: (i) extensive genetic heterogeneity, where variants in different genes yield overlapping phenotypes; and (ii) phenotypic pleiotropy, where variants in the same gene produce divergent clinical presentations. Retrospective studies in pediatric movement disorders report diagnostic yields of 51–56% using WES, gene panels, or targeted testing strategies[2,3], but vary by dominant phenotype: 60–70% for spasticity[4], 12.6–66.7% for dystonia, 33.2–55.2% for ataxia, and 9–26.9% for parkinsonism[1,5]. These findings highlight the dual challenge of undetected variants in known disease genes and the ongoing discovery of novel disease genes.

Whole genome sequencing (WGS) extends diagnostic potential by enabling the detection of complex structural variants, repeat expansions, deep intronic variants, small indels, and variants in regions poorly captured by WES. Short-read WGS (srWGS) remains the most widely implemented approach, offering cost-effectiveness, broad availability, and a low error rate (0.1–0.5%)[6]. Long-read sequencing (lrWGS) technologies generate much longer DNA fragments, enabling more accurate resolution of structural variants, repetitive regions,

and complex genomic rearrangements. This capacity has generated considerable enthusiasm for their potential to further improve diagnostic outcomes, particularly in disorders with heterogeneous genetic architectures. However, the added diagnostic yield and clinical impact of srWGS or lrWGS in pediatric movement disorders remain unknown.

Here, we evaluated the diagnostic contribution of srWGS in a real-world cohort of children and young adults with early-onset progressive movement disorders and prior nondiagnostic genetic testing. For a subset of unresolved cases, we further explore lrWGS. This study provides an in-practice evaluation of genome sequencing within a tertiary pediatric movement disorders program, highlighting both current capabilities and persisting limitations.

Materials and methods

Cohort Characteristics

This study was approved by the Institutional Review Board at Boston Children's Hospital (BCH; IRB-P00039630). Written informed consent was obtained from all participants or their legal guardians. One hundred individuals (<30 years) with childhood-onset progressive movement disorders of unclear etiology were enrolled in the Hereditary Spastic Paraplegia Genomic Sequencing Initiative (HSPseq; NCT05354622). The age cutoff of 30 years was chosen to capture the full spectrum of early-onset and young-adult progressive movement disorders seen in our pediatric and transitional clinics while maintaining a uniform cohort. Inclusion criteria were: (i) onset of a movement disorder in childhood, (ii) progressive clinical course, and (iii) absence of a confirmed genetic diagnosis. All participants were evaluated between March 2023 and May 2025 at a tertiary academic pediatric movement disorders program. Phenotypes were systematically documented using Human Phenotype Ontology (HPO) terms. Clinical evaluations included detailed histories, comprehensive neurological examinations, and review of prior medical records and genetic testing. Additional investigations comprised brain and spine MR imaging, EEG, cerebrospinal fluid analysis, and relevant laboratory studies, as indicated. Trios consisted of one affected proband and two unaffected parents in all cases unless otherwise specified. A small number of extended pedigrees with multiple affected siblings were sequenced as part of expanded trios or "trio + " analyses.

Sequencing and Variant Calling

This project utilized the workflow established by the Children's Rare Disease Collaborative (CRDC) (Suppl. Fig. 1A)[7,8]. Genome sequencing was conducted on genomic DNA derived from blood samples or buccal swabs. SrWGS was performed at GeneDx, a CLIA-certified laboratory, on the Illumina NovaSeq 6000 platform. For short-read data, the mean alignment coverage over the QC region was 44.4 ± 10.1, with 93.5% ± 2.4 of bases achieving ≥20× coverage, and a mean raw read length of 149.9 ± 0.8 (*Supplementary Table 1*). Variant calling for SNVs, CNVs, and SVs was conducted by the BCH Research Computing Department using the DRAGEN pipeline. A parallel analysis was performed using the GENESIS platform including the MAVERICK AI based variant prioritization tool[9,10]. Pathogenic repeat expansions were profiled with ExpansionHunter v5.0.0 on the DRAGEN-aligned CRAMs using the Illumina GRCh38 reference and companion hg38 STR variant catalog[11]. Candidate loci included *AFF2, AR, ATN1, ATXN1/2/3/7/8OS, CACNA1A, CBL, CNBP, CSTB, DIP2B, DMPK, FMR1, FXN, GIPC1, GLS, HTT, JPH3, NIPA1, NOP56, NOTCH2NL, PABPN1, PHOX2B, PPP2R2B, RFC1, TBP, TCF4*, and other genes relevant to movement disorders. Calls were filtered against established pathogenic/premutation thresholds. The realigned BAMs were sorted and indexed with samtools v1.17 and visualized with REViewer v0.2.7[12]. REViewer plots were inspected to confirm repeat size estimates, flanking-read support, and alignment integrity; only visually validated expansions with appropriate familial segregation were classified as pathogenic or likely pathogenic.

Long-Read Sequencing

Cases were prioritized for long-read sequencing based on (i) strong clinical suspicion of a monogenic etiology, (ii) candidate structural variants (copy-number, repeat, or other complex rearrangements) not fully resolved by srWGS, or (iii) familial recurrence suggestive of an undetected segregating variant. lrWGS was performed for selected unsolved trios through the CRDC - Broad Institute collaboration using the Pacific Biosciences HiFi platform and associated alignment and variant-calling pipeline. For long-read data, the mean sequencing depth was 27.6 ± 7.5, the mean read quality was 33.3 ± 1.4, and the mean read length was 15,581.7 ± 1,877.3 (*Supplementary Table 2*). PacBio HiFi data were processed with SMRT Link v12.0 and pbsv for variant calling, followed by tertiary analysis using Sequence Miner and Gregor on the GeneDx Discovery Platform.

Variant Prioritization and Classification

SNVs and small indels were prioritized in Emedgene, Wuxi and GENESIS based on criteria including population allele frequency (gnomAD v.4.1.0., gnomAD SVs v.4.1.0., gnomAD CNVs v.4.10., Database of genomic variants), prior ACMG classification (ClinVar), gene-phenotype associations, gene constraint scores (gnomAD v.4.1.0.), *in silico* prediction tools (CADD, REVEL, SpliceAI, MAVERICK, AlphaMissense, PolyPhen, phyloP, Pangolin, ClinGen Dosage Sensitivity), protein function, tissue expression databases (GTex, Human Protein Atlas), and segregation analysis. Cases were reviewed by an interdisciplinary team (movement disorder specialists, neurologists, geneticists, bioinformaticians, researchers, and genetic counselors; Suppl. Fig. 1B). Variants were classified as:

i) No variant of interest: No variant met restrictive or permissive filter criteria for quality, segregation, allele frequency, deleteriousness, or phenotypic overlap.

ii) Candidate variant: Rare and predicted deleterious by *in silico* tools, partial phenotypic concordance.

iii) Diagnostic variant: Met clinical diagnostic standards and variants were independently confirmed at a CLIA-certified diagnostic laboratory (GeneDx).

For analytic purposes, we define *exome-level analysis* of srWGS data as variant calling restricted to exonic regions within the srWGS dataset, directly comparable to WES, whereas *genome-level analysis* refers to the inclusion of non-coding, intronic, and structural variant calls.

Statistical Analysis

Analyses were performed using GraphPad Prism v10.0. Fisher's exact test assessed associations between categorical variables. Figures were created in GraphPad Prism.

Results

Cohort Characteristics

All referrals were for second-opinion evaluation at the Boston Children's Hospital Movement Disorders Program. SrWGS was performed for 100 individuals and, where available, parents and additional relatives; lrWGS was undertaken in 14 trios (see below). A

mixed phenomenology was common (49%, 49/100), and only 51% (51/100) presented with a single dominant movement disorder (Fig. 1A). Spasticity predominated (41%, 41/100), followed by dystonia (22%, 22/100) and ataxia (8%, 8/100); in 15%, a leading phenomenology could not be assigned (Fig. 1B) due to a complex mixed clinical presentation.

The cohort comprised 45 females and 55 males (median age [IQR]: females 9 [5–17] years; males 11 [6.5–15] years) (Fig. 1C). Trio data were available for 61% (61/100, with additional relatives in 5%, 5/100); duos comprised 22% (22/100, including duo plus additional relative 2%, 2/100), and singletons 12% (12/100, including singleton additional relative 1%, 1/100) (Fig. 1D). Self-reported background was largely non-Finnish European (46%, 46/100), with 14% mixed background (14/100) and 12% Hispanic (12/100); other backgrounds were also represented (Fig. 1D). Consanguinity was reported in 8% (8/100). Prior clinical genetic testing was typical of clinical practice in the United States: Most had undergone WES (67%, 67/100), alongside single-gene tests (20%, 20/100), multigene panels (37%, 37/100), and chromosomal microarray (42%, 42/100) (Fig. 1G–H).

Diagnostic Yield of srWGS

A molecular diagnosis was established in 27% (27/100, Supplementary Table 3); an additional 33% (33/100) harbored candidate variants (Fig. 2A, Supplementary Table 4). Of solved cases, 81.5% (22/27) were identifiable from exome level analyses, whereas 18.5% (5/27) required genome-level analyses. Genome-level findings included repeat expansions in *HTT* and *FXN*, an intragenic duplication in *MECP2*, an *ATM* Alu insertion, and a deletion in *FA2H* (Fig. 2B,F; Supplementary Table 3). The incremental yield attributable to genome-level analysis was therefore 5% (5/100). At the genome level, candidate variants (CNVs, STRs, deep intronic variants) were identified in 21.2% (7/33) of the candidate variant cohort (Fig. 2B; Supplementary Table 4).

Parental availability influenced outcomes: Trio analyses were associated with higher rates of diagnosis or candidate identification versus incomplete parental data (Fisher's exact, p=0.0024; Fig. 2C). In contrast, neither the analysis level (WES vs srWGS) nor the number of movement disorders present in an individual (as a proxy for disease complexity) was associated with outcome (Fisher's exact, p>0.99 and p=0.305, respectively; Fig. 2D). Patients who previously underwent WES, as opposed to other non-WES genetic tests, had no different diagnostic yield (26.9% vs. 27.3%, Fisher's exact test, p=0.100).

Diagnostic Yield of lrWGS

Long-read whole-genome sequencing was performed in 14 unsolved or candidate trios with a focus on SVs, CNVs, and repeat expansions. In all cases, lrWGS confirmed findings obtained with srWGS, but no additional diagnoses or candidate variants were identified. These results highlight current limitations, including enhanced pathogenicity predictions for non-coding variants, improved structural variant calling, incomplete normative datasets for rare structural changes, and the need for further refinement of lrWGS analysis software.

Illustration of cases solved on WGS level

Intragenic MECP2 duplication

This 13-year-old female was first evaluated in our program for a complex mixed hyperkinetic movement disorder characterized by generalized chorea, limb dystonia, segmental upper-body myoclonus, orofacial dyskinesia, and prominent hand stereotypies (Video_01: axial hypotonia and weakness and dystonic posturing with sustained fisting of the hands and curling of the toes). She received prior care outside of the United States and had a history of early global developmental delay with regression, acquired microcephaly, and medically refractory epilepsy. Previous investigations, including MR imaging of the brain and cerebrospinal fluid (CSF) analyses, were unremarkable. Clinical genetic testing prior to the referral, including WES, karyotype analysis, chromosomal microarray, and an intellectual disability gene panel, was non-diagnostic. Trio srWGS identified a 16.7 kb intragenic duplication in *MECP2* affecting exons 3 and 4 (Suppl. Fig. 2A). This finding established a diagnosis of *MECP2*-related disorder (OMIM #300005), with the variant classified as pathogenic. The patient was referred to a specialized clinic, where the patient is now monitored and cared for by an expert team, and the family was connected with *MECP2*-specific resources.

Ataxia-telangiectasia due to an Alu transposable element

A 19-month-old female was evaluated for suspected ataxia-telangiectasia (AT, OMIM #607585) after newborn screening indicated severe combined immunodeficiency and alpha-fetoprotein levels were found to be elevated. Trio WES identified two paternally inherited *ATM* variants in *cis* - a pathogenic nonsense variant (NM_000051.3: c.5644C>T; p.(Arg1882Ter)) and a VUS (c.7135C>G; p.(Leu2379Val)) - but no variant in *trans*. Functional studies confirmed absent ATM activity across all lymphocyte subsets. Targeted srWGS review revealed abnormal read alignment and increased coverage in exon 50, suggesting a

structural variant. Soft-clipped read analysis identified a maternally-inherited 283 bp AluYa5 insertion within exon 50 (Suppl. Fig. 2B), enabling CLIA confirmation and classification as pathogenic[13]. Heterozygous Alu insertions in *ATM* have been implicated in cancer predisposition syndromes, highlighting clinical relevance[14]. Although targeted antisense oligonucleotide therapy was not deemed feasible, the diagnosis guided supportive management, cancer surveillance, and genetic counseling for the patient and family.

Diagnostic Yield of Exome-Level Reanalysis

Of the 27 solved cases, 81.5% (22/27) were resolved through exome-level analysis of srWGS data. These variants were not counted toward the incremental yield of WGS since that could have been detected on WES. Most (96.3%, 26/27) had prior genetic testing; 66.7% (18/27) had undergone clinical WES between 2018–2024. In 33.3% (9/27) of solved cases, the causative variant had been reported previously but not recognized as diagnostic. In two cases, multicenter collaborations established novel disease–gene associations (*LHX2, RBBP5*)[15,16]. Five additional cases involved atypical presentations reclassified from VUS to likely pathogenic or pathogenic based on emerging literature. Variants in established disease genes were identified in 12 patients with typical presentations (*GCH1, KIF5A, MECP2, DNM1, ATM, FA2H, FGF14, SLC2A1*; *Supplementary Table 3*; Fig. 2E). In two additional patients with typical presentations, prior clinical WES failed to detect exonic repeat expansions (*FXN, HTT*) that were identified by our analysis. Furthermore, nine patients harbored variants in known disease genes but presented with expanded or atypical phenotypes (*XPNPEP3, SOX6, RARB, LONP1, EIF2AK2, RINT1, COQ4, TUBA4A, FBXO31*; *Supplementary Table 3*).

Novel Disease Genes

RBBP5-related disease

An 18-year-old female with early-onset epilepsy–dyskinesia syndrome presented with generalized dystonia, chorea, lower limb spasticity, microcephaly, bilateral sensorineural hearing loss, dysphagia, growth failure, and retinal dystrophy. At 16 years of age, she experienced acute regression characterized by an exacerbation of her dystonia and chorea with loss of ambulation. At the time of evaluation, her movement disorder consisted mainly of orofacial dyskinesia, limb chorea, segmental myoclonus, and stereotypies (Video_03: progression to generalized dystonia with fulltime wheelchair use). MR imaging of the brain

revealed slightly small, rounded thalami, mild cerebral atrophy, under-rotated hippocampi. Extensive prior testing was nondiagnostic except for a heterozygous nonsense *RBBP5* variant (NM_005057.4: c.762G>A; p.(Trp254Ter)), classified as a VUS. Duo srWGS confirmed this finding with no additional compelling variants identified. *RBBP5* encodes a core component of the KMT2B-WDR5 complex which is highly intolerant of predicted loss-of-function variant (pLI=1, LOEUF=0.14). Methylation analysis showed a global methylation pattern consistent with *KMT2B*-related disorder[17], we therefore deemed the *RBBP5* variant diagnostic. Clinical overlap with *KMT2B*-related dystonia suggests deep brain stimulation may be beneficial, though the family declined this intervention at this time.

Notable Molecular Findings in Established Disease-Associated Genes

FGF14-associated ataxia

A 4-year-old male presented with global developmental delay, tremor, and feeding difficulties. Pregnancy, delivery, and early postnatal history were unremarkable. He pulled to stand at 18 months and walked at 24 months with notable balance issues and an ataxic gait. At age two, a low-amplitude, high-frequency postural tremor of the hands emerged without functional impact. Feeding was limited by choking on solids and saliva, prompting thickened liquid recommendations. Brain MRI at 1 year revealed a right frontal cranial defect suggestive of sinus pericranii. Neurologic exam demonstrated mild hypotonia and ataxic gait. Chromosomal microarray (CMA) detected a heterozygous paternally inherited 13q33.1 deletion (212 kb) involving *FGF14* (NM_004115.3), confirmed by srWGS (Suppl Fig. 2D). The variant was classified as likely pathogenic. Pathogenic coding variants in FGF14 cause spinocerebellar ataxia type 27A (SCA27A, OMIM #193003), typically adult-onset, though early-onset and more severe phenotypes have been associated with structural variants[18–22]. The proband's father, who carries the same deletion, manifests only mild dysarthria. Given emerging therapeutic avenues - 4-aminopyridine and deep brain stimulation[23] - the deletion may hold future treatment relevance.

NTN1-associated congenital mirror movements

An 8-year-old male presented for evaluation of prominent congenital mirror movements, in the setting of global developmental delay, autism spectrum disorder, and ADHD. He sat at 11 months and walked at 21 months; mirror movements started in early infancy and persisted, impairing bimanual coordination. Additional features included toe-walking, frequent falls, sensory-seeking behaviors, poor safety awareness, and episodic mutism when overstimulated. Brain MRI was normal. Trio srWGS identified a paternally inherited likely pathogenic missense variant in *NTN1* (NM_004822.3: c.1132C>T, p.(Arg378Cys)).

NTN1 encodes netrin-1, critical for axon guidance and synaptogenesis[24], and pathogenic variants cause congenital mirror movements type 4 (OMIM #618264). The proband's father did not exhibit mirror movements, consistent with reduced penetrance[25]. Neurodevelopmental phenotypes beyond mirror movement have not been described thus far and warrant further confirmation.

FA2H-associated hereditary spastic paraplegia (SPG35)

This 29-year-old male presented for a second opinion evaluation in the setting of progressive lower limb spasticity since his early 20s, later complicated by balance issues, impulse control changes, and depressive symptoms. Examination revealed hyperreflexia, Babinski signs, sustained ankle clonus, and a spastic gait. Brain MRI showed thinning of the corpus callosum, posterior ventriculomegaly, cerebellar and pontine atrophy, and stable symmetric T2 hyperintensities. Trio srWGS detected biallelic *FA2H* variants: a maternally inherited missense change (NM_024306.4: c.896 C>G, (p.Thr299Ser), classified as VUS) and a paternally inherited ~2 kb deletion encompassing exon 1 (classified as pathogenic). Prior clinical WES had been performed but failed to detect the exon 1 deletion due to limitations in CNV calling. Biallelic *FA2H* variants cause SPG35 (OMIM #611026), a complex HSP with variable onset and features including cerebellar ataxia, dysarthria, dysphagia, and cognitive decline[26]. This adult-onset presentation underscores phenotypic variability and highlights the importance of developing more robust tools to call structural variants, including copy-number variants.

Notable Phenotype Expansions

LONP1-related mitochondrial cytopathy

A 7-year-old male presented with developmental delay, regression, ataxia, tremor, oculomotor apraxia, mild ophthalmoplegia, and distal muscle atrophy. Brain MRI showed cerebellar hypoplasia. At the time of evaluation, he required a walker for ambulation due to significant truncal and gait ataxia (Video_04: dysmetria and slow, ataxic gait requiring one-person assistance). Genomic analysis prioritized compound-heterozygous variants in *LONP1* (NM_004793.4: c.857C>T, p.(Thr286Met) / c.397C>G, p.(Pro133Ala)), a gene associated with CODAS (*Cerebral, Ocular, Dental, Auricular, and Skeletal Anomalies Syndrome*), given emerging evidence of a broader phenotypic spectrum[27]. Muscle biopsy revealed mitochondrial inclusion bodies, supporting the diagnosis, despite absent ocular features, again broadening the *LONP1*-associated phenotype beyond CODAS (Suppl. Fig. 2C). However, both variants remain classified as VUS. The patient was started on mitochondrial supplements, and the family was advised to avoid prolonged fasting and

medications known to impact mitochondrial function. Baseline screening, including an echocardiogram, revealed no abnormalities. A gene therapy for *LONP1*-related disorder is in preclinical studies[28].

RARB-related disorder without ophthalmologic abnormalities

A 10-year-old male presented with progressive spasticity, generalized chorea, orofacial dyskinesia, tics, and neurodevelopmental comorbidities. Chorea had been present since infancy, worsening in intensity, frequency, and duration, particularly with emotional stressors. Lower extremity spasticity led him to required assistance with walking (GMFCS III-IV) (Video_04: choreatiform movements of the upper and lower extremities, with lower extremity weakness and spasticity). Brain MRI at 8 and 11 months of age was normal, and CMA and a neuromuscular disease gene panel were non-diagnostic. Clinical WES revealed a *de novo* heterozygous VUS in *RARB* (c.836T>C (p.(Phe279Ser)), now classified as pathogenic, which was deemed inconclusive at the time. SrWGS reprioritized the *de novo* *RARB* variant as the most significant finding. This private variant, located in the AF2 region of the ligand-binding domain, was predicted to be damaging based on multiple *in silico* tools. *RARB* encodes the retinoic acid receptor beta, and variants in *RARB* have been implicated in syndromic microphthalmia 12 (*MCOPS12*, OMIM: 615524). A literature review revealed a recent report of a *de novo* *RARB* variant (p.Phe279Val) at the same amino acid position, shown to have a gain-of-function effect[18]. Additionally, a recent case series described 52 individuals with *RARB* variants, 15 of whom did not have microphthalmia and 4 of whom had no ophthalmologic abnormalities, thus expanding the phenotypic spectrum[18]. Following consultation with experts in MCOPS12, consensus was reached that the variant should be considered diagnostic.

De novo variant in FBXO31 mimicking cerebral palsy

A 13-year-old male with spastic diplegia (GMFCS III), intellectual disability, and MRI findings of “ears of the lynx,” thin corpus callosum, and periventricular white matter changes presented for a second opinion evaluation. Clinical WES performed in 2015 and 2024 were non-diagnostic. Trio srWGS identified a *de novo* missense variant in *FBXO31* (NM_024735.5, c.1000G>A, p.(Asp334Asn)), which was classified as pathogenic. The variant has not been reported in population databases and was predicted deleterious (AlphaMissense 0.995, CADD 26.5, SIFT 0). Recent publications have reported three individuals carrying *de novo* variants *FBXO31* with a cerebral palsy-like phenotype[29], including two unrelated individuals carrying the same *de novo* variant[30]. Accurate genetic testing enabled diagnostic closure and anticipatory guidance.

Notable Clinical Implications of Genomic Findings

Cases with clinical diagnoses were evaluated for their implications in patient care. A genetic diagnosis was deemed actionable based on ClinGen-derived categories as suggested by Lewis et al.[31]. Among the 27 solved cases, 40.7% (11/27) were determined to have direct clinical implications defined as impact on i) treatment options (*FXN, GCH1, MECP2, COQ4, EIF2AK2, SLC2A1*) or ii) option for targeted surveillance and prevention (*XPNPEP3, RINT1, LONP1, COQ4, ATM*) (Fig. 2E).

XPNPEP3-related disorder

This 8-year-old male was referred for a second opinion regarding progressive lower limb spasticity and gait impairment. Examination revealed lower extremity spasticity (GMFCS I–II), bilateral ankle clonus, a positive Babinski sign, and hyperreflexia. Trio srWGS identified biallelic variants in *XPNPEP3* - a frameshift (NM_022098.4: c.1215del, p.(Lys406ArgfsTer42)) and a canonical splice-site variant (c.856-2A>G), both classified as likely pathogenic. *XPNPEP3* encodes X-Prolyl Aminopeptidase 3, a mitochondrial protein associated with autosomal recessive nephronophthisis-like nephropathy 1 (OMIM: 613553). Reported features include renal insufficiency, tremor, developmental delay, seizures, sensorineural hearing loss, and gout[32]. Given the potential risk of nephropathy, the patient now undergoes regular surveillance.

DNM1-related disorder

This 10-year-old male presented with early hypotonia, progressive spastic quadriparesis, refractory epilepsy, and visual impairment. Brain MRI demonstrated delayed myelination. Prior CMA and clinical WES were non-diagnostic. Trio srWGS identified a *de novo* pathogenic splice-site variant in *DNM1* (NM_001288737.2, c.1197-8G>A), a gene associated with developmental and epileptic encephalopathy 31A (OMIM: 616346) via a dominant-negative mechanism[33]. Recent mouse models of dominant-negative DNM1 variants have shown therapeutic benefit from a knockdown-replace approach using AAV-based gene therapy[34], raising the possibility of future trial eligibility for this patient.

Incidental findings

Incidental findings meeting the criteria established by the American College of Medical Genetics and Genomics (ACMG) were identified in two cases. Detection of a maternally inherited pathogenic *ATM* variant discussed above led to identification of heterozygous carriers within this at-risk family. In a separate family, we identified a pathogenic deletion

encompassing *PMS2*, associated with Lynch syndrome 4 (OMIM 600259) in the proband and her mother. Given the associated cancer predisposition, these findings enable targeted surveillance and early intervention[35,36].

Discussion

In this study, we evaluated the diagnostic utility of srWGS in a real-world cohort of 100 individuals with childhood-onset movement disorders and previously non-diagnostic clinical genetic testing. A molecular diagnosis was established in 27% of cases. In five individuals (5%), the diagnosis was made based on genome-level findings, including repeat expansions, structural variants, CNVs, and a transposable element insertion, highlighting the incremental yield of srWGS beyond exome-based methods and other standard clinical genetic tests (eg. CMA). This finding is consistent with prior studies in rare disease cohorts, which estimate an added yield of 5–10% for genome sequencing over WES[37].

Our results emphasize several key insights. Most diagnoses (81.5%) were achieved through exome-level analysis, reaffirming the utility of WES while emphasizing the need to refine genome interpretation pipelines. Over one-quarter of solved cases involved variants previously reported but not deemed diagnostic, reflecting the evolving nature of variant interpretation and the value of systematic reanalysis. Illustrating this, seven diagnoses were established through reclassification or confirmatory testing rather than technical novelty of srWGS. These include *RBBP5* (methylation analysis), *FGF14* (confirmatory CMA and clinical interpretation), and several cases where literature re-evaluation or phenotypic correlation reclassified prior VUS. This shows the value of integrated multidisciplinary interpretation. Notably, 92.6% (25/27) of solved cases involved known disease genes, 40.5% (11/27) represented phenotypic expansions, and only 7.4% (2/27) implicated novel genes, reinforcing the importance of precise genotype–phenotype correlation and the involvement of movement disorder specialists and clinical geneticists in variant interpretation.

Key illustrative cases underscore the power of integrating deep clinical phenotyping with advanced analytic methods. In one instance, manual visualization identified a transposable element insertion in *ATM* that had been missed by standard pipelines. In another, abnormal methylation patterns suggestive of perturbations in the *KMT2B* pathway supported a targeted search for related genes, confirming the prioritization of a pathogenic variant in *RBBP5*. Together, these examples highlight the value of coupling detailed

phenotypic characterization with sophisticated bioinformatics pipelines, and of leveraging multidisciplinary expertise to optimize variant interpretation.

Despite comprehensive genomic analysis, 40% (40/100) of cases remained unsolved. Contributing factors likely include the continued emphasis on coding regions, unrecognized non-Mendelian inheritance (digenic/oligogenic), reduced penetrance, and the inherent limitations of srWGS for certain variant types. Short-read sequencing relies on DNA fragments typically 100–150 base pairs in length, which can make it difficult to resolve complex structural variants, repetitive elements, or variants in poorly mapped regions of the genome. lrWGS), in contrast, generates much longer sequence reads (often >10 kilobases), allowing more contiguous assembly of genomic regions and more accurate detection of tandem repeat expansions, structural variants, mobile element insertions, and methylation patterns. These features have fueled enthusiasm for lrWGS as a potentially transformative tool in rare disease genomics. In our small subset of 14 unsolved cases, however, lrWGS did not increase diagnostic yield - likely reflecting immature analytic tools and limited reference datasets rather than a lack of utility or lack of biological relevance. Ongoing efforts to optimize algorithms and to systematically profile tandem repeats, SVs, and methylation signatures in long-read genomes are expected to enhance future diagnostic performance, as demonstrated by recent studies[38].

Clinical reclassification and variant interpretation remain significant challenges in practice. VUS in established disease genes are increasingly identified and demand careful clinical correlation. In our cohort, detailed phenotypic assessment was essential for prioritizing candidate variants and guiding reanalysis. Notably, phenotypic complexity - quantified by the number of movement disorder phenotypes - was not associated with higher diagnostic yield, suggesting that rarity or severity alone does not reliably predict genetic etiology. Trio-based analysis significantly improved yield ($p = 0.007$), reflecting the critical role of parental DNA in phasing compound heterozygous variants, confirming segregation, and filtering *de novo* events. Conversely, absence of parental samples limited interpretation, even in cases with strong clinical suspicion.

A genetic diagnosis carried actionable implications in 40.7% (11/27) of solved cases, including targeted treatments (e.g., ubiquinone supplementation in *COQ4*-related disease), preventive surveillance (e.g., *ATM*-related cancer predisposition), and eligibility for interventions such as deep brain stimulation (*EIF2AK2*-related dystonia). Beyond clinical impact, diagnosis facilitated genetic counseling, informed reproductive decisions, and connected families to support networks.

Recent benchmarking studies and cost analyses have refined our understanding of the cost-effectiveness and optimal implementation of srWGS in rare disease diagnostics. As

sequencing costs continue to decline and analytical workflows mature, srWGS is increasingly recognized as a cost-effective first-line test when compared to serial or tiered approaches involving chromosomal microarray and exome sequencing. Beyond capturing all classes of single-nucleotide and structural variation in a single assay, srWGS offers the added value of streamlined logistics, improved turnaround time, and reduced downstream testing costs. However, its implementation should be guided by clinical prioritization favoring patients with high suspicion for monogenic disorders, heterogeneous phenotypes, or inconclusive prior evaluations. The recent multicenter study by Wojcik *et al.*[37] provides strong empirical framework for this model, showing that srWGS yields an incremental diagnostic benefit of approximately 8% over exome sequencing, largely driven by structural, deep intronic, and repeat expansion variants, and can replace existing tiered testing strategies when integrated into comprehensive clinical workflows.

Our study has several limitations that warrant consideration. First, the cohort size - although representative of a real-world rare disease population - was modest, which may limit statistical power for subgroup analyses and reduce the generalizability of certain findings. Exemplifying this point, the nature and extent of prior clinical genetic testing varied across the cohort, in some cases due to limited insurance coverage. Second, despite using advanced bioinformatics pipelines, residual technical challenges persist in detecting or prioritizing specific variant classes, including deep intronic mutations, mobile element insertions, low-level mosaicism, and complex structural rearrangements, which may have contributed to missed diagnoses. Third, the analysis primarily focused on monogenic mechanisms and did not systematically assess polygenic risk factors, oligogenic interactions, or epigenetic modifications that may contribute to disease pathogenesis. Fourth, while reanalysis was performed in select unsolved cases, comprehensive longitudinal reanalysis was not uniformly applied across the entire cohort, potentially missing variants that have since been reclassified based on emerging evidence. Finally, the underrepresentation of certain populations - particularly individuals from diverse ancestries or those lacking parental DNA for trio-based sequencing - may introduce bias in diagnostic yield estimates and limit the applicability of findings to all patient populations.

In summary, srWGS is a valuable diagnostic tool for childhood-onset movement disorders, especially when combined with rigorous phenotyping and expert review. Its incremental yield over WES is modest but clinically meaningful, particularly for variant classes inaccessible to exome methods. Maximizing the impact of genome sequencing will require continued investment in long-read and multi-omic approaches, improved variant interpretation frameworks, systematic reanalysis, and broad, inclusive cohort representation.

Data availability

Data are available from the corresponding author upon reasonable request.

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Competing interests

There are no conflicts of interest.

Supplementary material

Supplementary material is available at *Brain* online.

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Figure legends

Figure 1 Cohort overview. (A) Distribution of movement disorder phenomenology: 49% of individuals presented with a single movement disorder, while 51% exhibited mixed phenomenology with two or more. **(B)** Predominant motor disorders included spasticity (41%), dystonia (22%), ataxia (8%), chorea (7%), other (7%), and unclear or mixed presentations (15%). **(C)** Pedigree structure showed that 61% were enrolled as trios; the remainder included trios with additional members (5%), duos (20%), duos with additional members (2%), singletons (11%), and singletons with additional members (1%). **(D)** Self-reported ancestry was Non-Finnish European in 46%, mixed ancestry in 14%, Hispanic in 12%, African or African American in 6%, Middle Eastern in 6%, South Asian in 5%, East Asian in 4%, and not reported in 4%. **(E)** Prior clinical genetic testing revealed that 13% had no prior testing, 29% had one test, 29% had two, 14% had three, and 14% had four or more.

(F) Among those with one prior genetic test, 51% underwent whole exome sequencing (WES), 20.7% a gene panel, 13.8% chromosomal microarray (CMA), 10% whole genome sequencing (WGS), and 3.4% single-gene testing. For those with two tests, 41% had WES, 25% CMA, 22% a gene panel, 3% WGS, and 6.9% another genetic test. Among those with three tests, 33.3% had WES, 22.2% CMA, 22.2% another test, 13.3% a gene panel, and 8.9% WGS. Of those with four tests, 21.9% each had WES, CMA, and a gene panel; 18.7% another test; 9.4% single-gene testing; and 6.3% WGS. For those with five tests, 20% each had WES, CMA, and single-gene testing; 16.7% a gene panel and another test; and 6.7% WGS.

Figure 2 Overview analysis outcomes. A) Diagnostic categories across the cohort showed that a confirmed genetic diagnosis was established in 27% of cases, a candidate variant of interest in 33%, and no variant of interest in 40%. Candidate variants were defined as rare variants with predicted deleteriousness and phenotypic overlap. **(B)** Among solved cases, 81.6% (22/27) of diagnoses were made based on exome-level findings, whereas 18.5% (5/27) required genome-level analysis. **(C)** The availability of parental DNA was significantly associated with diagnostic outcome (Fisher's exact test, p = 0.0024): with incomplete parental data, 6% of cases were solved, 18% had candidate variants, and 10% remained unsolved; in contrast, when both parents were available, 21% were solved, 15% had candidate variants, and 30% were unsolved. **(D)** Diagnostic outcomes stratified by movement disorder complexity showed that among solved cases, 51.9% (14/27) had a single movement disorder, 18.5% (5/27) had two, and 29.6% (8/27) had three or more. In the candidate variant group, 51.5% (17/33) had a single movement disorder, 21.2% (7/33) had two, and 30.3% (10/33) had three or more. Unsolved cases included 50% (20/40) with a single movement disorder, 35% (14/40) with two, and 15% (6/40) with three or more. **(E)** Classification of solved cases by gene and phenotype revealed that most diagnoses involved known disease genes with typical clinical presentations (e.g., *FXN*, *GCH1*, *MECP2*), while others represented phenotypic expansions (e.g., *RARB*, *COQ4*, *LONP1*) or newly implicated genes (*LHX2*, *RBBP5*). Genes with clinical implications are highlighted in bold and with an asterisk. **(F)** A flowchart summarizes diagnostic outcomes by level of analysis and variant type: of 100 individuals with childhood-onset movement disorders and prior nondiagnostic testing, 27 were solved; 22 were identifiable at the exome level, whereas 5 required genome-level analysis, including detection of transposable element insertions, tandem repeat expansions, CNVs, and intragenic duplications.

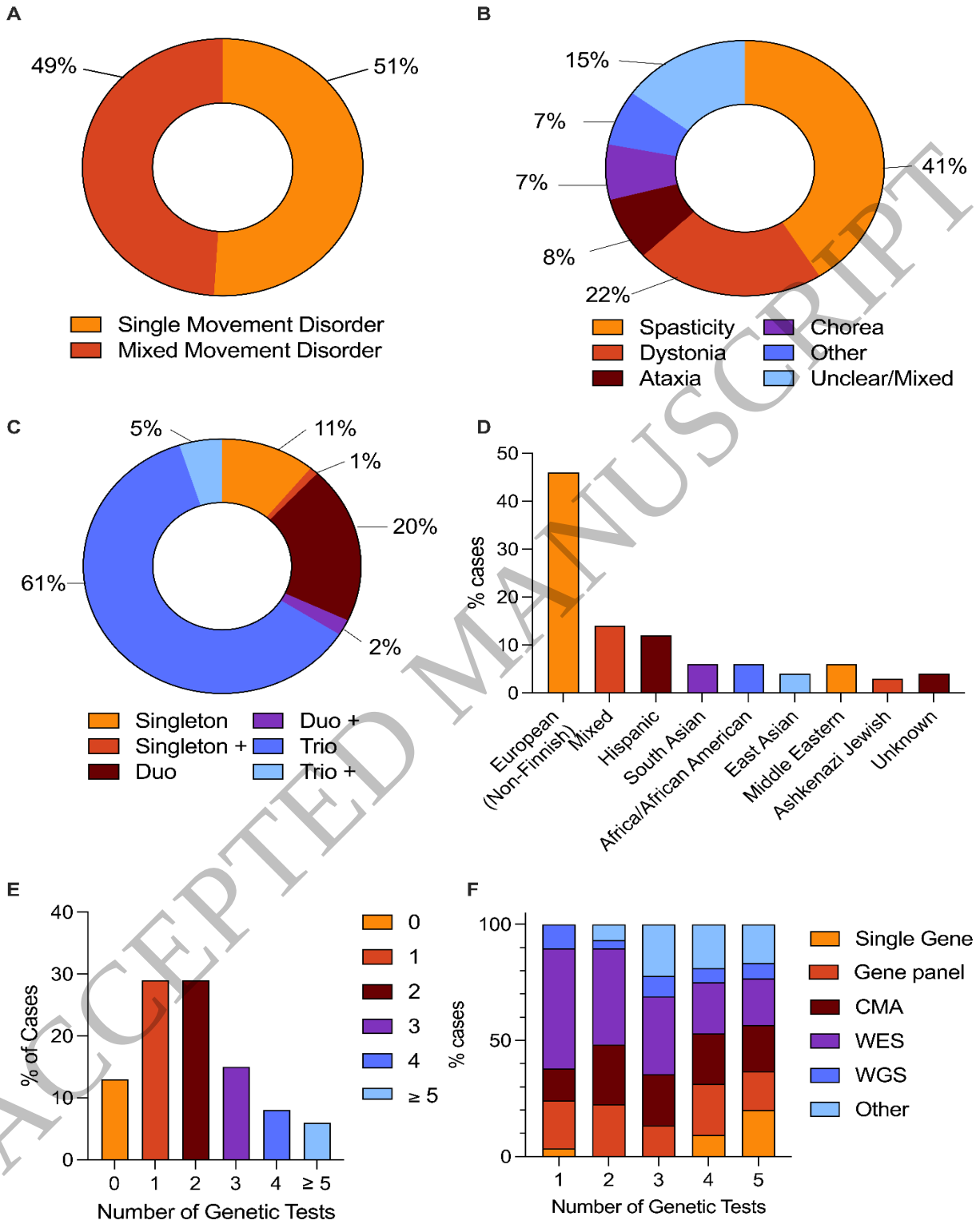

Figure 1
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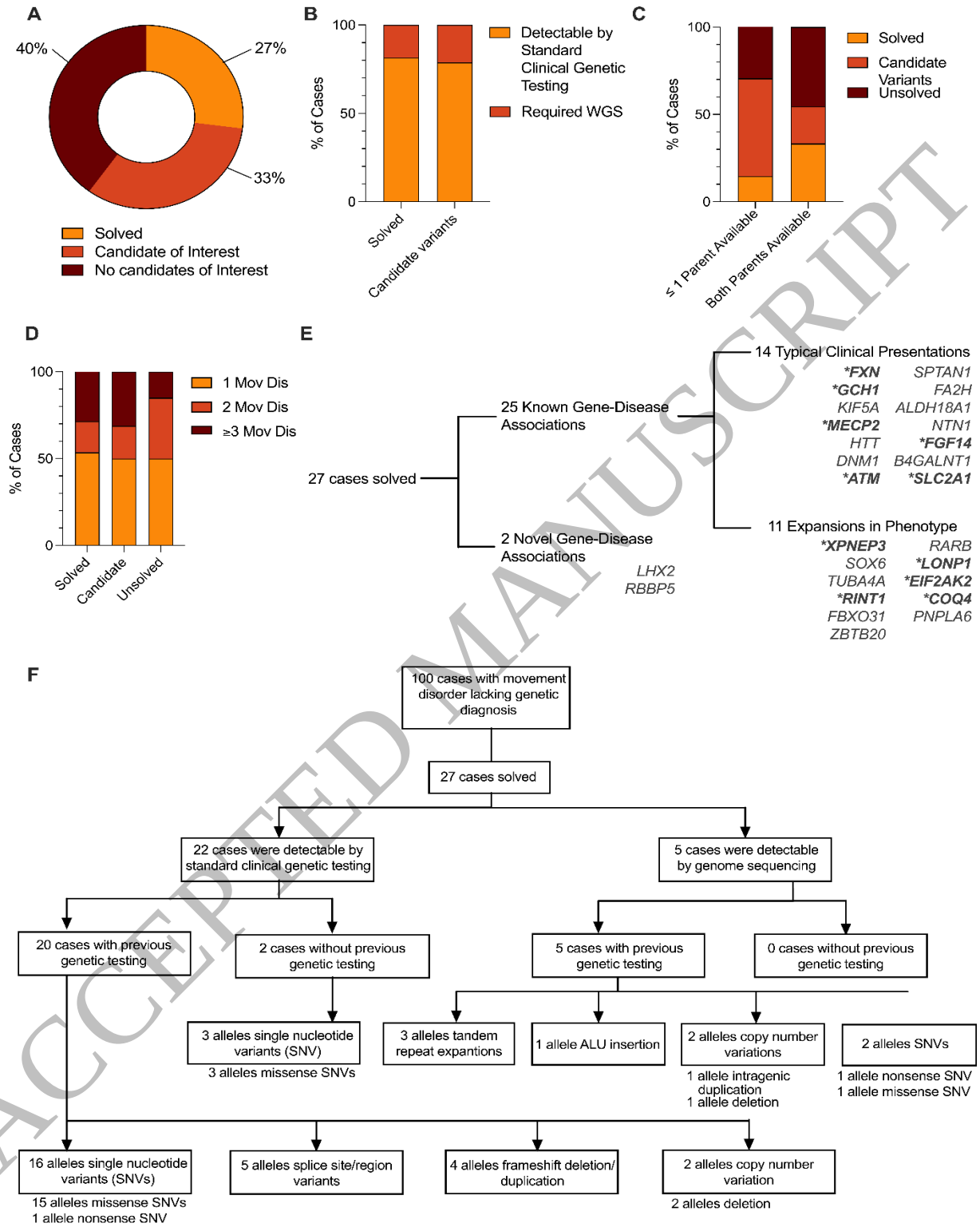

Figure 2
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