

Nanopore Sequencing Technology in Spinal Muscle Atrophy

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Abstract

Nanopore sequencing is a laboratory technique for determining the exact sequence of nucleotides, or bases, in a DNA molecule. Third generation sequencing method has been advanced and resulting data have been examined using bioinformatics to decrease error rate. Spinal Muscular Dystrophy is a Genetic neuromuscular disorder which is recognized as spinal muscular Atrophy caused by mutation or deletion in the SMN1 gene located on 5q13, which encodes the survival motor neuron (SMN) proteins, the region is highly unstable rich in duplicated sequences. A closely related gene SMN2, modifies disease severity depending on its copy number. Nanopore sequencing plays very important role in SMA as it provides accurate differentiation of SMN1 and SMN2, detection structural variants (deletion, duplications and hybrid gene), detection of point mutations (missense and nonsense mutations) and determine copy number. Motor neuron loss occurs before development of clinical symptoms. Newborn screening helps in detection of cases early and diagnosis early. Early diagnosis effects directly on treatment which significantly improves the outcome of disease. Carrier screening and genetic counselling (families with history of SMA) also plays an important role in decreasing the incidence of Spinal Muscle Atrophy. The diagnosed cases are treated with disease –modifying therapies such as nusinersen, risdiplam and onasemnogene abeparvovec. Nanopore sequencing technology highly contributes in early diagnosis and carrier screening and are very helpful in personalized treatment strategies. Its long-read and real-time sequencing properties make it one of the most promising technologies for the future diagnosis and management of Spinal Muscular Atrophy. As accuracy, affordability and clinical validation continue to improve, nanopore sequencing is likely to become an integral component of SMA diagnosis, newborn screening, carrier detection and personalized therapeutic management.

Keywords: Spinal Muscular Atrophy, Nanopore sequencing, Bioinformatics, genetic, mutation, deletion, therapies.

INTRODUCTION

Nanopore DNA sequencing is a laboratory technique for determining the exact sequence of nucleotides, or bases, in a DNA molecule. Nanopore Technology was developed by Oxford Nanopore technologies in 1990. This technique theoretically has much less errors as it does not involve the use of enzymes, amplification or fluorescently tagged nucleotides. The technology works by passing nucleic acid molecules through protein nanopores embedded in a membrane. Changes in electrical current are measured and translated into nucleotide sequences in real time.

A large –scale sequencing of genetic material of the human genome has been a subject of interest with Human Genome Project. The large amount of data is analyzing, which is more reliable, cost effective and less time consuming is best done by Next generation sequencing technologies. The basis of genome sequencing was established with the first-generation method which is based on two methods – chain termination and chemical degradation. Second generation include Roche 454, Illumina, Solid and Ion Torrent Platform, were used to reduce cost and save time. Third generation sequencing method has been advanced and resulting data have been examined using bioinformatics to decrease error rate. Third generation DNA sequencing Methods are divided into two techniques:- Pacific Bioscience and Nanopore technology.

Nanopore sequencing biophysical mechanism and integrated workflow

Molecular mechanism of single-molecule sensing: the core sensing system includes an encasing lipid bilayer and an arranged protein (e.g., MspA, α -HL, or CsgG derivatives). Driven by a constant transmembrane voltage, the target

original nucleic acid molecule in the cis chamber (cathode) is translocated through the nanopore toward the trans chamber (anode). The steric interaction of the polymer within the pore restricts the steady-state flow of ions, generating transient ionic current blockades with base-specific amplitudes and biophysical characteristics; ionic current-based decoding: the dynamic recording of raw electrical signals (in picoAmperes) over time is illustrated. Canonical nucleotides (A, C, G, T) and epigenetic modifications (e.g., 5-methylcytosine, 5mC) exhibit discrete current blockade levels and distinct dwell times as they pass through the sensing region of the pore. Utilizing real-time, deep learning-based basecalling algorithms (e.g., RNN or Transformer architectures), the system enables high-accuracy direct decoding of native DNA or RNA sequences, thereby eliminating the need for reverse transcription or polymerase chain reaction (PCR) amplification. Integrated “sample-to-result” workflow: this panel illustrates the practical implementation of the technology for point-of-care testing and clinical diagnostics. The highly integrated workflow encompasses the standardized acquisition of clinical body fluid specimens (e.g., whole blood, urine, cerebrospinal fluid), optimized rapid library preparation chemistries (typically amplification-free transposase-based or rapid ligation methods), and device loading onto portable, decentralized sequencing platforms. Combined with standardized bioinformatics pipelines, real-time data acquisition facilitates the rapid generation of comprehensive reports—including metagenomic pathogen identification, antimicrobial resistance (AMR) profiling, and virulence factor detection—providing immediate and precise data support for clinical decision-making.

Spinal Muscular Dystrophy is a Genetic neuromuscular disorder which is recognized as spinal muscular Atrophy caused by mutation or deletion in the SMN1 gene located on 5q13, which encodes the survival motor neuron (SMN) proteins, the region is highly unstable rich in duplicated sequences. A closely related gene SMN2, modifies disease severity depending on its copy number. Homozygous loss-of-function of *SMN1* causes spinal muscular atrophy (SMA), a severe neuromuscular disease.

SMN1 Gene:

The gene produces full length survival motor neuron protein, which is essential of motor neuron survival and RNA processing. Homozygous deletion of exon 7 of for approximately 95% of SMA cases.

SMN2 Gene:

This gene differs from SMN1 only by few nucleotides. A critical nucleotide substitution in exon 7 leads to exon

skipping during mRNA splicing, resulting in reduced production of SMN protein.

Disease severity can be assessed by the number of SMN2 copies. The severity of SMA ranges from prenatal onset and neonatal death (SMA type 0) to adult onset and a normal life expectancy (SMA type 4). The number of copies is directly proportional to production of functional SMN protein but inversely proportional to disease phenotype i-e;

1. 1-2 copies: severe phenotype
2. 3 copies: intermediate phenotype
3. 4 or more: milder phenotype

Therefore, accurate determination of SMN2 copy numbers is of significant clinical importance, so clinically it is classified into five types (0-5)-most severe to mild. The importance of role of SMN2 in SMN protein synthesis is more highlighted in cases with complete absence of both SMN1 and SMN2 leading embryonic lethality.

Clinical type	SMN2 Copy Number	Clinical Features
SMA Type 0	1	Onset before birth with reduced fetal movement in late pregnancy. At birth, infants show severe muscle weakness and hypotonia. Except for eye movement, there is almost no activity of the limbs, trunk, or facial muscles. No sucking reflexes, presence of congenital joint contractures, muscle atrophy, and absence of reflexes. Respiratory support is required immediately after birth. Congenital heart defects may also be present. Most infants die within weeks.
SMA Type 1	1 or 2	Onset before 6 months of age. The most common and severe infantile-onset form. Affected infants typically present as "floppy infant syndrome" with severe generalized hypotonia. Due to weakness of the tongue, facial, and chewing muscles, there is difficulty in sucking and swallowing. Tongue atrophy and fasciculation are common. Motor weakness primarily manifests in the lower extremities, with greater proximal musculature involvement than distal muscle groups. Infants cannot control head movements, roll over, or sit independently. Respiratory compromise arises early due to severe intercostal muscle weakness, resulting in paradoxical abdominal breathing and the development of a bell-shaped thorax. As the disease progresses, voluntary motor function continues to decline. The majority of patients develop respiratory failure secondary to recurrent pulmonary infections, with approximately 80% dying before one year of age. A small proportion may survive beyond two years with intensive supportive care.
SMA Type 2	3	Onset between 6 and 18 months. Normal development during the first 6 months, followed by stagnation of motor development. The hallmark feature is progressive, symmetric muscle weakness and hypotonia, with greater involvement of proximal than distal muscles and lower limbs more severely affected than upper limbs. Children can usually sit independently but are unable to stand or walk unaided. Clinical examination reveals limb weakness, decreased or absent deep tendon reflexes, and characteristic signs such as tongue atrophy and fasciculations. Postural hand tremors are observed in approximately 50% of patients. Over time musculoskeletal complications become more prominent. Progressive scoliosis, large joint contractures, and restrictive pulmonary insufficiency are common, often requiring orthopedic and respiratory interventions. Despite the severity of motor impairment, cognitive and language development remain normal. With comprehensive multidisciplinary care, most individuals with SMA Type 2 survive into adolescence or early adulthood, though respiratory complications are a leading cause of morbidity and mortality.
SMA Type 3	3 or 4	Onset between 18 months and 10 years of age, typically after a period of normal early motor development. Symptoms begin in childhood or adolescence, with initial symptoms often including difficulty running, climbing stairs, or rising from the floor. Patients can walk independently but experience gradually progressive proximal muscle weakness, more in the lower limbs than the upper limbs. In the early stages, weakness may be segmental. Spinal deformities, joint abnormalities, and respiratory insufficiency may occur in later stages. Despite the progressive motor impairment, intellectual and cognitive development remain intact. Life expectancy is near normal, with most individuals surviving into mid-to-late adulthood, especially with appropriate supportive care and access to disease-modifying therapies.
SMA Type 4	4 or more	Adult onset typically occurs between 30 and 60 years of age. Characterized by slowly progressive, predominantly proximal muscle weakness, with greater involvement of the shoulder and pelvic girdle musculature. Symptoms may include difficulty with activities requiring proximal strength, such as climbing stairs, rising from a seated position, or lifting objects overhead. Bulbar and respiratory muscles are usually spared, and functional mobility is often preserved for many years. The disease course is indolent, and while it may cause disability over time, it does not significantly impact life expectancy. Cognitive function remains normal. Due to its subtle and late onset, Type 4 may be under diagnosed or misattributed to other adult neuromuscular disorders.

The disease is characterized by the degeneration of anterior horn cells in the spinal cord which leads to muscle weakness, muscle wasting, muscle twitching and decreased motor functions. SMA is the second most common autosomal recessive disease and most common genetic cause of infant death. SMA is estimated to impact about 1 in 10,000 live births. About 1 in 40 people in the general population are believed to carry a copy of faulty SMN1 gene. Nanopore sequencing has the potential for universal implementation in SMA carrier screening and diagnostic program. Nanopore sequencing plays a very important role in SMA as it provides accurate differentiation of SMN1 and SMN2, detection of structural variants (deletion, duplications and hybrid gene), detection of point mutations (missense and nonsense mutations) and determine copy number.

Clinical Types of Spinal Muscular Atrophy

The principle of Nanopore sequencing depends on the movement of DNA molecule through Nanopore present within the synthetic membrane. The ionic current is created by applying voltage across the membrane. As nucleotides pass through the pore, each nucleotide combination produces characteristic disruptions in current. These electrical signals are recorded and translated into nucleotide sequences using Algorithms.

The process includes: -

1. Sample collection from tissue or blood. Sequencing adapters and motor proteins are attached to DNA molecule.
2. Nanopore translocation- motor protein controls the movement of DNA.
3. Signal detection.
4. Base calling – conversion of electrical signals into DNA sequences.
5. Data analysis- bioinformatics analyze the sequences of mutations, deletions, copy number variations and structural rearrangements.

Advantages of Nanopore Sequencing In SMA

1. Long read length: - entire SMN gene can be sequenced in single reads.
2. Real-Time analysis: - Data become available during sequencing.
3. Comprehensive Variant detection.
4. Portable devices such as MinION.
5. RNA sequencing without reverse transcription.
6. Epigenetic profiling: - detects methylation patterns directly.

Disadvantages

1. Sequencing errors: slightly higher than short – read length.
2. Bioinformatics requirement : requirement of specialized software and skilled personnel.
3. Expensive issues.

Motor neuron loss occurs before development of clinical symptoms. Newborn screening helps in detection of cases early and diagnosis early. Early diagnosis effects directly on treatment which significantly improves the outcome of

disease. Carrier screening and genetic counseling (families with history of SMA) also plays an important role in decreasing the incidence of Spinal Muscle Atrophy. The diagnosed cases are treated with disease –modifying therapies such as nusinersen, risdiplam and onasemnogene ABEPRVOVEC.

Future Prospectives of Nanopore Sequencing Technology in SMA

The future is targeting nanopore assays integrating into screening programs newborn pathways and low resource diagnostic settings. Early diagnosis is important as disease – modifying therapies are most effective any motor neuron loss.

Newborn screening could provide rapid genetic confirmation of SMA, early identification in high-risk infants, faster initiation of treatment and improved long term better clinical outcome.

The next gain will probably come from better automation, simpler analysis pipelines and broader external validation across laboratories. As nanopore strength is long-read, real-time sequencing also helps in diagnosis of highly complicated genetic disorders. It could also help in monitoring gene therapy outcomes as gene therapies have transformed SMA management and also evaluate changes in SMN transcript expression. Might help in analysis of multiple neuromuscular disorders. As accuracy, affordability and clinical validation continue to improve, nanopore sequencing is likely to become an integral component of SMA diagnosis, newborn screening, carrier detection and personalized therapeutic management.

CONCLUSION

Nanopore sequencing technology is highly important in spinal muscle atrophy because it gives comprehensive analysis of SMN gene region, detects mutation copy number accurately, identification of pathogenic variants and characterization of complex haplotypes. All these things contribute in early diagnosis and carrier screening and are very helpful in personalized treatment strategies. Its long-read and real-time sequencing properties make it one of the most promising technologies for the future diagnosis and management of Spinal Muscular Atrophy. As accuracy, affordability and clinical validation continue to improve, nanopore sequencing is likely to become an integral component of SMA diagnosis, newborn screening, carrier detection and personalized therapeutic management.

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