REVIEW

Long-Read Sequencing: The Third Generation of Diagnostic Testing for Dystonia

Thomas Wirth, MD,[1,2,3] Kishore R. Kumar, MBBS, FRACP, PhD,[4,5,6,7*] and Michael Zech, MD[8,9,10]

ABSTRACT: Long-read sequencing methodologies provide powerful capacity to identify all types of genomic variations in a single test. Long-read platforms such as Oxford Nanopore and PacBio have the potential to revolutionize molecular diagnostics by reaching unparalleled accuracies in genetic discovery and long-range phasing. In the field of dystonia, promising results have come from recent pilot studies showing improved detection of disease-causing structural variants and repeat expansions. Increases in throughput and ongoing reductions in cost will facilitate the incorporation of long-read approaches into mainstream diagnostic practice. Although these developments are likely to transform clinical care, there is currently a discrepancy between the potential benefits of long-read sequencing and the application of this technique to dystonia. In this review we highlight current opportunities and limitations of adopting long-read sequencing methods for the investigation of patients with dystonia. We provide examples of long-read sequencing integration into diagnostic evaluation and the study of pathomechanisms in individuals with dystonic disorders. The goal of this article is to stimulate research into the application and optimization of long-read analysis strategies in dystonia, thus enabling more precise understanding of the underlying etiology in the future. © 2025 The Author(s). *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: long-read sequencing; PacBio; Oxford Nanopore; dystonia; structural variants; repeat expansions; long-range haplotype phasing

Significant progress in genomic methodologies has led to the identification of a broad spectrum of genetic defects linked to dystonia.[1] Current advances in sequencing approaches have improved our ability to uncover unique and recurrent variations underlying different dystonic syndromes, resulting in a growing catalog of molecularly defined monogenic entities.[2] Despite this, at least half of patients with a suspected inherited form of dystonia remain undiagnosed.[3] Broadly, conditions that manifest with dystonia can be categorized into two groups: one group in which dystonia is the sole clinical abnormality, and another group in which dystonia is combined with additional movement, non-motor neurological, and/or systemic disorders.[4] For both groups, present gold standards of clinically-oriented sequencing with short-read sequencing (SRS) technology including short-read exome sequencing (srES) and short-read genome sequencing (srGS) have made it possible to discover a range of

[1]Neurology Department, Strasbourg University Hospital, Strasbourg, France; [2]Institute of Genetics and of Molecular and Cellular Biology (IGBMC), INSERM-U964/CNRS-UMR7104/Strasbourg University, Illkirch-Graffenstaden, France; [3]Strasbourg Translational Medicine Federation (FMTS), Strasbourg University, Strasbourg, France; [4]Translational Neurogenomics Group, Genomics and Inherited Disease Program, Garvan Institute of Medical Research, Darlinghurst, New South Wales, Australia; [5]Faculty of Medicine and Health, University of Sydney, Sydney, New South Wales, Australia; [6]Department of Neurology and Molecular Medicine Laboratory, Concord Repatriation General Hospital, Concord, New South Wales, Australia; [7]School of Clinical Medicine, UNSW Medicine & Health, UNSW Sydney, Sydney, New South Wales, Australia; [8]Institute of Human Genetics, Technical University of Munich, School of Medicine and Health, Munich, Germany; [9]Institute of Neurogenomics, Helmholtz Munich, Neuherberg, Germany; [10]Institute for Advanced Study, Technical University of Munich, Garching, Germany

*Correspondence to: Asst. Prof. Kishore R. Kumar, Molecular Medicine Laboratory, Hospital Road, Concord Repatriation General Hospital, Concord, NSW 2139, Australia. E-mail: kkum4618@uni.sydney.edu.au

Relevant conflicts of interest/financial disclosures: The authors report no disclosures regarding the present work.

Funding: No specific funding.

Received: 23 December 2024; **Revised:** 14 March 2025; **Accepted:** 2 April 2025

Published online in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.30208

pathogenic variants, including single-nucleotide variants (SNVs), small insertions and deletions (indels), and copy-number variants (CNVs).[5] Especially for the second group, an additional array of genomic alterations such as pathogenic short tandem repeats (STRs) and other structural variants (SVs) are believed to play a role, although evidence comes from a few case studies only.[5–7]

Intrinsic limitations of srES and srGS hinder the process of obtaining a comprehensive view of causal genetic variation in dystonia. This is associated with inherent uncertainties in precise diagnosis and the paucity of therapies targeting pathophysiological mechanisms.[1] Moreover, molecular testing in dystonia often continues to rely on tiered diagnostic strategies (eg, srES followed by srGS, or combinations of srES/srGS and chromosomal microarray or STR screening), with prolonged diagnostic journeys and avoidable cost-ineffectiveness.[1]

Long-read sequencing (LRS) methods including targeted LRS and genome-wide LRS are emerging as new promising tools to decipher the genetic underpinnings of rare disorders in a high-throughput fashion, potentially capable of replacing existing testing strategies by virtue of their great accuracy.[8–10] LRS has proven utility for the identification of diverse types of genetic changes in previously undiagnosed diseases.[8–10] Although LRS is expected to outperform conventional srES/srGS workflows, its role in dystonia has not yet been explored.

In this review we focus on potential applications and predicted benefits of LRS in dystonia. We summarize diagnostic outcomes of srES/srGS studies, and we explain shortcomings of srES/srGS with regard to detection of variants that can cause dystonic disorders. We describe technical details of the two main LRS platforms, Pacific Biosciences' (PacBio) single-molecule real-time (SMRT) sequencing and Oxford Nanopore Technologies (ONT) nanopore sequencing. Additionally, we review instances where LRS has already demonstrated impact on the accurate molecular diagnosis of dystonia, with further emphasis on prospects of integrating DNA-methylation and RNA analyses. We also recognize existing pitfalls for broad routine implementation of LRS assays. Finally, we highlight ongoing initiatives that study the potential of LRS, aiming at optimizing the genetic diagnostic pipeline. We hope that the aspects covered herein will provide a greater understanding of the latest developments in sequencing diagnostics and guide clinicians in future precision resolution of genomic variants that can be found in individuals with dystonia.

SRS Approaches: Diagnostic Yield and Limitations

SRS approaches are currently first-tier tests for genetic studies of dystonia. Different approaches can be used in clinical and in research settings including targeted gene panels, srES, and srGS. A summary of key published studies can be found in Table 1. The cohorts examined in these studies were characterized by heterogeneous types of dystonia, with preselection of participants who were more likely to have genetic disorders (eg, enrichment for cases with early disease onset, generalized symptom distribution, coexisting features, and/or history of consanguinity) in some of these studies (Table 1). Overall, the diagnostic yield is relatively low (9% to 41.7%, Table 1). The yield depends on the characteristics of the study sample (eg, age at onset, family history, clinical phenotype), as well as the genomic methodologies. A recent next-generation sequencing gene panel study which included 1207 participants with dystonia found a diagnostic rate of 9% (109/1207).[11] Other major gene panel studies of dystonia have also shown a diagnostic yield below 20%.[12,13,15] A major limitation of this approach is that it is limited to a panel of preselected genes,[12] which will miss clinically-relevant variants in genes outside of the panel.

By contrast, srES involves sequencing of the majority of protein-coding regions of a genome. A European multicenter study of 728 families with dystonia who were sequenced with srES found a diagnostic yield of 19% (135/728).[2] Through this study a scoring algorithm was developed to predict the diagnostic success rate of srES. The algorithm assigned higher likelihood for achieving a srES-based molecular diagnosis to those with younger onset (<21 years), generalized/segmental dystonia (versus focal), and those with combined dystonia or dystonia plus non-movement disorder-related neurological symptoms (versus isolated dystonia). In another study from France, srES was applied to a cohort of 32 individuals with early-onset or familial dystonia after gene panel sequencing, with an overall diagnostic rate of 34.4%.[16] The diagnostic rate was higher among patients with coexisting features as compared with patients with isolated dystonia.

Furthermore, srGS can provide sequencing information on nearly the entire genome including coding and non-coding regions. An early study of srGS identified a diagnostic yield of 11.7% for a cohort of 111 participants (13/111).[22] This diagnostic yield improved to 18.9% (21/111) following genomic reanalysis and gene discovery efforts, showing the added value of genomic reanalysis to boost the diagnostic rate in SRS experiments.[5]

Despite these results, there is a major diagnostic gap for genetic testing in dystonia (58.3–91%). This diagnostic gap may be explained by unknown disease genes and the limitations of SRS technology. For example, SRS is unable to sequence long stretches of DNA. Fragments of DNA are amplified and then assembled into a continuous sequence. This can introduce biases and there may be failure to generate a significant overlap between DNA fragments. This results in inaccurate

TABLE 1 *Summary of key studies for genetic testing in dystonia comparing the diagnostic yield for existing short-read sequencing genetic testing methods*

Study	Participants with dystonia	Selection criteria	Diagnostic approach	Diagnostic yield
Thomsen et al.[11]	1207 patients	Dystonia registry recruitment	Gene panel analysis	109/1207 (9%)
Van Egmond et al.[12]	61 patients	Tertiary referral for evaluation of dystonia	Gene panel analysis	9/61 (14.8%)
Wu et al.[14]	318 patients	Individuals with isolated or combined dystonia recruited from movement disorder clinics	Gene dosage analysis and next-generation sequencing panel	40/318 (12.6%)
Montaut et al.[13]	135 patients	Tertiary referral for evaluation of dystonia	Gene panel analysis	21/135 (15.6%)
Ma et al.[15]	65 patients	Dystonia as the single or main clinical feature	Targeted gene capture	12/65 (18.5%)
Zech et al.[2]	764 individuals (728 families)	Recruited through movement disorder and neuropediatric specialty centers, different types of dystonia including dystonic cerebral palsy	srES	135/728 (19%)
Zech et al.[6]	953 unrelated patients	Various types of dystonia	CNV detection from srES dataset	14/953 (1.5%)
Wirth et al.[16]	32 patients from 24 families	Early-onset or familial dystonia	srES	11/32 (34.4%)
Atasu et al.[17]	42 families	Increased rates of consanguinity (38%)	srES	11/42 (26%)
Powis et al.[18]	189 patients	Dystonia with high proportion of combined dystonia (57%)	Clinical diagnostic srES	41/189 (21.7%)
Dhar et al.[19]	65 patients	Patients with dystonia presenting as the predominant motor feature	srES	15/65 (23.1%)
Holla et al.[20]	48 patients	Retrospective review of patients with dystonia who had undergone clinical srES	Clinical srES	20/41 (41.7%)
Ahn et al.[21]	43 patients	Young-onset dystonia	srES	9/43 (20.9%)
Fellner et al. [5], Kumar et al.[22]	111 probands	Tertiary referral for evaluation of dystonia	srGS	11.7% (13/111); increased to 18.9% with reanalysis

Abbreviations: srES, short-read exome sequencing; CNV, copy number variant; srGS, short-read whole-genome sequencing.

genome assembly and difficulty detecting large SVs, STRs, and variants located in hard-to-sequence regions including highly repetitive areas.[23] These genetic changes may be relevant to the genetic diagnosis in a notable portion of patients with dystonia, and could be addressed through the advent of LRS.[24]

Methods of LRS

The limitations of srES and srGS drove the development of LRS, also known as third-generation sequencing. SRS typically has read lengths of 150 base pairs (bp).[9] In comparison, LRS technologies can generate sequence reads of tens of thousands of bases in length that can be sequenced in real time. The two main technologies for LRS are PacBio SMRT sequencing[25] and ONT nanopore sequencing[26] (Fig. 1). Broadly speaking, PacBio captures sequencing information by monitoring a polymerase as it replicates a circular target DNA molecule; ONT measures changes in ionic currents as a single-stranded DNA passes through a specifically designed nanopore protein.

Nanopore sequencers include MinION, GridION, and PromethION.[27] Their flowcells incorporate a membrane that has a grid of fixed nanopores; the membrane separates two ionic solutions allowing an electrical current to flow through the nanopores.[9] During the library preparation, protein adapters are linked to polynucleotides, which are then directed to nanopores, allowing for the initiation of sequencing. A template strand is passed through the pore by the potential difference across the membrane. As the polynucleotide advances

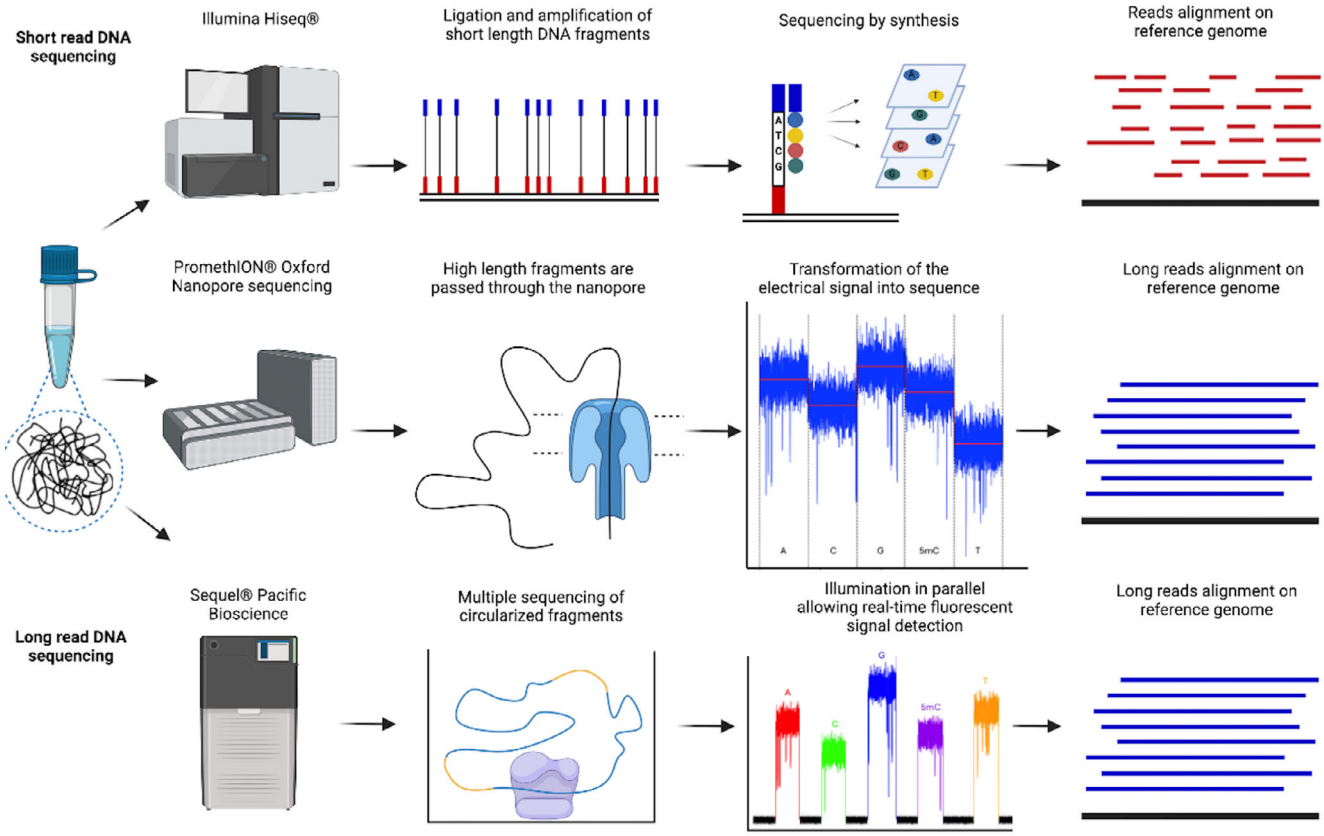

FIG. 1. Illustration of short-read sequencing (SRS) methods (example of Illumina Hiseq technology) and long-read sequencing (LRS). Examples of PromethION technology from Oxford Nanopore (ONT) sequencing and Sequel Pacific Biosciences' (PacBio) single-molecule real-time (SMRT) sequencing. In SRS, the DNA is fragmented, repaired, polyadenylated, and linked to indexed adapters which will allow their fixation on a solid support, the flowcell. Sequencing by chemical synthesis will generate short-reads (75–150 base pairs), which can be mapped and aligned by bioinformatics processing on the reference genome. Some complex regions of the genome will remain poorly covered. In LRS using nanopore sequencing, high molecular weight DNA fragments pass through a nanopore. The passage of these long fragments modifies the transmembrane electric current at the level of the flowcell, and depending on the nature of the base passing through the nanopore, the modification of the signal will be different, and can ultimately be reconstituted in the form of a sequence of long-reads (>10 kb). In LRS using SMRT HiFi, high molecular weight DNA fragments are circularized using specific adapters. Fluorescent dinucleotides are added by a polymerase, their fluorescence being recorded in real time. The DNA sequence is then reconstituted. For both technologies, the resulting long-reads are easily aligned to the reference genome with excellent coverage of target regions. Created with BioRender.com.

through the pore, there is a unique disruption in current flow for each consecutive dimer of nucleotides.[9,27] The electric signal is subsequently recorded and processed to reconstitute the DNA fragment sequence.[27] An interesting aspect of ONT sequencing is adaptive sampling, which leverages real-time analysis of the sequencing data to dynamically adjust the sequencing parameters, focusing sequencing efforts on regions of interest.[9] This technique improves the efficiency of data generation by targeting specific genomic regions, allowing for deeper coverage and more effective utilization of sequencing resources.[9] ONT sequencing additionally allows for the identification of base modifications at the DNA level.[27] Modified bases such as 5-methylcytosine in DNA can be detected based on differences in the current flow between modified and unmodified bases, paving the way for direct and unbiased methylome analysis in parallel with DNA sequencing.[28]

SMRT technology implemented by the PacBio Sequel and Revio platforms utilizes parallel systems of polymerase which are bound to DNA fragments previously circularized by sequencing adaptors.[29] The incorporation of labeled bases by the polymerase enables the resulting DNA to fluoresce. This fluorescence is subsequently detected in real time using a zero-mode waveguide and a charge-coupled device camera.[25] The fluorescent signal is then processed to generate reads of tens of kilobases in length or more. A significant limitation of this technology is its error rate, which is 4 to 20 times higher than SRS.[9] This issue has been addressed by PacBio with the introduction of HiFi reads, which are based on the circular consensus sequencing concept.[30] Each circularized DNA fragment from the SMRT library is sequenced multiple times, and since single-pass errors are distributed randomly, they eventually disappear upon building consensus as the sequencing process is repeated.[30] The latest SMRT platforms also allow for base methylation calling.[31]

Advances in Variant Discovery and Beyond

LRS strategies are becoming increasingly powerful tools for the examination of genetic aberrations that are difficult to analyze with conventional srES or srGS approaches (Fig. 2, Table 1).[8–10]

The improved performance of LRS for discovery of various types of SVs, such as deletions, duplications, and insertions as well as inversions and translocations, is being recognized for different clinical indications including somatic and constitutional diseases.[33] SVs are emerging as an important class of molecular pathologies underlying movement disorders including dystonia.[5,6] A recent comprehensive literature review identified dystonia as one of the most common movement abnormalities in patients with pathogenic deletion CNVs detected by chromosomal microarray.[34] Similarly, published srES and srGS analyses in larger cohorts of individuals with dystonia demonstrated that CNV screening can effectively increase the diagnostic yield, although discovery power was likely to be limited in these SRS studies.[5,6] LRS is uniquely capable of providing maximum precision for SV detection by generating high-confidence reads spanning genomic stretches that contain corresponding SV breakpoints and/or entire SV events.[35] A comparative study investigating the efficiency of accurate SV identification illustrated that standard srGS processed as per Illumina methodology failed to uncover 47% of deletions and 78% of the insertions that were found by PacBio-based LRS, even though different combinations of modern calling algorithms were employed.[36] In particular, insertions larger than 50 bp are considered one of the hardest-to-detect variant types in conventional short-read experiments.[9] Intriguingly, an early key result from the application of LRS was the discovery of a SINE-VNTR-Alu retrotransposon insertion in intron 32 of *TAF1* as the cause of X-linked dystonia-parkinsonism (XDP), which had escaped multiple prior mapping attempts through conventional approaches.[37] The finding enabled researchers to study fundamental aspects of dystonia pathophysiology including mechanisms of transcriptional perturbation and neurodegeneration.[38] In addition, a recently established LRS workflow provided insights into the nature of a hexanucleotide repeat motif within the XDP-causing insertion element, paving the way for enhanced understanding of modifiers of dystonia onset and expressivity.[39]

LRS has also led to the discovery of other complex SV events in patients with etiologically unresolved conditions with dystonic features. For example, Daida and colleagues used ONT technology to decipher the presence and orientation of a previously unannotated 7-Mb inversion affecting large parts of the coding region of *PRKN* in siblings with dystonia and parkinsonism, establishing the diagnosis of *PRKN*-linked early-onset Parkinson's disease and the molecular basis for efficacious treatment of dystonic features with levodopa.[40] The report exemplified the utility of LRS in a situation where only a single heterozygous pathogenic variant was found for a suspected recessive disorder, highlighting that the method can comprehensively complement inconclusive pre-screening results.

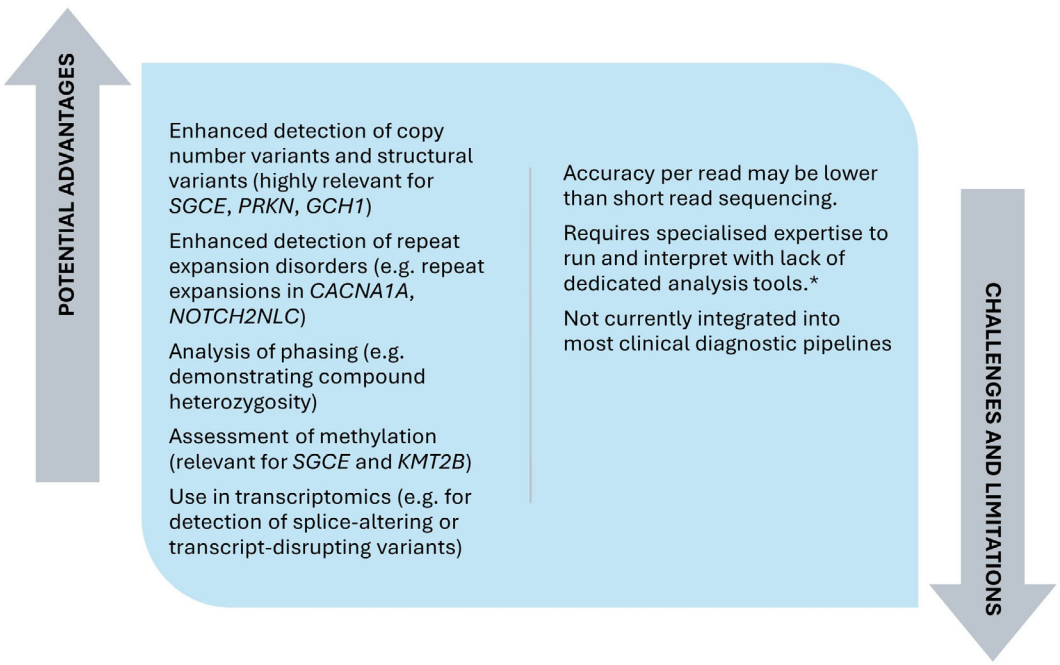

FIG. 2. Potential advantages versus challenges and limitations of long-read sequencing versus short-read sequencing with examples relevant to dystonia. *Amarasinghe et al.[32]

Another prime advantage of LRS is the possibility of systematically assessing STR pathologies with high accuracy.[33] Especially for patients with dystonia and accompanying neurological signs, LRS is expected to emerge as a solid alternative approach to laborious polymerase chain reaction (PCR)-based techniques or Southern blot for the identification of expanded STR alleles in genes that have been linked to syndromes that can manifest with dystonia and comorbid ataxia or epilepsy such as *CACNA1A*, *CSTB*, *FGF14*, *FXN*, and *TBP*.[41] LRS offers unprecedented abilities to precisely determine expansion lengths, irrespective of GC-content and additional variation within the STR interval.[33]

A study by Ardui and colleagues described successful implementation of a PacBio LRS assay for evaluation of AGG interruptions within the *FMR1* CGG expansion responsible for Fragile X-associated tremor/ataxia syndrome (FXTAS), gaining sensitivity in the construction of disease-related STR architectures[42]; knowledge about the number of interrupting AGG triplets in *FMR1* is important for estimating the individualized risk of transmitting pathogenic repeat sizes to future generations. Notably, dystonia has been observed in a growing number of FXTAS-affected female individuals, mostly in the form of an associated feature in the context of ataxic, tremulous, and/or neuropathic presentations, indicating that the counseling of patients with dystonia on the basis of *FMR1* STR pathology may become an increasing clinical need.[43,44] Although the implication of neuropathogenic STRs in isolated dystonia is largely unknown, accurate profiling of STR expansions by ONT technology has recently been used to detect a disease-causing GGC expansion in the 5'UTR of *NOTCH2NLC* in a patient affected by adult-onset cervical dystonia without other symptoms.[45] *NOTCH2NLC* GGC expansions,[45] originally linked to neuronal intranuclear inclusion disease in LRS studies in 2019, are causative for heterogeneous neurodegenerative phenotypes ranging from parkinsonism and ataxia to dementia and diffuse leukoencephalopathy.[46] It is conceivable that future broader applications of LRS methods in patients with dystonia will uncover new, unanticipated roles for pathogenic STRs in different types of dystonic syndromes, including the group of poorly resolved late-onset focal dystonias.

LRS is also well suited to overcoming challenges related to pseudogenes. Pseudogenes are DNA intervals with high sequence similarity to annotated genes but without protein-coding function.[47] They complicate the investigation of their functional counterparts because they often trigger mis-mapping of short-reads, resulting in false-negative (or false-positive) results.[48] The use of LRS with de novo assembly can eliminate alignment errors in ambiguous genomic regions and holds potential for improved evaluation of clinically relevant genes with pseudogenes.[8] A very recent study highlighted the usefulness of PacBio-based LRS for comprehensive identification of SNVs in *GBA1*, whose sequence interrogation is known to be challenging due to the presence of the highly homologous pseudogene *GBAP1*.[49] Except for some rare reports linking *GBA1* biallelic variants to complex dystonic syndromes,[50,51] there is no current direct evidence for an association between *GBA1* variants and the risk of developing dystonia; however, accumulating data suggest a role for lysosomal impairment including functionally altered GBA1 in individuals with dystonia, emphasizing the need for accurate genotyping at this locus.[52]

Given its ability to span entire genes with unique alignments, LRS can be of further value for the phasing of SNVs/indels.[8] In the absence of information on maternal and paternal chromosomes, the method can help to determine whether two identified heterozygous variants are located in trans or on the same haplotype.[8] Importantly, the demonstration of a compound heterozygous status of two variants in a recessive disease gene is a key criterion for the assignment of pathogenicity according to the American College of Medical Genetics and Genomics (ACMG) recommendations.[53] Difficulty in showing biparental inheritance is a common situation in genetic testing in adults with dystonia, as well as in affected pediatric individuals for whom DNA of both parents is not available. A proof-of-principle study by Castellotti and colleagues used LRS to demonstrate the biallelic location of two heterozygous *POLG* alterations, one of which had occurred de novo, in a patient with paroxysmal dystonia, enabling the (re)classification of the variants as (likely) pathogenic and securing the diagnosis after a long diagnostic journey.[54]

Finally, LRS drives our increasing ability to perform high-throughput screening for disease-related molecular aberrations beyond DNA variations.[8,10,55] The access to measurements of DNA modifications such as methylation is an additional core benefit of LRS and may become especially relevant for the diagnosis of dystonic conditions in which deregulation of transcriptional control plays a role.[55] For example, Lüth and colleagues determined CpG methylation of DNA sequences around the transcription factor gene *TAF1* in brain-derived samples from patients with XDP by ONT analysis, revealing hypermethylation states potentially related to disease pathogenesis.[39] A promising field of application for combined variant and DNA-methylation analysis could be the study of patients with *KMT2B*-related dystonia. Disease-causing variants in the epigenetic regulator *KMT2B* are associated with genome-wide changes in DNA methylation, and these CpG markers are powerful indicators for pathogenicity of an identified sequence change in this gene.[56,57] The use of standard point-of-care bisulfite sequencing for methylation analysis may become obsolete with future advancements in the analytical approaches of LRS,

paving the way towards implementation of a single test for thorough diagnostic evaluation on different molecular layers.

LRS can also be applied to transcriptome profiling.[58] Although LRS-based analysis of full-length RNA isoforms needs to be further evaluated in the clinical setting, this strategy could lead to important discoveries of previously unrecognized transcript pathologies in patients with monogenic dystonia. The importance of comprehensive RNA integrity evaluation in individuals with dystonia is underlined by the substantial proportion of splice-altering and other transcript-disrupting alleles among cataloged dystonia-causing variants.[2,59] An example with immediate clinical impact has been reported in a recent transcriptomics approach, describing a family with a causative homozygous variant in an untranslated regulatory region of the dystonia-linked gene *SPR*. This variant, prioritized based on its effect on RNA, highlighted the indication for etiology-directed dopaminergic therapy.[60]

Limitations of LRS

Although LRS is emerging as a promising approach for the detection of different types of variants in dystonic syndromes, the methods are still facing several challenges.

One of the most significant limitations of LRS is the accuracy per read, which may be lower than in SRS.[9] In the case of SMRT, the circular consensus sequence quality is dependent on the depth of sequencing of the individual SMRT molecule, defined by the number of times the molecule is read by the polymerase, which is itself correlated with the length of the original fragment and the longevity of the polymerase. An estimated four passes are required to provide a consensus circular sequence with 99% accuracy and nine passes for 99.9% accuracy.[30] The quality of ONT is heavily dependent on the length of the DNA fragments, which are only read once, conversely to SMRT.[27] Read quality relies on achieving optimal translocation speed of the nucleic acid through the pore, which decreases over time as the sequencing process advances, altering the quality of sequencing.[9,27] The error rate in ONT ranges between 1% and 3%, which also remains higher than SRS.[27] Several developments have been proposed to tackle this significant issue in both SMRT and ONT LRS, either based on available SRS data (hybrid correction) or using solely the information contained in LRS data (self-correction).[61] Importantly, these computational error correction algorithms represent promising strategies for the reduction of error rates without the need for modification of experimental protocols, thereby lowering costs. The future application of LRS in diagnostic settings will depend largely on the optimization of approaches to circumvent error rates.

Another hurdle to current broad implementation of LRS platforms in research and diagnostics is the high "cost per base". This metric is used to compare the cost of sequencing genomes across various platforms.[9] The cost per base of ONT's PromethION and the most recent Revio platform offers competitive LRS at around US$30/Gb while the most recent SRS Illumina platform can achieve costs below US$10/Gb. However, this metric does not appreciate the inherent value of LRS data, which are superior to SRS and carry additional information, allowing the detection of causal variants that remain uncovered with SRS. Additionally, portable ONT sequencers can be easily adopted without the need for a large financial investment such as is required for Illumina or PacBio sequencing, mitigating the impact of the apparent elevated cost of sequencing.[9,27] Eventually, it can be anticipated that the cost of sequencing will decrease as the LRS chemistries become increasingly mature, as has been the case for SRS technologies.

LRS yields lower data throughput compared with SRS platforms, the process for generating long-reads take significantly more time compared with the rapid output of high-throughput SRS, affecting the speed of large projects.[9] For instance, with a high-density SMRT Cell, up to four SMRT Cells per run, and 24-hour run times, the Revio system with the most recent chemistry delivers up to 480 Gb of HiFi reads per day, corresponding to four human genomes with a depth of coverage of 30X. In comparison, the most recent SRS platform yields 2400–3000 Gb per run, corresponding to more than 20 human genomes. Improvements in chemistry and refinement of the sequencing protocols may also this limitation to be overcome and accelerate LRS projects in the future.

While selecting the most appropriate LRS approach is crucial for the success of individual research projects, we are currently unable to propose a recommendation which would prioritize one LRS platform over another with regard to dystonia; ONT and PacBio platforms have only been compared in a limited number of studies, none of them focusing specifically on molecular diagnosis for rare neurogenetic conditions. ONT could be more suitable for research projects that focus on throughput rather than higher accuracy per sequencing read. However, additional benchmarking studies and comparative analyses will be required to determine an optimal LRS approach for different research directions. This will be also important for the decision about the preferable LRS-based RNA/methylation analysis framework; it may be hypothesized that PacBio could outperform ONT in the context of high-quality RNA isoform analysis.

The platform is not the sole limiting factor for the length of the sequenced DNA, which is also affected by the library preparation and DNA extraction procedure.

Production of long-reads heavily relies on using high-quality DNA of high molecular weight.[62] Not all clinically accessible tissues are deemed suitable for LRS projects, and the library preparation protocols for LRS require high molecular weight DNA from blood or other invasive tissue collection, excluding saliva. As the DNA extracted using classical methods is no longer bound and protected by its histones, simple disruptions can lead to fragmenting of the DNA strands.[9] Specific DNA extraction kits are consequently required to obtain high molecular weight DNA, directly increasing LRS framework cost, and thus limiting the widespread use of LRS. This also prevents automated extraction of DNA, which has been widely implemented for SRS.[63]

While LRS allows for the direct calling of methylation, it has been demonstrated that coverage affects the consistency of CpG methylation measurements in LRS data.[28,31] A minimum depth of coverage of 20X is advised for the calling of methylated bases with clinically relevant accuracy. Such depth of sequencing can be costly and time-consuming to obtain.[55]

LRS requires tailored analysis tools and bioinformatics approaches to take full advantage of this technology. While various tools for base or methylation calling, error correction, mapping, and phasing using long-read data are being developed, we are still at the beginning of the application of LRS,[9] currently preventing its widespread use in a clinical setting. Compared with cost limitations, the insufficient availability of powerful data evaluation and storage infrastructure may emerge as obstacles that are more difficult to overcome. The large output data volumes and the often non-standardized secondary and tertiary analysis pipelines hinder LRS from becoming established as a mainstream diagnostic application. Bioinformatics assessment tools have mostly been optimized for SRS, and it will be an important future task to adapt and comprehensively test the analytic strategies for LRS data.

It is important to highlight that improved variant-detection capacity alone may not be sufficient to unravel 'all' the missing heritability of dystonia. Indeed, it is noteworthy that several neurogenetic conditions characterized by dystonia are caused by incompletely penetrant variants.[64,65] In these situations, causal pathogenic variants inherited from an asymptomatic relative may be detected by SRS or LRS but will be excluded by segregation analysis based on a too strict assumption of Mendelian inheritance. Case–control studies through collapsing burden analysis that can take advantage of LRS data will be necessary to address this limitation.[66]

Lastly, it is important to consider that LRS can be adopted using a targeted or genome-wide approach. Genome-wide LRS will provide more information, cover clinically relevant variants in genes outside of restrictive gene panels, and serve as a potential resource for future gene discovery efforts. However, genome-wide LRS currently remains prohibitively expensive, and many studies have instead used a targeted LRS approach.[67,68] Further research is needed to explore the role of targeted versus unbiased genome-wide LRS, in addition to studies showing the benefit of LRS in individual dystonic indications.

Conclusions and Perspectives

Methodologies for conducting LRS studies have matured substantially and are playing increasingly important roles in the assessment of clinically significant genetic variations across different fields of medicine.[8–10] Moreover, LRS approaches hold promise for offering the capacity for parallel evaluation of complementary biological layers, including DNA sequence- and RNA-level information as well as methylation status and other base modifications. Nevertheless, despite the discussed opportunities and the herein illustrated examples of discovery power in the field of dystonia, systematic applications of LRS have yet to demonstrate the ability to enhance the precision of molecular diagnoses in research and clinical settings on a cohort-wide scale. The argument for an increased diagnostic yield through application of LRS remains to be confirmed. To date, the two major LRS platforms, ONT and PacBio, have advanced our understanding of the genetic causes and disease pathogenesis in selected patients with dystonic syndromes, reaching outstanding performances in the detection of SVs, the characterization of repeat expansions, and simultaneous long-range variant-phase inference.[40,45,54] Given that LRS addresses many of the shortcomings of SRS strategies, we speculate that the methodology may be able to offer an added diagnostic yield of several percentage points following unyielding standard-of-care genetic testing in dystonia. LRS may also provide advantages in the analysis of mitochondrial DNA, in particular by allowing for the detection of structural alterations such as large-scale deletions and rearrangements, which may be diagnostically important given the relevant contribution of mitochondrial DNA defects to dystonia.

When considering the implementation of LRS in genomic diagnostics for daily use, four main factors should be addressed: accuracy and yield in a given indication (eg, isolated dystonia versus more complicated forms of dystonia with coexisting neurological features), throughput, and cost. We envision that further developments of LRS-based analytic workflows will continue to improve rapidly, making it possible to overcome current challenges in the comprehensive analysis of causative variants in patients with dystonia at a reasonable cost. Given the clear indications that LRS is superior to srES/srGS by virtue of the exceedingly high levels of continuity in genome assembly and variant-

calling quality,[69] it is likely that LRS will become commonplace in the diagnostic workflow of patients with suspected genetic diseases within the next few years. We, as a dystonia research community, should keep pace with the activities of modern genomic methodology drivers in order to ensure that the patients we treat can benefit from the most effective technologies for achieving a definitive diagnosis. Well-designed studies and robust data should be available to judge for which dystonic indications LRS could serve as a truly holistic test that can overcome inefficient sequential diagnostics paradigms. We expect that the methodology could be capable of boosting diagnostic yields under different analytical protocols, ranging from first-line analysis of preselected cases with a higher suspicion of an underlying monogenic disease to undiagnosed individuals with negative testing results from SRS, including patients with isolated late-onset dystonia. Encouragingly, a number of efforts utilizing LRS in groups of patients with different types of dystonia are underway, and several collaborative initiatives – for example, PreDYT (https://www.ejprarediseases.org/predyt/) (Germany, Italy, France, Switzerland, Luxembourg), GenoDYT (France), Ainsworth 4 Dystonia Genetic Research Mission (Australia) – have announced the goal of advancing knowledge about the analytical validity of LRS in dystonia. Consequently, we may be embarking on an era in which challenging genetic variations and thus far intractable molecular constellations with significant clinical implications can be resolved, refining diagnostic processes and bolstering precision clinical management. Furthermore, with the ability to accurately recognize mutational configurations in pathogenic aberrations such as repeat expansions, we may be able to uncover mechanisms underlying variable expressivity and reduced penetrance in monogenic disorders with dystonia.[70] The growth of high-resolution data generated by LRS promises an enormous capacity to enhance insights into genomic, epigenetic, and transcriptomic variations and their link to dystonia. A careful appreciation of the role of LRS in dystonia research and care can help us to drive greater progress in the discovery of etiology and pathomechanisms. ■

Author Roles: (1) Review Article: A. Conception, B. Design, C. Execution, D. Analysis; (3) Manuscript Preparation: A. Writing of the First Draft, B. Editing of the Final Version.
T. W.: 1A, 1B, 1C, 1D, 2A, 2B.
K.R.K.: 1A, 1B, 1C, 1D, 2A, 2B.
M.Z.: 1A, 1B, 1C, 1D, 2A, 2B.

Acknowledgments: M. Z. received research support from the German Research Foundation (DFG 458949627; ZE 1213/2-1). M. Z. acknowledges grant support from the EJP RD (EJP RD Joint Transnational Call 2022) and the German Federal Ministry of Education and Research (BMBF, Bonn, Germany), awarded to the project PreDYT (PREdictive biomarkers in DYsTonia, 01GM2302), and from the Federal Ministry of Education and Research (BMBF) and the Free State of Bavaria under the Excellence Strategy of the Federal Government and the Länder, as well as by the Technical University of Munich – Institute for Advanced Study. M. Z. is a member of the Medical and Scientific Advisory Council of the Dystonia Medical Research Foundation and a member of the Governance Council of the International Cerebral Palsy Genomics Consortium. M.Z.'s research is supported by a "Schlüsselprojekt" grant from the Else Kröner-Fresenius-Stiftung (2022_EKSE.185). T.W. received research support from the French Ministry of Health through the "Projet Hospitalier de Recherche Clinique" grant awarded for the project GenoDYT. T. W. acknowledges research grants from the French Ministry of Health, the "Société Française de Neurologie", the Fondation Planiol, the APTES and the France Parkinson organizations. K.R.K. is supported by the Ainsworth 4 Dystonia Genetic Research Mission and the Medical Research Future Fund. Open access publishing facilitated by University of New South Wales, as part of the Wiley - University of New South Wales agreement via the Council of Australian University Librarians.

Full Financial Disclosures for the Previous 12 Months: Nothing to report.

Data Availability Statement

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

References

1. Thomsen M, Lange LM, Zech M, Lohmann K. Genetics and pathogenesis of dystonia. Annu Rev Pathol 2024;19:99–131.
2. Zech M, Jech R, Boesch S, Škorvánek M, Weber S, Wagner M, et al. Monogenic variants in dystonia: an exome-wide sequencing study. Lancet Neurol 2020;19(11):908–918.
3. Zech M, Jech R, Boesch S, Škorvánek M, Necpál J, Švantnerová J, et al. Scoring algorithm-based genomic testing in dystonia: a prospective validation study. Mov Disord 2021;36(8):1959–1964.
4. Albanese A, Bhatia K, Bressman SB, Delong MR, Fahn S, Fung VSC, et al. Phenomenology and classification of dystonia: a consensus update. Mov Disord 2013;28(7):863–873.
5. Fellner A, Wali GM, Mahant N, Grosz BR, Ellis M, Narayanan RK, et al. Genome sequencing reanalysis increases the diagnostic yield in dystonia. Parkinsonism Relat Disord 2024;124:107010.
6. Zech M, Boesch S, Škorvánek M, Necpál J, Švantnerová J, Wagner M, et al. Clinically relevant copy-number variants in exome sequencing data of patients with dystonia. Parkinsonism Relat Disord 2021;84:129–134.
7. Depienne C, Mandel JL. 30 years of repeat expansion disorders: what have we learned and what are the remaining challenges? Am J Hum Genet 2021;108(5):764–785.
8. Mantere T, Kersten S, Hoischen A. Long-read sequencing emerging in medical genetics. Front Genet 2019;10:426.
9. Oehler JB, Wright H, Stark Z, Mallett AJ, Schmitz U. The application of long-read sequencing in clinical settings. Hum Genomics 2023;17(1):73.
10. Mahmoud M, Huang Y, Garimella K, Audano PA, Wan W, Prasad N, et al. Utility of long-read sequencing for all of us. Nat Commun 2024;15(1):837.
11. Thomsen M, Marth K, Loens S, Everding J, Junker J, Borngräber F, et al. Large-scale screening: phenotypic and mutational spectrum in isolated and combined dystonia genes. Mov Disord 2024;39(3):526–538.
12. van Egmond ME, Lugtenberg CHA, Brouwer OF, Contarino MF, Fung VSC, Heiner-Fokkema MR, et al. A post hoc study on gene panel analysis for the diagnosis of dystonia. Mov Disord 2017;32(4):569–575.
13. Montaut S, Tranchant C, Drouot N, Rudolf G, Guissart C, Tarabeux J, et al. Assessment of a targeted gene panel for identification of genes associated with movement disorders. JAMA Neurol 2018;75(10):1234–1245.
14. Wu MC, Chang YY, Lan MY, Chen YF, Tai CH, Lin YF, et al. A clinical and integrated genetic study of isolated and combined dystonia in Taiwan. J Mol Diagn 2022;24(3):262–273.

15. Ma J, Wang L, Yang YM, Wan XH. Targeted gene capture sequencing in diagnosis of dystonia patients. J Neurol Sci 2018;390:36–41.
16. Wirth T, Tranchant C, Drouot N, Keren B, Mignot C, Cif L, et al. Increased diagnostic yield in complex dystonia through exome sequencing. Parkinsonism Relat Disord 2020;74:50–56.
17. Atasu B, Simón-Sánchez J, Hanagasi H, Bilgic B, Hauser AK, Guven G, et al. Dissecting genetic architecture of rare dystonia: genetic, molecular and clinical insights. J Med Genet 2024;61(5): 443–451.
18. Powis Z, Towne MC, Hagman KDF, Blanco K, Palmaer E, Castro A, et al. Clinical diagnostic exome sequencing in dystonia: genetic testing challenges for complex conditions. Clin Genet 2020; 97(2):305–311.
19. Dhar D, Holla VV, Kumari R, Yadav R, Kamble N, Muthusamy B, et al. Clinical and genetic profile of patients with dystonia: an experience from a tertiary neurology center from India. Parkinsonism Relat Disord 2024;120:105986.
20. Holla VV, Neeraja K, Stezin A, Prasad S, Surisetti BK, Netravathi M, et al. Utility of clinical exome sequencing in dystonia: a single-center study from India. J Mov Disord 2022;15(2): 156–161.
21. Ahn JH, Kim AR, Park WY, Cho JW, Park J, Youn J. Whole exome sequencing and clinical investigation of young onset dystonia: what can we learn? Parkinsonism Relat Disord 2023;115:105814.
22. Kumar KR, Davis RL, Tchan MC, Wali GM, Mahant N, Ng K, et al. Whole genome sequencing for the genetic diagnosis of heterogenous dystonia phenotypes. Parkinsonism Relat Disord 2019;69: 111–118.
23. Owusu R, Savarese M. Long-read sequencing improves diagnostic rate in neuromuscular disorders. Acta Myol 2023;42(4):123–128.
24. Espinosa E, Bautista R, Larrosa R, Plata O. Advancements in long-read genome sequencing technologies and algorithms. Genomics 2024;116(3):110842.
25. Eid J, Fehr A, Gray J, Luong K, Lyle J, Otto G, et al. Real-time DNA sequencing from single polymerase molecules. Science 2009; 323(5910):133–138.
26. Clarke J, Wu HC, Jayasinghe L, Patel A, Reid S, Bayley H. Continuous base identification for single-molecule nanopore DNA sequencing. Nat Nanotechnol 2009;4(4):265–270.
27. Wang Y, Zhao Y, Bollas A, Wang Y, Au KF. Nanopore sequencing technology, bioinformatics and applications. Nat Biotechnol 2021; 39(11):1348–1365.
28. Liu Y, Rosikiewicz W, Pan Z, Jillette N, Wang P, Taghbalout A, et al. DNA methylation-calling tools for Oxford Nanopore sequencing: a survey and human epigenome-wide evaluation. Genome Biol 2021;22:295.
29. McCarthy A. Third generation DNA sequencing: pacific biosciences' single molecule real time technology. Chem Biol 2010;17(7): 675–676.
30. Wenger AM, Peluso P, Rowell WJ, Chang PC, Hall RJ, Concepcion GT, et al. Accurate circular consensus long-read sequencing improves variant detection and assembly of a human genome. Nat Biotechnol 2019;37(10):1155–1162.
31. Flusberg BA, Webster D, Lee J, Travers K, Olivares E, Clark TA, et al. Direct detection of DNA methylation during single-molecule, real-time sequencing. Nat Methods 2010;7(6):461.
32. Amarasinghe SL, Su S, Dong X, Zappia L, Ritchie ME, Gouil Q. Opportunities and challenges in long-read sequencing data analysis. Genome Biol 2020;21(1):30.
33. Mastrorosa FK, Miller DE, Eichler EE. Applications of long-read sequencing to Mendelian genetics. Genome Med 2023;15(1):42.
34. Soliani L, Alcalá San Martín A, Balsells S, Hernando-Davalillo C, Ortigoza-Escobar JD. Chromosome microarray analysis for the investigation of deletions in pediatric movement disorders: a systematic review of the literature. Mov Disord Clin Pract 2023;10(4): 547–557.
35. Conlin LK, Aref-Eshghi E, McEldrew DA, Luo M, Rajagopalan R. Long-read sequencing for molecular diagnostics in constitutional genetic disorders. Hum Mutat 2022;43(11):1531–1544.
36. Chaisson MJP, Sanders AD, Zhao X, Malhotra A, Porubsky D, Rausch T, et al. Multi-platform discovery of haplotype-resolved structural variation in human genomes. Nat Commun 2019;10(1): 1784.
37. Aneichyk T, Hendriks WT, Yadav R, Shin D, Gao D, Vaine CA, et al. Dissecting the causal mechanism of X-linked dystonia-parkinsonism by integrating genome and transcriptome assembly. Cell 2018;172(5):897–909.e21.
38. Capponi S, Stöffler N, Penney EB, Grütz K, Nizamuddin S, Vermunt MW, et al. Dissection of TAF1 neuronal splicing and implications for neurodegeneration in X-linked dystonia-parkinsonism. Brain Commun 2021;3(4):fcab253.
39. Lüth T, Laβ J, Schaake S, Wohlers I, Pozojevic J, Jamora RDG, et al. Elucidating hexanucleotide repeat number and methylation within the X-linked dystonia-parkinsonism (XDP)-related SVA retrotransposon in TAF1 with nanopore sequencing. Genes (Basel) 2022;13(1):126.
40. Daida K, Funayama M, Billingsley KJ, Malik L, Miano-Burkhardt A, Leonard HL, et al. Long-read sequencing resolves a complex structural variant in PRKN Parkinson's disease. Mov Disord 2023;38(12):2249–2257.
41. Chintalaphani SR, Pineda SS, Deveson IW, Kumar KR. An update on the neurological short tandem repeat expansion disorders and the emergence of long-read sequencing diagnostics. Acta Neuropathol Commun 2021;9(1):98.
42. Ardui S, Race V, de Ravel T, Van Esch H, Devriendt K, Matthijs G, et al. Detecting AGG interruptions in females with a FMR1 premutation by long-read single-molecule sequencing: a 1 year clinical experience. Front Genet 2018;9:150.
43. Ros-Castelló V, Latorre A, Álvarez-Linera J, Martinez-Castrillo JC, Bhatia KP, Pareés I. Dystonia in a female Fragile X premutation carrier. Mov Disord Clin Pract 2021;8(5):797–799.
44. Alvarez Bravo G, Fernández-Carril JM, López-Zuazo I, Yusta Izquierdo A, Abrol T, Alsinaidi O. A novel clinical phenotype of Fragile X-associated tremor/ataxia syndrome. Mov Disord Clin Pract 2018;5(4):430–432.
45. Williams LJ, Qiu J, Ong TL, Deveson IW, Stevanovski I, Chintalaphani SR, et al. NOTCH2NLC GGC repeat expansion presenting as adult-onset cervical dystonia. Mov Disord Clin Pract 2023;10(4):704–706.
46. Sone J, Mitsuhashi S, Fujita A, Mizuguchi T, Hamanaka K, Mori K, et al. Long-read sequencing identifies GGC repeat expansions in NOTCH2NLC associated with neuronal intranuclear inclusion disease. Nat Genet 2019;51(8):1215–1221.
47. Afonso Ribeiro J, Sousa M, Alonso I, Moreira F, Pereira R, Palavra F. PRKRAP1 pseudogene complicating the diagnosis of young-onset dystonia due to PRKRA gene disease-causing variants (DYT-PRKRA). Mov Disord Clin Pract 2022;9(5):703–706.
48. Magrinelli F, Lohmann K. PRKRAP1 and other pseudogenes in movement disorders: the troublemakers in genetic analyses are more than genomic fossils. Mov Disord Clin Pract 2022;9(5):698–702.
49. Pachchek S, Landoulsi Z, Pavelka L, Schulte C, Buena-Atienza E, Gross C, et al. Accurate long-read sequencing identified GBA1 as major risk factor in the Luxembourgish Parkinson's study. NPJ Parkinsons Dis 2023;9(1):156.
50. Hertz E, Lopez G, Lichtenberg J, Haubenberger D, Tayebi N, Hallett M, et al. Rapid-onset dystonia and parkinsonism in a patient with Gaucher disease. J Mov Disord 2023;16(3):321–324.
51. Imbalzano G, Ledda C, Romagnolo A, Covolo A, Lopiano L, Artusi CA. Neurological symptoms in adults with Gaucher disease: a systematic review. J Neurol 2024;271(7):3897–3907.
52. Calakos N, Zech M. Emerging molecular-genetic families in dystonia: endosome-autophagosome-lysosome and integrated stress response pathways. Mov Disord 2025;40(1):7–21.
53. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 2015;17(5):405–424.
54. Castellotti B, Gellera C, Caputo D, Danti FR, Messina G, Corbetta M, et al. Paroxysmal non-kinesigenic dyskinesias

associated with biallelic POLG variants: a case report. Mov Disord 2024;39(12):2300–2302.

55. Sigurpalsdottir BD, Stefansson OA, Holley G, Beyter D, Zink F, Hardarson MÞ, et al. A comparison of methods for detecting DNA methylation from long-read sequencing of human genomes. Genome Biol 2024;25(1):69.
56. Mirza-Schreiber N, Zech M, Wilson R, Brunet T, Wagner M, Jech R, et al. Blood DNA methylation provides an accurate biomarker of KMT2B-related dystonia and predicts onset. Brain 2022; 145(2):644–654.
57. Oexle K, Zech M, Stühn LG, Siegert S, Brunet T, Schmidt WM, et al. Episignature analysis of moderate effects and mosaics. Eur J Hum Genet 2023;31(9):1032–1039.
58. Bayega A, Wang YC, Oikonomopoulos S, Djambazian H, Fahiminiya S, Ragoussis J. Transcript profiling using long-read sequencing technologies. Methods Mol Biol 2018;1783:121–147.
59. Lange LM, Junker J, Loens S, Baumann H, Olschewski L, Schaake S, et al. Genotype–phenotype relations for isolated dystonia genes: MDSGene systematic review. Mov Disord 2021;36(5):1086–1103.
60. Murdock DR, Dai H, Burrage LC, Rosenfeld JA, Ketkar S, Müller MF, et al. Transcriptome-directed analysis for Mendelian disease diagnosis overcomes limitations of conventional genomic testing. J Clin Invest 2021;131(1):e141500.
61. Zhang H, Jain C, Aluru S. A comprehensive evaluation of long read error correction methods. BMC Genomics 2020;21(6):889.
62. Trigodet F, Lolans K, Fogarty E, Shaiber A, Morrison HG, Barreiro L, et al. High molecular weight DNA extraction strategies for long-read sequencing of complex metagenomes. Mol Ecol Resour 2022;22(5):1786.
63. Lee JH, Park Y, Choi JR, Lee EK, Kim HS. Comparisons of three automated systems for genomic DNA extraction in a clinical diagnostic laboratory. Yonsei Med J 2009;51(1):104.
64. Steel D, Zech M, Zhao C, Barwick KES, Burke D, Demailly D, et al. Loss-of-function variants in HOPS complex genes VPS16 and VPS41 cause early onset dystonia associated with lysosomal abnormalities. Ann Neurol 2020;88(5):867–877.
65. Wirth T, Roze E, Delvallée C, Trouillard O, Drouot N, Damier P, et al. Rare missense variants in KCNJ10 are associated with paroxysmal kinesigenic dyskinesia. Mov Disord 2024;39(5):897–905.
66. Zech M, Winkelmann J. Next-generation sequencing and bioinformatics in rare movement disorders. Nat Rev Neurol 2024;20(2): 114–126.
67. Miller DE, Sulovari A, Wang T, Loucks H, Hoekzema K, Munson KM, et al. Targeted long-read sequencing identifies missing disease-causing variation. Am J Hum Genet 2021;108(8):1436–1449.
68. Stevanovski I, Chintalaphani SR, Gamaarachchi H, Ferguson JM, Pineda SS, Scriba CK, et al. Comprehensive genetic diagnosis of tandem repeat expansion disorders with programmable targeted nanopore sequencing. Sci Adv 2022;8(9):eabm5386.
69. Marx V. Method of the year: long-read sequencing. Nat Methods 2023;20(1):6–11.
70. Trinh J, Lüth T, Schaake S, Laabs BH, Schlüter K, Laβ J, et al. Mosaic divergent repeat interruptions in XDP influence repeat stability and disease onset. Brain 2023;146(3):1075–1082.