

Current Development in Treatment of Spinal Muscular Atrophy

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Abstract. Spinal muscular atrophy (SMA) is an autosomal recessive genetic disease that affects the alpha motor units and leads to muscular atrophy. The survival motor neuron-1 (SMN1) mutation, which is found on chromosome 5q13, is responsible for the majority of SMA cases. SMA spectrum range from type 0 to type 4, with various severity, longevity, and symptoms. Usually, the more early onset of the disease is, the more severe the symptoms are. Therefore, early detection is vital since treatment can be implemented as soon as possible. Currently, diagnosis methods include newborn screening, point mutation testing, diagnostic testing, and carrier testing, each with a different purpose. Although there has been no cure for SMA so far, some medications could help to relieve the symptoms and allow patients to achieve a higher quality of life, like Onasemnogene abeparvovec-xioi, Nusinersen, and Evrysdi approved by the FDA. However, these treatments are costly. Additional support for SMA patients comes from physical therapy and careful daily management. Current research aims to identify more biomarkers of SMA to maximize therapeutic success and provide more precise therapeutic doses. This review provides a literature review of the pathogenesis, testing, and treatment of spinal muscular atrophy.

Keywords: Spinal Muscular Atrophy, DNA Sequencing, Treatment.

1. Introduction

Spinal muscular atrophy is a genetic disorder characterized by the degeneration of motor neurons resulting in muscle weakness. The SMN1 gene's homozygous deletion at 5q13 is the root cause of 95% of SMA cases. The incidence is estimated at 1 in 11000, and carriers of mutated SMN1 gene are around 1 in 40. The incidence varies by race as 0.89 in 100,000 for black ethnicity and 0.96 in 1000,000 for mixed ethnicity [1].

The spectrum of SMA ranges from type 0 to type 4, each with different severity and longevity. For SMA 0, the age on onset is during prenatal, and infants often die before 1-month-old. Symptoms include severe muscle weakness, lack of reaction to stimuli, and heart defects. The most common type, SMA 1 or Werdnig-Hoffmann disease, accounts for 50% of all cases. The onset age is before six months old, characterized by breathing stress, inability to sit independently, and weak cries. Generally, SMA 1 patients do not survive beyond two years. SMA 2 or Dubowitz disease usually begins between the ages of 7 and 18 months. At this stage, lower limbs are more affected than upper limbs, and facial features are generally unaffected. Patients cannot stand without assistance but can sit without support. However, deep tendon reflexes are absent. SMA 3, or Kugelberg-Welander disease, is milder and occurs after 18 months of age. At this stage, patients have reached most of their development motor milestones and can walk independently. Symptoms vary by individual. As the disease progress, some may need a wheelchair to assist mobility, while others only have a minor muscular weakness. However, complications such as obesity and osteoporosis might develop in those with mobility problems. Death usually occurs during adulthood. The last type, SMA 4, has adult onset and only accounts for 5% of the incidence. Patients could have an average lifespan and is able to maintain mobility throughout life. Overall, the early the onset of SMA, the more severe the symptoms are, and the less chance of survival [1,2].

There has still been no approved cure for SMA nowadays, but some treatments are available to prevent further complications of SMA. Currently, the studies on the mechanism of treating SMA are usually focused on three aspects. The first is to increase the quantity of endogenous fl-SMN proteins, which includes enhancing the expression of the SMN2 gene, changing the splicing of SMN2 transcripts, and increasing the activity of SMN protein. The second is to provide gene therapy and

stem cell treatment, but most of these methods are under clinical trials. The third is focused on improving the survival rates of motor neurons by exercising regularly, applying neurotrophic factors and neuroprotective agents [3].

2. DNA Sequencing and Early Detection

2.1. Mutated Gene

Based on genotype and clinical characteristics, SMA is divided into types 0–4, and the number of SMN 2 genes present affects how severe the condition is. For example, SMA-1 has only one copy of SMN 2, and death occurs within weeks of birth. On the contrary, SMA-4 has four or more copies of SMN 2 genes, and the condition does not interfere with lifespan.

Most SMA cases are caused by the mutation in survival motor neuron-1 (SMN1) located on chromosome 5q13. SMN 2 is a human-specific copy gene with 99% of the identical nucleotides as SMN 1 except for a few silent nucleotides. SMN 1 and SMN 2 contain nine exons that code for 2294 proteins. The difference lies in a C-to-T transition on exon 7, where SMN 2 cause an alternatively spliced mRNA isoform that disrupts an exonic splice enhancer in the exon. So, the transcript product of SMN 2 lacks the 16 amino acids produced by exon seven and cause incomplete SMN protein. Therefore, about 10% of SMN2-produced proteins are functional and identical to SMN 1 [4].

SMN protein is a 38 -KD protein that involves numerous cellular processes and biochemical pathways like assembling snRNP complex in the cytoplasm. Homozygous deletion of SMN 1 exon 7, absence of SMN1, point mutation, and SMN 1 gene conversion is expected in 95% of SMA patients. However, SMN 2 is the current target for treating SMA, but it also provides enough proteins to cause the development of SMA. By analyzing the expression of SMN1 and SMN2 in patients with SMA-1, SMN 2's strength of producing functional SMN protein is stronger in unaffected tissues such as muscle and kidneys. These results indicate that SMN2 may be a potential treatment for SMA [5].

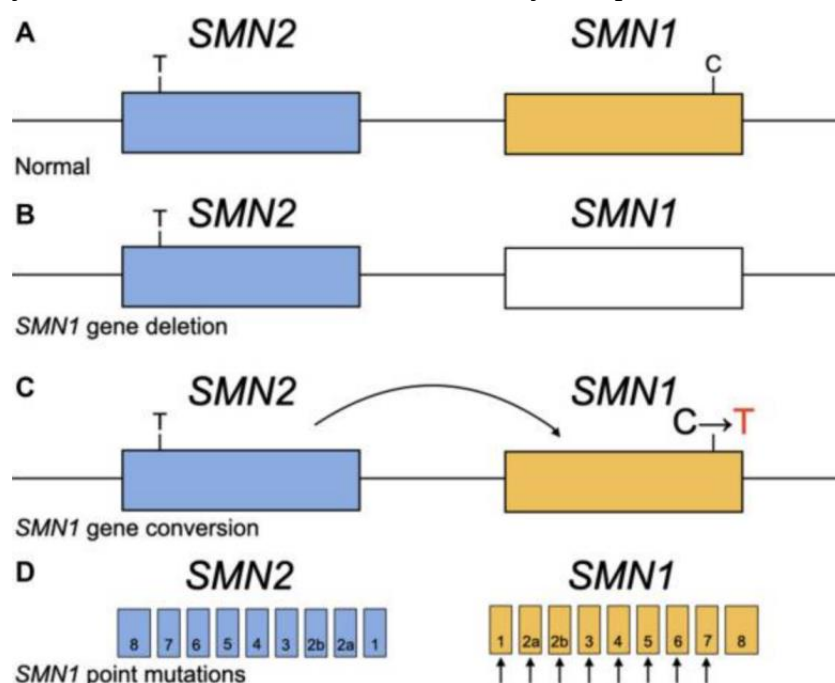


Figure 1. Examples of mutated SMN genes [6]

2.2. Testing

2.2.1. Diagnostic Testing

A standard DNA sequencing technique is to use quantitative polymerase chain reaction (qPCR) or multiplex ligation-dependent probe amplification (MLPA) DNA assays to find missing SMN1 exon

7 in patients [7]. Prenatal testing for SMN1 deletion is for fetuses with abnormal ultrasound detection, like decreased fetal movements or previous family history. DNA is extracted either from amniotic fluids or the chorionic villus. If the mother's blood contaminates the sample, it may cause a false-negative test result. The test is highly sensitive, and patients could receive the result within 24 hours after testing [4].

2.2.2. Point Mutation Testing

The whole-exome sequencing can be used by both the parents and the child to detect SMN1 and SMN2 point mutation [7]. For patients who tested negative for the homologous deletion test, the Sanger sequence could be used to identify mutations due to SMN1's small size. There is about 5% of SMA patients with SMN1 mutation. Mutation types include splice site mutations, nonsense mutations, insertions, and deletion on a single SMN1 on one allele [4].

2.2.3. Newborn Screening

To use newborn screening to test for SMA, blood is required from the newborn's heels. Newborns usually do a DNA sequence test for SMN1 exon 7. This is important because SMA-1 patients start to lose motor units within weeks after birth [4]. The earlier the treatment is, the higher chance of improving the survival rate and achieving a higher quality of life. After receiving an SMA diagnosis, parents should immediately contact the baby's doctor or health providers and implement treatment [8]. According to a study conducted by Kay et al., the pre-symptomatic infants who received early treatment remained no symptoms and indicated the importance of early detection [9].

2.2.4. Carrier Testing

A carrier test is beneficial for those who want to use a blood sample to assess their risk of having a child with SMA and have a family history of the condition. The test could identify the carrier with one copy of SMN1 from non-carriers who have two or more copies of SMN2. Although the test can determine whether the scanner is a carrier or not, it cannot predict how severe the SMA is if the child inherits the gene. Usually, one parent is tested first. If the first parent is a carrier, then there is a need to scan the second parent. If both parents are carriers, it is recommended that during pregnancy, the mother should have amniocentesis or chorionic villus sampling to see if the child inherited the gene [10].

3. Symptomatic Treatment

3.1. Physical Therapy

Physical therapy for SMA includes positioning and bracing, supported standing, and physical exercises. For patients who need help sitting, seating systems, including molded pillows and supine positioning with rolls, are required for daily use. Cervical bracing is used for patients who have reduced head control. Components include cervical jackets to maintain the stability of the head and a neck collar to fix the bracing. Knee immobilizers and hand splints could help when the patients want to stretch. Helping SMA patients stand could benefit spinal alignment, lung, and gastrointestinal functions, especially for children lacking walking ability. In addition, physical exercises could improve motor function and stimulate SMN gene expression [11]. According to a study by Dr. Sneha Buttepatil and Dr. Nilashri Naik, patients with SMA-2 on Hammersmith Functional Motor Scale showed physical enhancement in sitting, kneeling, unsupported standing, squats, muscle power, and extremity after physical therapy. It was safe for patients to join the rehabilitation program and improvement appeared after 18 months of training [12].

3.2. Management

SMA-1 and -2 could cause respiratory insufficiency and infections in infants and children. Respiratory management requires non-invasive ventilation for early onset and mechanical ventilation for infants with respiratory failure. Early sleep-related hypoventilation is common in childhood, and

non-invasive ventilation may help. However, airway clearance and ventilation support are needed if a severe pulmonary infection happens. In addition, young patients' intake of calories, nutritional supplements, and food should be constantly supervised, their ability to breathe, feed and swallow, and constipation should be elevated routinely. Complications are usually experienced in early childhood, and if poor intake happens, a nasogastric tube or percutaneous gastric tube is suggested as management. Besides, orthopedic management is essential for SMA-2 patients because scoliosis often occurs. For scoliosis, spinal fusion or the placement of spinal growing rods is recommended. SMA-1 patients usually do not exhibit scoliosis because most infants cannot sit. Other management includes consulting psychologists to relieve depression and anxiety caused by SMA for patients and their family members [13].

4. Drug Treatment

4.1. Nusinersen

Nusinersen is a drug approved by the US Food and Drug Administration (FDA) to treat all types of SMA to increase the expression of SMN protein. Since the SMN2 gene is responsible for producing protein to modulate the severity of SMA, SMN2 is chosen, especially antisense oligonucleotides (ASOs), as the therapeutic target [13]. Nusinersen is an SMN-2-directed ASO that alters the splicing of SMN2 pre-mRNA and blocks splicing factors [14]. Nusinersen is injected into the cerebrospinal fluid since it cannot cross the blood-brain barrier (CSF). Therefore, the applied dosage should remain the same regardless of age and weight [13]. A clinical trial conducted by Finkel et al. reported that the administration of Nusinersen to the onset of SMA in infants between three weeks and seven months old showed safety and tolerability, and the results were in accordance with the described mechanisms and clinical efficacy [6]. However, adverse effects still exist. Common adverse events include infections in lower and upper respiratory tract, constipation, and pyrexia [13]. Moreover, further complications caused by drug-like nephrotoxicity, blood coating disorders, and thrombocytopenia should be considered [14]. Although Nusinersen is effective, the drug's high cost remains a concern. An injection of Nusinersen costs about \$125,000, and it is estimated that it would cost around \$750,000 for a one-year treatment. Therefore, the debate about whether Nusinersen should be covered under health insurance is ongoing [13,14].

4.2. Onasemnogene abeparvovec-xioi

Onasemnogene abeparvovec-xioi, or AVXS-101, is an adeno-associated virus vector-based gene therapy aiming to deliver functional SMA gene to motor neuron 1 (SMN1 gene) and enhance SMN protein production [15]. Onasemnogene abeparvovec is a biological drug composed of AAV9 virus capsid containing SMN1 transgenic and synthetic promoters that goes into a complete life with the help of a helper virus and undergoes transduction in neurons [16]. FDA has approved this gene therapy for children under two years old with mutations in the SMN1 gene to enhance muscle movement and increase their survival rate [17]. A global phase 3 clinical trial called Gene Replacement Therapy Clinical Trial recruited patients with SMA type 1 who are younger than six months of age from the United States, Europe, and Asia Pacific and found that 91% of patients had an event-free survival at 14 months of follow up [16]. Patients treated with AVXS-101 also showed improved motor abilities such as head control, reaching the infant's motor milestones, and sitting without help. However, side effects do exist. Common adverse events include elevated aminotransferases, vomiting, and severe liver toxicity or injury, and the long-term efficacy and risks are still being determined. Like Nusinersen, the cost remains high [15-17]. For the first year of treatment, the estimated cost is \$750,000, and the subsequent year of treatment costs about \$375,000 annually. The one-time dose cost \$2.125 million [16]. Luckily, the United States and other developed countries have covered the partial cost of AVXS-101 under insurance and would provide partial rebates if the treatment is ineffective [15].

4.3. Evrysdi

Evrysdi is an SMN2 mRNA splicing modifier oral medication approved in August 2020 by the FDA to treat all ages of SMA patients to increase SMN protein levels rapidly. Evrysdi regulates SMN2 splicing to include exon 7 in mRNA transcripts, thereby increasing the expression of full-length SMN proteins from SMN2 genes [18]. Although Evrysdi is a daily oral and life-long medication, it is the first and only at-home treatment for SMA. Trials were tested on mice, infants and adults, and patients who have received SMA treatment before. The results were statistically significant compared to the placebo group [19]. However, adverse reactions exist, such as fever, lower respiratory tract infection, rash, diarrhea, vomiting, and constipation. Evrysdi may also cause embryofetal harm to pregnant and breastfeeding women and damage to male fertility [20]. Evrysdi's cost varies a bit across pharmacies, but the general price is \$12,360 for 80 mL oral powder (0.75 mg/mL). The daily dosage is 5 mg for people two years of age and older who weigh at least 20 kg [21].

Table 1. Promising Treatment Currently Undergoing Clinical Trails [22]

Name	Phase	Study Type	Primary Purpose	Status
Hydroxyurea	I, II	Interventional	Treatment	Completed
Valproic Acid and Levocarnitine	I,II			
Sodium Phenylbutyrate (NaPB)	I,II			
Whole Body Vibration Therapy	Not applicable			
Reldesentiv	II			
Olesoxime	II			
Risdiplam	II, III			
Amifampridine Phosphate	III			

5. Conclusions

SMA is not a curable disease, and the mutated SMN genes on chromosome 5 cause most incidences. The types of SMA range from 0 to 4, with type 0 being the most severe. In general, the earlier SMA manifests, the more severe its symptoms are and the lower the survival chance. Current treatment could only help patients to relieve symptoms and extend their longevity. Treatment such as Onasemnogene abeparvovec-xioi (gene therapy) and Nusinersen (injection) 's prices are staggering. At the same time, Evrysdi (oral medication) has a relatively low cost, but it must be taken daily. Therefore, early detection and screening are helpful. Doing the carrier testing for both parents could relieve the risks of having a child with SMA. Because the parents are aware that their child may inherit the mutated SMN genes, detection of the child during pregnancy is vital. Other tests can help to diagnose SMA and start the treatment as early as possible to maximize the chance of survival and maintain a high-quality life.

A challenge of SMA treatment involves limited effects since most patients are diagnosed during the advanced stages of motor neuron degeneration. Therefore, the evidence of drugs and therapies is often limited to a specific disease spectrum or a particular age group. Furthermore, since only a few volunteers could participate in clinical studies, shreds of evidence are often inconclusive. This leads to the problem of giving the same dose to all age groups and may cause over-dose toxicity. In addition, the screening process is still advancing since not all children display symptoms immediately after birth, which may delay treatment [23].

Despite these challenges, more research and clinical studies are working to unravel the pathophysiology of SMA. For example, the search for the biomarker of SMA is currently under active investigation to better reflect disease progression and adjust therapeutic doses. Some proposed biomarkers include electrophysiological measurement and SMN protein levels in peripheral blood and are supported by preclinical trials on mice. Further investigation is working on the correlation between radiologic markers and motor function in SMA, as this relationship is already demonstrated in Duchenne muscular dystrophy [24]. Matsaers et al. identified a potential SMA biomarker,

Calreticulin and GrP75/Mortalin, present in neurodegenerative diseases, and more research is going on to identify more precise SMA biomarkers [25]. Despite the identification, many of the biomarker substrates have not been proven to reflect SMA disease progression [24]. However, with the new discoveries and the advancement of genetic coding, medications, and nanorobots, it is possible that one day SMA could be treated.

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