A Review on Spinal Muscular Atrophy - A Fetal Autosomal Neurotransmitter Recessive Disorder

By

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A project submitted to the School of Pharmacy in partial fulfilment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

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Declaration

It is hereby declared that

1. The project submitted is my own original work while completing a degree at BRAC University.

2. The project does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.

3. The project does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.

4. I have acknowledged all main sources of help.

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Approval

The project titled "A Review on Spinal Muscular Atrophy - A Fetal Autosomal Neurotransmitter Recessive Disorder" submitted by Nafiza Tabassum (19146029), of Spring, 2023 has been accepted as satisfactory in partial fulfilment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

This study did not involve any human participants, human specimens or tissue, vertebrate animals or cephalopods, vertebrate embryos or tissues and field research.

Abstract

Spinal muscular atrophy (SMA) is the degradation of anterior horn cell which prevent the motor signal to travel throughout skeletal muscle, hence resulting in degradation of spinal motor neuron leading to muscle weakness and atrophy. This genetic disorder is caused by the production of SMN2 gene when SMN1 gene is mutated, which is less effective to produced SMN protein essential for motor neuron functioning. Symptoms depend on the severity and types of disease. In infants they can manifest as progressive muscle weakness, difficulties in respiratory system, breathing, swallowing and motor impairment. Genetic testing is done through blood and saliva for diagnosis purpose. Creatinine kinase, nerve conduction studies, EMG, muscle biopsy are the additional tests. The most recent medicine to be licensed for use in the treatment is Nusinersen of Spinraza, administering through lumber puncture. This antisense oligonucleotide medication targets the backup gene SMN2 to produce and synthesize full-length SMN protein.

Keywords: Spinal muscular atrophy, SMN1, SMN2, Genetic testing, EMG, Nusinersen, SMN protein.

Dedication

This work is dedicated to my parents and supervisor for their love and constant support.

Acknowledgement

I am grateful to almighty Allah for providing me the opportunity to work with such wonderful people from the school of pharmacy who have always been idealistic and encouraging throughout my journey.

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SMA	Spinal Muscular Atrophy
SMN1	Survival of Motor Neuron 1
SMN2	Survival of Motor Neuron 2
EMG	Electromyography
HRQoL	Health Related Quality of Life
NMJ	Neuromuscular Junction
LMNs	Lower Motor Neurons
AchR	Acetylcholine Receptor
RNPs	Small Nuclear Ribonucleoprotein complexes
snRNAs	Small Nuclear RNAs
hnRNP	Heterogenous Nuclear Ribonucleoprotein
BN	Ballooned neurons
CMAP	Compound Muscle Action Potential
MUNE	Motor Unit Number Estimation
ASO	Antisense Oligonucleotide
AAV9	Adeno-Associated Virus 9

Chapter 1

Introduction

1.1 Background

SMA refers to spinal muscular atrophy. It is a neuromuscular disease containing a series of genetic disorders which are characterized by the degradation of anterior horn cells (it is an alpha motor neuron that sends out motor signals to skeletal muscles) ultimately causing muscle atrophy and weakness. It was originally identified in children by doctors Guido Werdnig and Johan Hoffman in the early 1890s. About a century later, it was discovered that SMN2 (Survival of Motor Neuron 2) gene is brought on by a mutation in the SMN1 (Survival of Motor Neuron 1) gene. SMN1 gene helps to assemble small nuclear ribonucleoprotein complexes (snRNPs), and hence plays a significant role in ensuring that motor neurons are able to perform their functions correctly (Aslesh & Yokota, 2022). Without the proper functioning SMN1 gene, the body relies on the backup gene SMN2 which is less effective to produce a significant amount of SMN protein (Wirth et al., 2020).

A lower amount of SMN protein is produced as a consequence of the deletion or mutation of the SMN1 gene, which ultimately leads to the death of motor neurons and gradual deterioration of muscle tissue. Which means the nerve cells that control the muscle tissue get destroyed and eventually resulting in damaging in spinal cord after birth. Muscle weakness, stiffness of neck, disability, respiratory complication, congenital heart disease and even death might result fromthe lack of SMN protein in the nervous system (Burghes & Beattie, 2009). But they process normal intelligence and intellectual ability as SMA do not have effect on sensory neurons (Arnold et al., 2015a).

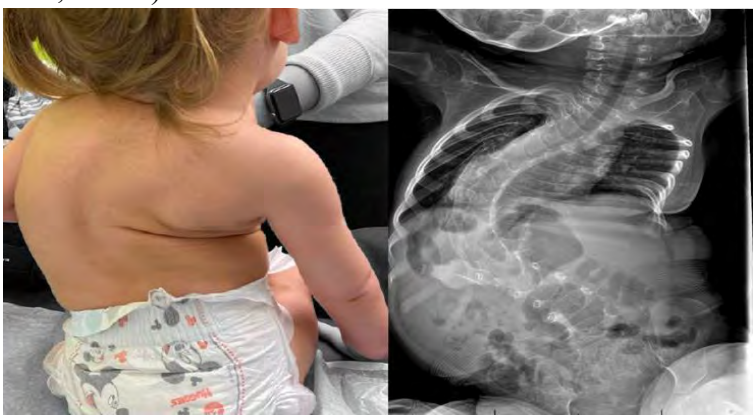

Figure 1: A patient with spinal muscular atrophy, seen here in a clinical image and on an anteroposterior radiograph (*Miqad A. | Lawrence G. Lenke, MD*, n.d.).

A case with severe scoliosis and type 2 spinal muscular atrophy is shown in a clinical picture and anteroposterior x-ray. Different types of spinal muscle atrophy have different life expectancies. Most people with type 1 spinal muscle atrophy die before they are 2 years old. People with Type 2 might be expected to live until they are young adults. People with type 3 have a life expectancy that is about average. Improvements in care and support for breathing have helped to make these people more likely to live longer (Miqad A. | Lawrence G. Lenke, MD, n.d.).

1.2 Function of SMN protein

The whole protein that contains SMN has a molecular weight of 38 and is 294 amino acids long. It is located in the development cones of motor neurons, as well as the nucleus, cytoplasm, and gems (a unique nuclear structure) (Farooq et al., 2013). Proteins involved in motor neuron survival (SMN) are found only in nerve cells. and the general roles of these cells were studied. SMN controls the biogenesis, maturation, and recycling of small nuclear ribonucleoprotein (snRNP) in Gemini of Cajal bodies and Cajal bodies, and ribosome synthesis in the nucleolus are all located in the astrological sign of Gemini, mRNA transport in axons, snRNP biogenesis and actin dynamics in the cytoplasm, and actin dynamics and vesicle release at the synapses (Bowerman et al., 2017).

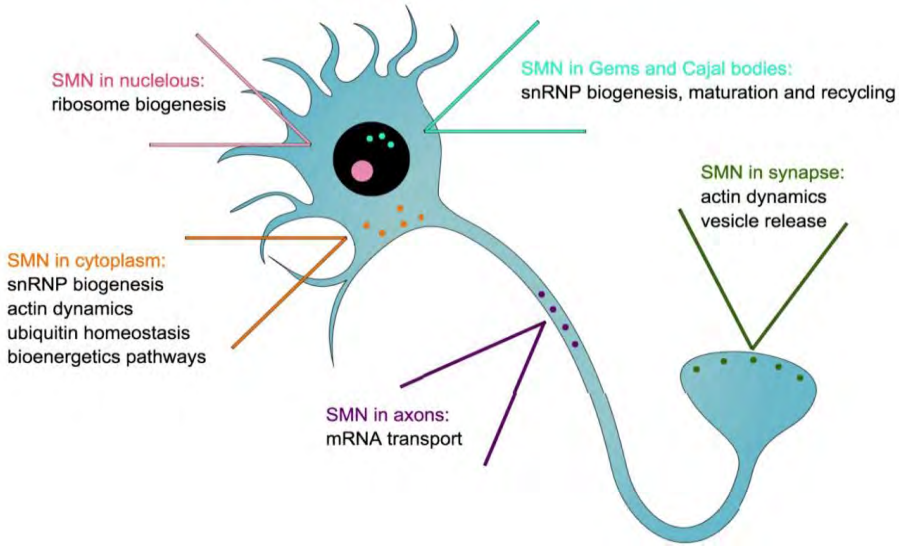

Figure 2: Localization of SMN (Spinal Motor Neuron) protein (Bowerman et al., 2017).

It appears that SMN expression is required for cell survival in all cell types. SMN assists in binding Sm proteins on small nuclear RNAs (snRNAs) during the splicing of pre-mRNA. They

play an essential role in placing together other groups of ribonucleoproteins (RNPs) (Burghes & Beattie, 2009). As a result of the SMN complex's ability to directly recognize and bind to both the protein and the RNA components of the ribonucleoproteins, as well as to enhance their interaction with one another. The assembly process for small nuclear ribonucleoproteins ensures by a high degree of specificity (Kolb et al., 2007). When there isn't enough SMN protein, the growth of the neuromuscular junction (NMJ) stops after birth. This makes it hard for acetylcholine receptor (AChR) groups to mature into "pretzels". Defects in the presynaptic circuitry, such as insufficient terminal arborization and aggregation of the intermediate filament, could potentially be used as a diagnostic biomarker. Functional abnormalities at the NMJ, shown as intermittent breakdowns in neurotransmission, are a direct result of these failures (Kariya et al., 2008) .

1.3 Significance

There is no ultimate cure for SMA along with that the symptoms gets worsen over time. Complications might develop with the bones, joints, spine, and breathing system as time passes. There are treatments and methods available that can assist to manage and take control of the symptoms. Treatments have been approved by regulators that helps to raise SMN expression through different molecular processes, administration routes, and organ biodistributions. These therapies not only enable people who have never been sat or walked before learn how to do so, but they also help newborns to survive longer who would have died before they turned two years old.

1.4 Epidemiology

Spinal muscular atrophy, also known as SMA, is the second most prevalent autosomal recessive neuromuscular illness. It is characterized by the loss of motor neurons from the anterior horn of the spinal cord. SMA is the second most common neuromuscular condition. This loss of motor neurons causes musculoskeletal symptoms such as weakness, hypotonia, and finally atrophy of the muscles (Farooq et al., 2013). The rate of 5q-SMA is 0.27 per 10,000 live births for type 1 and 0.08 per 10,000 live births for type 2 across the world in cases where genetic testing was able to definitively diagnose the condition (Ito, M. et al. (2022).

1.5 Prevalence

The prevalence of SMN1 mutation is estimated to be 2-3% (1 in 40) in the general population, with a SMA incidence of 1 in 6000-11000. The number of cases varies by racial background. For example, 8 cases per 100,000 white people, 0.89 cases per 100,000 black people, and 0.96 cases per 100,000 people of mixed ethnicity have been recorded (Burr & Reddivari, 2023a). SMA has a carrier frequency of 1:50 and a pan-ethnic incidence of 1:11,000 live births, making it one of the leading genetic causes of infant death throughout the entire world.

In a study that took place in Japan in 2017 stated that, 658 people were known to have SMA, and 79.5% of them had DNA tests done. The number of cases was found to be 1,478 with a 1.17 cases per 100,000 individuals and 0.51 cases per 10,000 live births represent the disease's prevalence and incidence, respectively (Ito, M. et al. (2022).

In Bangladesh, according to sources about 30 individuals with SMA are seen annually at the National Institute of Neurosciences and Hospital (NINH), with additional cases seen annually at Bangabandhu Sheikh Mujib Medical University, Shishu Hospital, and Dhaka Medical College Hospital, respectively.

1.6 Aim

The purpose of this review is to examine and understand different characteristics of SMA patients such as disease subtype, age at diagnosis, and severity of symptoms affecting their HRQoL (health related quality of life). Bringing together all we will be able to know about SMA from the phenotypic to the genetics to the treatments, highlighting the important gains that have been made thus far while also presenting some predictions about what the future holds for the diagnosis and treatment of SMA.

1.7 Objectives

The objectives of this review are:

- To compile information about existing and emerging consequences about SMA disease.
- To collect information about health status of SMA effected patients and the survival rate.

- Accurate information on the therapeutic approaches that may be used to raise the level of expression of the SMA protein in the appropriate cell at the appropriate time
- Explain in brief the phenotype, molecular pathogenesis, diagnostic approach, and treatment regimens

Chapter 2

Methodology

This review study is conducted using current and pertinent research papers and articles from high impacted journals. Peer-review journals, official papers and articles were all thoroughly searched. To enhance the review article, basic and supplementary information were gathered from numerous sources. Some search engines have been used to gather data for this review paper which are Scopus, Science-direct, Springer & PubMed. The major publications were focused on to needed data are- Nature, Frontiers, Meghtex etc. A thoroughly screening was done before focusing on the most recent and pertinent ones to create an ideal quality review on "Spinal Muscular Atrophy - A Fetal Autosomal Neurotransmitter Recessive Disorder".

Chapter 3

Body

3.1 Molecular Mechanism of the Disease (Splicing defect in SMA)

The SMN1 gene is mutated and depleted in individuals with SMA patients; consequently, the body relies on the SMN2 gene, which is very similar to the mutated SMN1 gene. In the end, it leads to a reduction in the production of SMN protein. Spliceosomes are complexes that play an important part in the construction and function of cells. This complex is responsible for mediating the process of splicing. The activity of this complex is determined by a number of different factors(Tariq et al., 2013). The SMN protein is an essential component of these complexes. Spliceosomes are responsible for eliminating non-coding parts from the genetic material that are referred to as introns. This makes it possible for the remaining sections, which are referred to as exons, to be put together to form a final functional messenger RNA (mRNA) molecule. Without enough SMN protein, spliceosomes can't put themselves together and work properly. This causes the wrong splicing of several genes that control how motor neurons work, including the SMN2 gene. In particular, both SMN1 and SMN2 are spliced by different cis- and trans-acting elements. The C-T transition that occurs at position 6 of exon 7 of the SMN2 gene causes exon 7 to be skipped because it either makes an exonic splice suppressor (ESS; recognised by hnRNP A1/A2) or inhibits the function of an exonic splice enhancer (ESE; recognised by SF2/ASF to support exon 7 inclusion) (Tariq et al., 2013). This ESS is used as a component in the construction of hnRNP A1, which is a protein that is known to suppress gene expression. The SMN2 axon 7 RNA is the only type of RNA that this protein binds to; SMN1 axon 7 RNA is inaccessible to this protein's binding (Kashima & Manley, 2003).

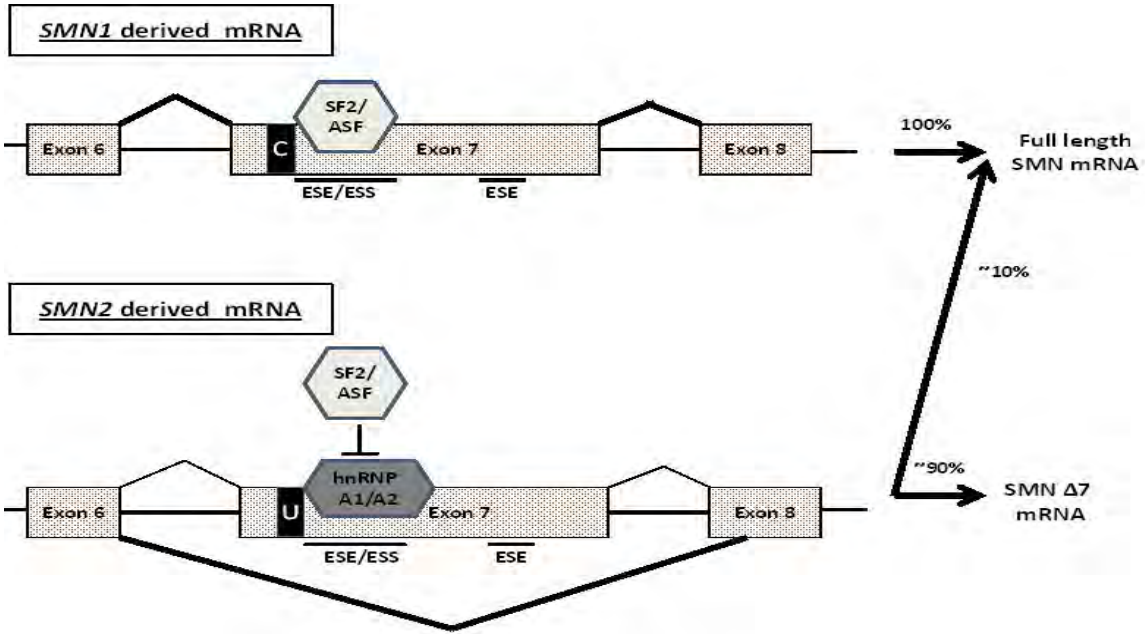

Figure 3: Splicing of SMA (Tariq et al., 2013)

SMN1 and SMN2 only differ by 5 nucleotides in their genomic sequences, but only one of these differences is functionally important. This is a CT alteration in an exonic splicing region of SMN2 that has no functional impact on the translated protein. Because of this change, exon 7 is often skipped when SMN2-derived transcripts are put together (Mercuri et al., 2022). Splicing of the SMN2 gene results in the production of a shortened form of the SMN protein. This form does not function as well as the full-length version that is produced by the SMN1 gene, and it is also less stable.

3.2.1 Genotype or Classification

Background

Spinal muscular atrophy was first seen in two infant boys by Guido Werdnig in 1891. Seven more cases were seen by Johann Hofmann between 1893 and 1900.Even though Werdnig-Hoffmann disease later became known as the severe infantile form of SMA, Werdnig and Hofmann's cases were actually of moderate severity. Sylvestre and Beevor described severe infantile SMA for the first time in 1899 and 1903, respectively.1 Wohlfart, Fez, and Eliasson didn't describe a weaker form of SMA in which patients could still stand and walk and lived longer until the 1950s. Kugelberg and Welander then went into more detail (Kolb, 2011).

Spinal muscular atrophy, also known as SMA, is a hereditary condition that weakens muscles and causes atrophy (wasting away) of the muscle tissue. In the brainstem and spinal cord, it affects the motor neurons. The severity of the condition, as well as the particular kind of SMA that individual possesses, can have an effect on the phenotypic, or observable characteristics, of a person who has spinal muscular atrophy (SMA). There are various subtypes of SMA, which are distinguished by the age at which symptoms first appear and the degree to which they manifest. From a physiological point of view, the extreme nature of SMA, is classified into the categories of "Non-sitters," "Sitters," and, less commonly, "Ambulant." Type 0/Type 1, Type 2, and Type 3 SMA are the corresponding terms., Type 4 and their subtypes respectively (Fitzgerald et al., 2018).

Type 0 or Prenatal-onset SMA:

The most extreme and uncommon form of SMA is called type 0. It is sometimes referred to as severe SMA or SMA that begins in utero. In most cases, symptoms appear in utero, or during pregnancy, or within the first weeks of a baby's life.

They face abnormalities in their brains that got worse over time that aren't usually seen in people with SMA. So, severe brain involvement may be the final sign of an already severe SMA phenotype that is caused by a significant decrease in the total amount of SMN protein found in the brain (Farrar et al., 2017). It frequently becomes apparent before birth, at some point during pregnancy, or not long after birth. Even while still in the womb, infants who are affected by this form of spinal muscular atrophy (SMA) may show symptoms, such as decreased fetal movement (Mendonça et al., 2019).

Type 1 or Werdnig-Hoffmann disease:

Werdnig-Hoffmann disease and infantile-onset spinal muscular atrophy are both names for the same phenotype 1 of spinal muscular atrophy (SMA). This version of the illness is the most serious kind, and it usually becomes apparent within the first few months of a person's life. Most of the time it appears within first 6 months. Typically, all cases are deadly by the age of 2; 50% of patients die by 7 months, and 90% by 12 months. The severity can be divided into 3 categories: (Bach, 2008)

- infants who will need non-oral nutritional supplementation and persistent mechanical ventilation before they turn 5 months old

- newborns who need nutritional supplementation outside of the mouth because they acquired acute respiratory failure from an ineffective cough brought on by an upper respiratory tract infection (URTI) before the age of 24 months.
- Approximately 10% of children with SMA type 1 do not require non-oral nutritional support until after the age of 24 months because they do not develop respiratory failure.

The observable signs and symptoms of this form of SMA are referred to as the phenotypic. Individuals who have this form of SMA have these characteristics and symptoms. In the first six months of life, a person with Spinal Muscular Atrophy Type 1 typically has weakness in the trunk and nearby limbs, and they are unable to roll over or sit up. Fasciculations of the tongue are visible at presentation, and deep tendon reflexes in the arms and legs are absent. Failure to thrive, hypoventilation, and acute respiratory presentations such viral bronchiolitis or aspiration pneumonia are common results of inadequate feeding and trouble swallowing (Darras, 2015). Most of the people approximately 90% with SMA Type 1, who don't get treatment die before they are 12 months old, and all of them die before they are 24 months old. In some cases, tracheostomy can help people live for more than 20 years, but people with tubes don't learn to speak and can't breathe from the point where the tracheotomy was done (Bach, 2008b). For Spinal Muscular Atrophy Type 1, the standard of care in Australia and the United Kingdom has traditionally been providing palliative care with the understanding that the patient will likely die within the first two years of life, despite the efforts of notable advocates of more proactive management such as the use of gastrostomy feeds and invasive ventilation (Finkel et al., 2016).

Type 2 or Intermediate SMA:

A form of spinal muscle atrophy (SMA) has been identified that is in between the Werdnig-Hoffmann (type I) and Kugelberg-Welander (type III) types in terms of severity. This is called intermediate or type II SMA, and it causes weakness in the muscles near the joints. It usually starts between 3 and 15 months and lasts for more than 4 years, generally until adolescence or later (Fried & Emery, 2008).

Children who have type 2 spinal muscular atrophy can typically sit up on their own without assistance, but they may have trouble standing up and walking. But also along with that individuals who are diagnosed with SMA Type 2 have, in general, a better outlook compared to those who have one of the more severe kinds of SMA. Many people who have SMA Type 2

can see an improvement in their quality of life and a reasonably stable progression of the disease if they receive the appropriate medical care, supportive therapies, and advancements in medications such as Spinraza (nusinersen) (Mercuri et al., 2016).

Type 3 or Juvenile SMA or Kugelberg-Welander Disease:

Spinal muscular atrophy type 3 is also known as Kugelberg-Welander SMA, after the authors who first wrote about it in 1956. They said that it was a "pseudomyopathic" form of spinal muscle atrophy that started between the ages of 2 and 17 years (Salort-Campana & Quijano-Roy, 2020). Type III SMA usually starts between the ages of 18 months and adulthood (the rate at birth is estimated to be around 13%. By definition, the patient can stand or walk without help, but as the disease gets worse, many patients lose these skills. They face muscle fatigue, increasing weakness, and atrophy of the lower limbs after the individual has learned to walk on their own. One can anticipate an ordinary lifespan and a change in the incidence of Type III in the SMA population can be noticed as it advances in age. It is dependent on the individual's natural history of the disease as well as the moment at which they received their diagnosis (Rouault et al., 2017).

The less severe types of SMA are SMA3 and SMA4, which affects adults. But a clinical identification is harder than it used to be because some of the symptoms are the same as those of more common myopathies. Complementary tests can show nerve damage, nerve growth problems, or a certain pattern of muscle participation (Salort-Campana & Quijano-Roy, 2020). They have 3 subtypes based on the severity:

- **Type 3a**: This form is sometimes called "mild" SMA Type 3. Onset usually happens between early childhood and puberty. People with Type 3a can walk on their own and reach standing and walking stages without any help.
- **Type 3b:** This form is sometimes called "intermediate" SMA Type 3. Symptoms usually start in late childhood or early teens. People with Type 3b might be able to walk on their own for a while, but their motor function declines more quickly than in Type 3a. As, the disease gets worse, they may lose the ability to walk and have to use gadgets to get around.
- **Type 3c**: This form is sometimes called "severe" SMA Type 3. Usually, it starts during youth or early adulthood. People with Type 3c have a major loss of motor function and

mobility. They might lose the ability to walk and need aids or a wheelchair to move around.

Type 4 or Adult onset SMA:

Spinal Muscular Atrophy (SMA) Type 4, also called Adult-Onset SMA, is the mildest form of SMA and usually shows up in adults. Type 4 SMA shows up later in life, unlike the more serious forms that show up in babies and young children (Shababi et al., 2014). Its age of onset is 10 to 30 years or more than 30 years. People with SMA Type 4 have a better than average chance of survival. Their life expectancy is normally normal, despite the fact that they may have difficulties due to muscle weakness. In comparison to more severe forms of SMA, this form often progresses slowly and has a milder effect on daily living (Zolkipli et al., 2012). They are able to walk or stand independently even without the help of any machineries or personnel.

3.2.2 Phenotype or Symptoms

Type 1 or Werdnig-Hoffmann disease:

Type 1, also known as Werdnig-Hoffmann disease, is the worst form of SMA as it's survival rate is least among other SMA types. It is usually diagnosed in infancy (in first few months of life) or early childhood.

Age of Onset: Evident at birth or within the first few months of life.

Signs of type 1 spinal muscular atrophy are(Darras et al., 2015a) (Arnold et al., 2015b):

i. **Muscle Weakness and Hypotonia:** Infants who have Spinal Muscular Atrophy (SMA) Type 1 generally show severe muscle weakness from the time of birth or shortly afterward. The weakness of their muscles prevents them from performing even the most fundamental movements, giving them a floppy, "hypotonic" appearance.
ii. **Limited movement:** Because of the weakness in their muscles, infants who have type 1 spinal muscular atrophy have a restricted range of motion and are unable to move their limb or sit up or turn over. They have a severe delay in the development of their motor skills.

iii. **Challenges in breathing and swallowing**: Weak muscles that control breathing and swallowing can cause serious breathing problems and make it hard to eat. Babies may have trouble sucking and swallowing, which makes it hard to feed them also there is a risk of filling the liquid in lungs.

iv. **Frequent infection**: Infections occur frequently, especially in the respiratory system because of how poorly it is functioning. Pneumonia and other respiratory illnesses are potentially fatal.

v. **Spinal curvature (Scoliosis):** As the disease advances, it can cause abnormal curvature of the spine (scoliosis) due to muscular weakness in the spine's supporting structures.

vi. **Poor head control:** Infants born with type 1 spinal muscular atrophy frequently have insufficient head control as a result of the weakness of the muscles in their necks, which makes it difficult for them to hold their heads up.

vii. **Weak cry:** Weakness impacts the muscles involved for vocalization, which includes sobbing. Therefore, newborns with type 1 SMA may cry weakly and in a way that is distinctive from conventional cries.

viii. **Lack of reflexes:** Infantile reflexes, such as the Moro reflex (startle reflex) and the grab reflex, may be missing or severely impaired in neonates with type 1 spinal muscular atrophy.

ix. **Delayed milestone:** Delays in reaching Motor Milestones As a result of their weakened muscles, infants who have type 1 SMA face considerable delays in reaching motor milestones such as sitting, crawling, and standing.

Type 2 or Intermediate SMA:

Age of onset: At the time of infancy or the early years of childhood. Symptoms are (Arnold et al., 2015b),:

i. **Muscle weakness:** The muscle weakness experienced by those with Type 2 SMA is less severe than that seen in those with Type 1 SMA. Because of this deficit, mobility may be restricted.

- **Muscle Twitching and Tremors:** People with Type 2 SMA can have muscle twitching (called fasciculations) and tremors because their muscles are weak and their motor neurons don't work well.

- **Contractures:** Joint contractures are constant tightening of the muscles and tendons around a joint. They can happen when muscles are out of balance or weak. This can make it hard for joints to move.

ii. **Delayed motor milestones:** Children with Type 2 SMA often take longer to do things like sit up, stand up, and walk. With help or special tools, some people may reach these goals in the long run.

iii. **Walking difficulties:** Although some people who have type 2 spinal muscular atrophy may eventually learn to walk, many people with this condition will have difficulties walking independently owing to muscle weakness. Some people could need assistance or mobility devices like braces, walkers, or wheelchairs. Others might be able to get around without them.

iv. **Problems with respiratory:** They are typically not as severe as they are in type 1 spinal muscular atrophy (SMA). People can be at risk of getting respiratory infections, and they might need to have their lung function checked at regular intervals.

v. **Scoliosis:** People who have Type 2 spinal muscular atrophy have an increased chance of developing scoliosis, which is an abnormal curvature of the spine, as their muscle weakness worsens.

vi. **Fatigue**: Muscle weakness can lead to fatigue, which can contribute to overall decreased levels of energy. It can occur with even the simplest of actions.

vii. **Challenges with Speech:** Weakness in the muscles can sometimes have an effect on the muscles that are utilized for speech, which can lead to difficulties with articulation and vocalization.

viii. **Concerns with nutrition:** It include difficulty swallowing and a lack of strength in the mouth muscles, which can make it difficult to eat and drink. This can impair one's nutrition as well as their ability to gain weight.

ix. Intellectual Function: In Type 2 SMA, intellectual ability is typically unaffected. However, difficulties caused by motor limits can have an effect on everyday life and limit the range of activities they can partake in.

Type 3 or Juvenile SMA or Kugelberg-Welander Disease:

Onset: later on throughout childhood or later on during adolescence. Symptoms are (Arnold et al., 2015b; Darras et al., 2015a):

i. **Muscle weakness:** People who have SMA Type 3 do suffer from muscle weakness, though it is typically not as severe as the muscle weakness seen in people with SMA Types 1 and 2. This weakness manifests itself largely in the muscles of the lower body.
   - **Muscle cramps and twitching:** Motor neuron dysfunction can cause muscle cramps and fasciculations, also known as muscle jerking**.**
   - **Contractures:** Muscle weakness and misalignment can lead to joint contractures, which can make it hard to move the joint.

ii. **Delayed motor milestones:** Children with Type 3 SMA often reach motor milestones like sitting and standing, but it may take them longer than their peers to do so.

iii. **Gait abnormalities:** Weak muscles can cause people to walk in a different way. Waddling or going on the tips of your toes are common ways to move.

iv. **Climbing stairs:** As muscle weakness gets worse, it can be hard to climb stairs or slopes.

v. **Frequent falls:** People with Type 3 SMA may fall down often, particularly when doing physical activities, because their muscles are weak and they have trouble keeping their balance.

vi. **Fatigue**: Weak muscles can make it harder to stay active for long periods of time.

vii. **Scoliosis:** As muscle weakness gets worse, there is a chance of scoliosis, which is a curvature of the spine. This is the same as with other types of SMA.

viii. **Respiratory function:** Compared to other types of SMA, the signs of Type 3 SMA tend to be less severe when it comes to breathing. But some people may have minor trouble breathing or get respiratory infections.

ix. **Normal intellectual functioning:** Type 3 SMA patients usually have normal intelligence. The disease impacts motor skills more than cognition.

x. **Sitting up:** Lower-limb muscle weakness might make it hard to sit up without help.

xi. **Adaptive strategies:** Type 3 SMA patients often develop adaptive techniques to compensate for muscular weakness. This may involve railings, assistance gadgets, or environmental changes.

Type 4 or Adult onset SMA:

Age of Onset: Adulthood.

Type 4 SMA symptoms are milder than others. Common Type 4 SMA symptoms (Arnold et al., 2015b),(Darras et al., 2015a):

i. **Muscle weakness:** Type 4 SMA causes mild to severe muscular weakness. This weakness is usually milder than in other SMA variants.
   - Muscle twitching and cramping can result from motor neuron dysfunction.
   - muscular weakness and imbalances can cause joint and muscular discomfort in Type 4 SMA patients.

ii. **Walking difficulties:** Type 4 SMA patients may have trouble walking, however many can walk independently. Later in life, some may have walking problems.

iii. **Gait abnormalities:** Muscle weakness might cause a small waddle or walking pattern modifications.

iv. **Fatigue:** Muscle weakness can cause fatigue, especially after exercise.

v. **Respiratory function:** Type 4 SMA has minor or no respiratory symptoms. People rarely experience breathing problems.

vi. **Scoliosis:** Type 4 SMA can cause scoliosis, but it is rare and mild.

vii. **Progression:** The course of Type 4 SMA is slow and gradual, with many maintaining functional abilities for years.

viii. **Normal intellectual functioning:** Type 4 SMA patients usually have normal intelligence. The disease rarely affects cognition.

ix. **Adaptive activities:** Many Type 4 SMA patients can live independently, while muscle weakness may make some everyday tasks harder. Muscle weakness can be managed and functional abilities maintained through adaptive techniques. This may require assistive technology or environmental changes.

Table 1: Classification and symptoms of subtype of Spinal Muscular Atrophy (Farrar et al., 2017).

Genotype	Age of onset	Motor milestone	Motor function and additional features	Prognosis
SMA 0	Before Birth	None	Severe hypotonia; unable to sit or roll	Respiratory insufficiency at birth; death within weeks
SMA 1	2 Weak 3 Months 6 Months	None	Severe hypotonia; unable to sit or roll	Death or ventilation by 2 years
SMA 2	6 to 18 months	Sitting	Proximal weakness or unable to walk independently	Survive into adulthood
SMA 3	> 3 Years (IIIa) < 3 Years (IIIb) > 12 Years (IIIc)	Walking	May lose ability to walk	Normal lifespan
SMA 4	10 to 30 years > 30 years	Normal	Mild motor impairment	Normal lifespan

3.3 Pathogenesis

All types of SMA are characterized by the loss of motor neurons in the lower brainstem and the anterior horn of the spinal cord (Murayama et al., 1991). Anterograde axonal atrophy is when the nerve ends of the myocytes in the motor unit die off. This can sometimes cause the muscle to get new nerves. This happens when undamaged motor neurons next to the damaged ones start to grow. This causes myocytes to group into fibers. When SMA muscle cells are looked at using histopathology, a lot of rounds, atrophic fibers can be seen. This is because the nerves in those muscles have been cut off. People used to think that SMA was mostly a neuronopathy (a problem with the cell body) with axon degeneration as a minor problem. But newer findings in the field have shifted attention away from the motor neuron cell body and

towards the distal axon and the potential of a synaptopathy deficiency in SMA (Simic, 2008). Lower motor neurons (LMNs) were found to be underdeveloped in patients of SMA, as revealed by histopathology. They had fine Nissl bodies that were only on the outside Comprising tiny spherical somata with few cell processes when they were still in the womb, and their profiles were small after birth. LMNs started to get smaller and fewer when the baby was still in the womb. After birth, ballooned neurons (BNs) and surviving LMNs appeared., got smaller over time. BNs were full of phosphorylated neurofilament protein and looked like normal chromatolytic neurons but were smaller. This is called an axonal response (Ito et al., 2011).In particular, the hypothesis of dysregulation of the presynaptic transcriptome has been put forward. That SMN is involved in the transport of functional mRNA is clearly demonstrated by results, such as the species that codes for beta-actin, from the brain to the periphery. No matter where in the cell SMN causes damage, since SMA is commonly believed to be a motor neuron disease, therapeutic strategies focus on the use of medicines that can penetrate the BBB and act on the central nervous system (CNS) (Farooq et al., 2013). The following is a list of 10 pathogenetic occurrences that could lead to SMA: (Simic, 2008)

1. Defects in the SMN1 gene, such as deletions or mutation.
2. Increased SMN mRNA decay and a decrease in full-length functional SMN protein.
3. Motoneuron axonogenesis and dendrogenesis were hampered.
4. Failure of motoneurons from the upper part of the brain to connect with corticospinal Wbers.
5. Motoneuron movement towards ventral spinal roots that isn't normal.
6. Inappropriate persistence of motoneuron apoptosis.
7. A lot of motoneurons are still moving in the wrong way (called "heterotopic motoneurons") and are going into the ventral roots.
8. The heterotopic motoneurons that make up the ventral root glial bundles, drew glial cells.
9. Impaired actin transport in the axons, which causes the remaining motoneurons to become chromatolytic.
10. Neuronal death due to apoptosis, heterotopy, or chromatolysis; apoptosis is the more rapid and prevalent of the three in the early stages and necrosis being the main cause of death in the later stages.

The pathological mechanism of SMA is given below: (Simic, 2008)

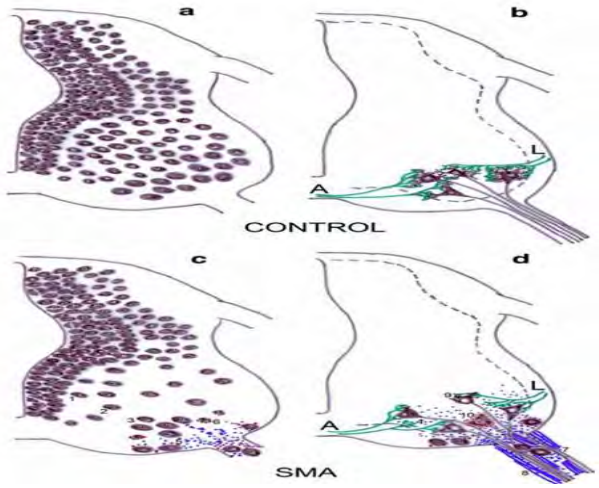

Figure 4: Parts a and b provide an overview of the processes involved in typical motoneuron development and parts c and d are provided so that they can be compared with SMA. Schemes a and c illustrate what may occur in the early stages of pregnancy, whereas schemes b and d illustrate what may occur in the later stages of pregnancy. The steps that are proposed to be involved in the pathogenetic process are numbered from 1 to 10 in the text, and those numbers correspond to the corresponding numbers on the figure. A lateral corticospinal tract Wbers are shown in green, while L anterior corticospinal tract Wbers are also shown in green. Red dots represent microglia, blue dots represent astrocytes, blue lines represent glial bundles, and SMA is also represented by red dots (Simic, 2008).

3.4 Diagnosis Tests

3.4.1 Genetic Testing

When it comes to diagnosing SMA, genetic testing, and more specifically molecular genetic testing, is considered to be the gold standard. As it offers the most precise and definitive diagnosis of SMA possible, in addition to details regarding the type of the disorder as well as the severity of its impacts. The following are the most common types of genetic tests:

- **Testing of the SMN1 Gene:** This test determines whether the SMN1 gene contains any mutations or deletions. People who have spinal muscular atrophy have mutations in this gene. The diagnosis is confirmed when there is either a lack of or a reduced copy number of functioning SMN1 gene copies (Ogino et al., 2002).

- **SMN2 copy number analysis:** People have more than one copy of the SMN2 gene, which makes some SMN protein that works. The SMN2 copy number is looked at to figure this out. There is a negative relationship between the number of copies of the SMN2 gene and how severe the disease is. Through genetic testing, one can find out how many copies of SMN2 they have.

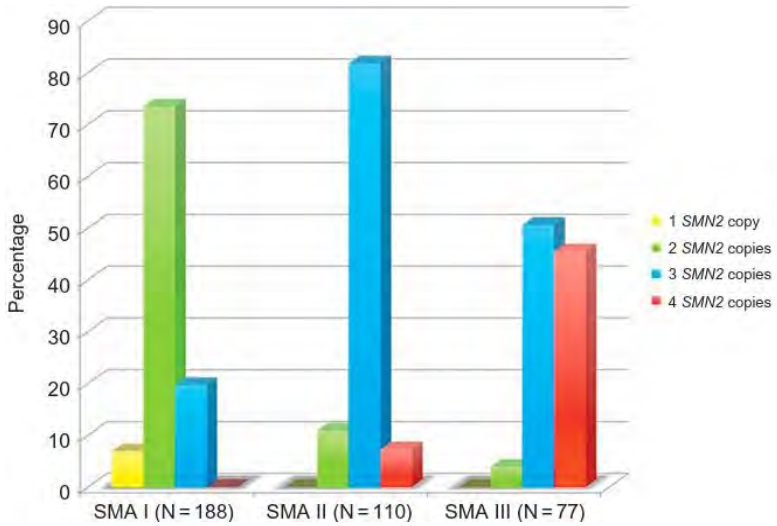

Figure 5: Number of cases with SMA types I, II, and III and SMN2 copy numbers. (Darras et al., 2015a)

The percentage of patients that have SMA type 1, 2, and 3, as well as SMN2 copy numbers. In SMA type 1, 80% of cases had 1 or 2 copies of the SMN2 gene. 82% of people with type 2 had 3 copies of SMN2, and 96% of people with type 3 had 3 or 4 copies (Darras et al., 2015a).

- **Paternal and preimplantation genetic testing:** It is possible to do chorionic villous sampling (CVS) on a specimen or amniotic fluid so as to identify homozygous lack of SMN1. In order to prevent getting misleading negative results because of maternal contamination, it is important to calculate how much maternal DNA is present in comparison to fetal DNA. In spite of the fact that CVS specimens are more frequently contaminated with maternal DNA than amniotic fluid specimens, such contamination is almost never reason for concern if that CVS is handled correctly (Ogino & Wilson, 2004).

3.4.2 Serum Creatinine Kinase

Creatine kinase in the serum that is elevated beyond the normal range may be an indicator of damage to the muscles. Despite the fact that it does not specifically address SMA, it can provide extra information to support the diagnosis. Both serum creatine kinase activity and serum

creatinine concentration are good methods to measure the severity of spinal muscle atrophy. They are also potential biomarkers that can be used to evaluate people with spinal muscular atrophy at different stages of the disease and also helps to anticipate how they will respond to treatment (Freigang et al., 2021).

3.4.3 Nerve Conduction Studies

Nerve conduction studies generally show that motor nerve axons are losing their connections over time, while action potentials stay the same in sensory nerves. This examination determines how quickly nerve signals reach the muscles after leaving the brain. Abnormal results may point to nerve damage, which is a symptom that may be present in some cases of spinal muscular atrophy (SMA). Although nerve conduction studies are not considered a key diagnostic technique for spinal muscular atrophy (SMA), they may be applied as a component of a more thorough evaluation in order to differentiate between SMA and other neuromuscular diseases that involve peripheral nerves. The nerve conduction speed (NCS) and electrical signal strength are both measured during an NCS examination. Conditions that primarily impact peripheral nerves, such as some forms of neuropathies, can be easier to detect and make distinctions with the use of this test (Arnold et al., 2015b).

3.4.4 Needle Electromyography (EMG)

Studies using electromyography, it has different signs of motor neuron and axon loss that are consistent with motor neurons losing their ability to work. Electrodiagnosis is an important diagnostic tool for unusual cases of SMA and SMA that is not linked to the 5q gene because it shows that the illness is neurogenic. Electrodiagnostic testing is commonly utilized early in the diagnostic process because of its potential use for diseases that show up later in life and have a wide range of possible causes. Active denervation and chronic compensating modifications of reinnervation and motor unit action potential expansion are detected by electromyography (EMG) and indicate the loss of motor neurons or motor axons. Most of the time, fibrillation potentials, which are abnormal random activity, can be seen (Arnold et al., 2015b). It is possible to monitor the health of the motor unit pool with electrophysiological tests like the compound muscle action potential (CMAP) and the motor unit number estimation (MUNE). These measures are especially important for motor neuron disorders. CMAP reaction measures how much work a certain muscle or group of muscles is doing. The size of the CMAP is based on the size and number of muscle fibers that have lost their charge after supramaximal nerve activation. MUNE is an electrophysiological way for figuring out how many motor units

supply a certain muscle (BUCHTHAL & OLSEN, 1970). Interactions between CMAP, MUNE, and EMG and various markers of motor function, clinical severity, and overall function have been demonstrated in cross-sectional investigations. (Table 2) (David Arnold et al., 2014). Electrophysiological results in spinal muscular atrophy patients after the development of significant clinical weakness (David Arnold et al., 2014).

Table 2: (SMA) spinal muscular atrophy; (CMAP) compound muscle action potential; MUNE, motor unit number estimation:

Fibrillations **SMA Type**	**CMAP**	**MUNE**	**Electromyography**		
			Decreased recruitment	**Enlarged motor units**	
Type 1	↓↓↓	↓↓↓	++	↑↑↑	↑
Type 2	↓↓	↓↓	+	↑↑	↑↑
Type 3	↓ or normal	↓	+/−	↑	↑↑↑

3.4.5 Muscle biopsy

The diagnosis of SMA does not require the use of muscle biopsy any longer. Even in patients with unusual symptoms or patients whose testing for SMN1 deletion or mutation was negative. A muscle biopsy is unable to differentiate between SMA subtypes in a definitive manner; nonetheless, some histological findings are connected with the severity of the disease (Arnold et al., 2015b). A muscle biopsy performed on an infant with type 1 or type 2 diabetes reveals extensive clusters of atrophy-associated fibres interspersed with fascicles of hypertrophied and normal fibres. Frequently, atrophic fibres are circular as opposed to angular and they can be either type 1 or type 2 in terms of their genetic makeup (Fenichel & Engel, 1963). When there is diagnostic doubt or when a muscle condition needs to be distinguished from SMA, one

option that could be considered is to perform a muscle biopsy. It is essential to keep in mind, however, that a muscle biopsy is not diagnostically definitive for SMA and can't provide a definitive diagnosis on its own. A tiny sample of muscle tissue is removed during a muscle biopsy so that it may be examined more closely under a microscope. This allows the clinician to look for anomalies in the muscle fibres, cellular structure, and other aspects of the muscle.

3.5 Therapeutic strategies

3.5.1 Primitive Treatment

Prior to the development of current medical advances and individualised treatment, there were few therapeutic options available for Spinal Muscular Atrophy (SMA). The following is a list of some of the more ancient or historical treatments that were used in the past but are no longer believed to be successful or have become obsolete as a result of developments in medical understanding (Darras et al., 2015a):

i. **Quinazoline Drug:** By increasing the activity of the SMN2 promoter, inhibiting the decapping scavenger enzyme (DcpS) increases FL-SMN2 RNA. DcpS inhibitor quinazoline (Repligen or RG3039) has been shown to increase the quantity of SMN protein in SMA mice and extend their lifespan. Despite the fact that RG3039 inhibited DcpS in the blood of patients, the amount of SMN protein did not change significantly, according to a phase 1b study. (Jędrzejowska & Kostera-Pruszczyk, 2020). So, the pharmaceutical company came to the conclusion that RG3039 would not help SMA patients, and the study was stopped (T.-H. Chen, 2020).
ii. **Supportive Care:** In the past, methods of supportive care such as physical therapy, occupational therapy, and orthopaedic therapies were utilised in order to alleviate patients' symptoms and enhance their quality of life. These interventions are still necessary in today's world, but they are now incorporated into a more comprehensive care plan that also makes use of more cutting-edge therapies.
iii. **Bracing and other Mobility Aids:** People who have spinal muscular atrophy have been known to need braces, splints, and other mobility aids (such as walkers or crutches) to assist in supporting their weakening muscles and maintaining their mobility. Even though traditional approaches are still utilised, contemporary treatments are being developed with the goal of more successfully addressing the underlying cause of SMA (T.-H. Chen, 2020).

iv. **Respiratory Support:** People who have spinal muscular atrophy often have problems with their respiratory system, including trouble breathing and difficulty clearing secretions, support for the respiratory system treatments, such as assisted coughing techniques and manual ventilation, have traditionally been used to treat respiratory symptoms (Darras et al., 2015a).

v. **Nutritional Support:** People who have spinal muscular atrophy may have received nutritional support in the form of customised diets or aid with feeding as a result of difficulty with swallowing and the possibility of problems related to their nutrition (Darras et al., 2015a).

vi. **Assistive Devices:** Devices like as communication boards, adaptive technologies, and customised utensils were utilised to assist individuals who have spinal muscular atrophy (SMA) in communicating and carrying out day-to-day activities.

SMA patients received some relief from these crude treatments, but they did not address the genetic aetiology. Recent medical advances have made gene therapies and targeted interventions like Spinraza and gene replacement therapies possible. These medicines target SMA's genetic and molecular underpinnings, which may enhance patients' quality of life and prognosis.

3.5.2 SMN Dependent

If treatments that rely on SMN can be given before symptoms appear, when the dysfunction of motor neurons may still be reversible, and if they can be given with a high level of effectiveness to significantly increase SMN levels in motor neurons in the spinal cord, then it is probable that the ongoing process of neurodegeneration will not be entirely stopped but rather than it will simply be slowed down. Patients who are treated for spinal muscular atrophy have the potential to have a delayed degradation of their neuromuscular system (Bowerman et al., 2017).

3.5.2.1 RNA Based Modulation

Nusinersen, which is marketed under the brand name Spinraza, is the RNA-based modulation therapy that is most widely used to treat Spinal Muscular Atrophy (SMA). It is the first splicing-modify treatment to be authorized for SMA. Nusinersen is a therapeutic antisense oligonucleotide (ASO) that is intended to target specific RNA sequences in order to stimulate an increase in the production of SMN protein that is functional (Torroba et al., 2023).

- **Nusinersen (Spinraza) :** It is an ASO treatment uses an antisense oligonucleotide to target a particular RNA. sequence in the SMN2 gene. SMN2 is a gene that is closely linked to SMN1, and it is the gene that makes SMN protein. Because of a small change in the SMN2 gene, it mostly makes a form of the SMN protein that is shorter and less useful. Nusinersen works by binding to a certain sequence in the SMN2 RNA, which changes the way the RNA is spliced. They work by making it more likely for exon 7 to be added to the SMN2 gene during RNA splicing. When Exon 7 is added, this change makes more full-length SMN protein, which helps make up for the lack of protein. Without medication, exon 7 is often skipped, which makes an SMN protein that is unstable and doesn't work. By changing how RNA is spliced, Nusinersen makes it more likely that exon 7 will be included. Hence resulting in making more functional SMN protein (T.-H. Chen, 2020).

  Side effect: As nusinersen is unable to penetrate through the blood-brain barrier (BBB), beyond which the targeted MNs that need to be rescued are present, there isn't a good way to give it other than intrathecally on a regular basis. Some of the risks of doing a lumbar puncture on a person with SMA are (T.-H. Chen, 2020):

  - making their breathing worse because they have to bend their knees to their chests during the process
  - getting a headache
  - having CSF leak out

Without the help of modern imaging, multiple intrathecal injections can be hard for some long-term SMA patients with a lot of scoliosis (Talbot & Tizzano, 2017).

So far, more than 8,000 SMA patients around the world have been treated with nusinersen. But it costs a lot—$750,000 for the first year of treatment. First, there seems to be a specific time in neuromuscular growth when increasing SMN levels work best, according to studies done before they are used in people (Kariya et al., 2014). The information from human studies also shows how important a therapeutic window is for a treatment that includes SMN. Unfortunately, SMA screening programmes for newborns have not yet been done all over the world or even all over the country. Contrasted with, Type 2 SMA patients who got sick later showed a big improvement in their motor skills after treatment with nusinersen (T.-H. Chen, 2020; Mercuri et al., 2018).

3.5.2.2 Trans splicing RNA

Another treatment idea for SMA is to stop the SMN2 gene from skipping over exon 7 so that it makes more full-length transcripts. HDAC inhibitors including sodium butyrate, TSA, and VPA appear to have two distinct effects on the synthesis of SMN mRNA. They appear to alter the splicing process in addition to opening up the chromatin structure and accelerating transcription. (Farooq et al., 2013).

- **Aclarubicin:** It has been demonstrated that the antibiotic aclarubicin can change the splicing process in vitro to result in increased levels of full-length SMN transcript (Farooq et al., 2013).
- **Antisense oligonucleotides:** ASOs, sometimes referred to as antisense oligonucleotides, have been created and are able to obstruct an intronic splicing suppressor element. Exon 7 is thus prevented from being skipped over.

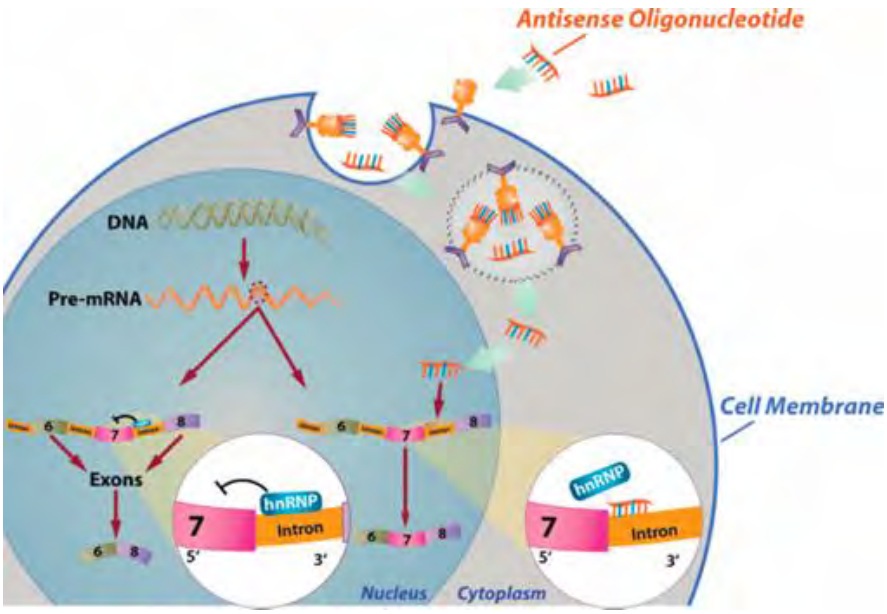

Figure 6: Mechanism of SMN antisense oligonucleotide.(Darras et al., 2015a)

Single-stranded anti sense oligonucleotides (ASOs) bind to proteins on cell surfaces and move into the cytoplasm. through a process called endocytosis. After that, they left the endosome and enter the nucleus, where they bind to the SMN2 pre-mRNA and inhibit the splicing of intron 7 by inhibiting an intronic splicing suppressor element. The hnRNP protein is moved by the ASO when it attaches to the RNA, which usually stops exon 7 from being spliced. This makes it easier for exon 7 to be included during splicing, which makes full-length SMN protein (Darras et al., 2015a).

3.5.2.3 SMN protein stabilization

- **Aminoglycoside Antibiotics:** Tobramycin, geneticin, and amikacin are all examples of aminoglycoside antibiotics, which belong to a family of medications that have been approved by the FDA. These antibiotics have the ability to either stabilize or raise the level of SMN in patient fibroblasts because they conceal premature stop codon mutations and promote read-through of exon 8. (T.-H. Chen, 2020).
- **Azitheromycin:** Azithromycin, which acts on stop codon read-through, was given FDA approval after it was discovered that it increased SMN in cell lines from SMA patients and to enhance motor performance and lifespan in a SMA animal model when intrathecally administered (Calder et al., 2016). The pre-clinical study was expanded so that it included another SMA mouse model, which also produced encouraging evidence (Cobb et al., 2013).
- **Bortezomib:** Ubiquitin proteasome inhibitors include bortezomib, that is commonly used to prevent the degradation of SMN proteins. It has been demonstrated that it raises SMN protein levels in cultured cells as well as in the peripheral tissues of SMA model mice. Animals that were given Bortezomib exhibited enhanced motor performance, which was connected to diminished pathology in the muscles and the spinal cord as well as increased neuromuscular junction size. However, the animal death rates remained unchanged (T.-H. Chen, 2020; Kwon et al., 2011).

3.5.2.4 Gene therapy

SMN1 Gene Replacement Therapy

In contrast to increasing SMN output by focusing on SMN2, another method of treating the condition is to introduce the SMN1 gene into the neural cells. The most hopeful gene-delivery system was revealed to be the self-complementary adeno-associated virus 9 (AAV9 vector because it is able to penetrate the BBB and infect roughly 60% of MNs. furthermore, human SMN1 expression over time makes it easier and faster to make SMN, which has been allowed for in in-vivo study.

- **Zolgensma (AVXS-101 or onasemnogene abeparvovec):** It is the first AAV9-SMN1 gene therapy, was given to 15 people with type 1 SMA. In a phase 1/2a study, the patients' motor skills got better and they lived longer. The follow-up study showed that

early treatment is very effective. Early results from a phase 3 open-label study of babies with type 1 SMA that is still going on continue to look good (Cobb et al., 2013). The FDA has recently suspended the intrathecal administration of Zolgensma to SMA patients older than 2 years and younger than 5 years due to safety concerns (T.-H. Chen, 2020).

- **Risdiplam:** Risdiplam (RG7916) showed effectiveness in both tests conducted in vivo and in vitro, as well as pharmacokinetic information from humans. Risdiplam is a powerful treatment for SMA as a whole-body disease because it has the ability to spread to both central and peripheral tissues (Sturm et al., 2019). Both the FIREFISH study for people with type 1 SMA and the SUNFISH trial for people with Branaplam (LMI070) has the ability to connect with U1 snRNP and promote the inclusion of exon 7 of the SMN2 transcript. As a result, the SMN protein levels increase and a longer time without a stroke (T.-H. Chen, 2020).
- **Branaplam:** To enable the inclusion of exon 7 of the SMN2 transcript, branaplam (LMI070) has the capacity to bind with U1 snRNP. The levels of SMN protein rise as a result. and an improvement in phenotypes. Branaplam came from pyridazine 2, which was a hit from a high-throughput phenotypic screening, and it changed over time through multiparameter lead optimization. In a mouse model of severe SMA, branaplam therapy increased full-length SMN RNA and protein levels while also extending the mice's lifespan (Cheung et al., 2018). The purpose of Branaplam's ongoing phase 1/2 clinical trial, which is an open-label, first-in-human study using oral administration, is to examine the treatment's safety and effectiveness in people with type 1 spinal muscular atrophy. The initial results showed a considerable improvement in the patients' motor capabilities after 86 days of treatment. Five patients' illnesses still improved after 127 days of therapy (T.-H. Chen, 2020).
- **Celecoxib:** In both cell and animal models of SMA, the cyclooxygenase 2 inhibitor celecoxib has shown the capacity to raise SMN levels. Due to its ability to cross the blood-brain barrier (BBB) and its acceptable safety profile in human tests, it is seen as a promising candidate for treating SMA. People with SMA types 2 and 3 are being eagerly sought to participate in a phase 2 trial. Celecoxib may be used as an extra treatment for SMA in the hopes that it will help, especially since it can be taken in low doses that are safe (T.-H. Chen, 2020).

3.5.3 SMN Independent Therapies

Approaches that depend on SMN are especially hard for people with chronic SMA (types 3 and 4), who are frequently diagnosed following the crucial treatment window. Focusing on the SMN-independent pathways that are damaged following SMN is especially crucial for persons with the chronic type of SMA who have lost a lot of MNs.

3.5.3.1 Stem cell therapy

Spinal Muscular Atrophy (SMA) is being studied and researched in the area of stem cell therapy. However, as of September 2021, the notable fact is stem cell therapy strategies for SMA are still in the experimental stages and has not yet become a standard treatment (T.-H. Chen, 2020).

Stem cell therapy is useful for the establishment of model systems for the development of drugs as well as a potential treatment for spinal muscular atrophy (SMA). A type 1 SMA patient and his mother were used to create pluripotent stem cells that could develop into motor neurons but did not express SMN1. This was done in the most recent few years. In the future, stem cell therapies for SMA treatment could be developed using this as a key model system for evaluating new drugs (Darras et al., 2015). The efficacy of cell therapy in the treatment of SMA is directly proportional to the capacity of stem cells to offer assistance to endogenously deteriorating MNs (Corti et al., 2008a). Two trials on stem cell transplantation for spinal muscular atrophy (SMA) are now available. Both of these researches revealed that the spinal cord engrafted with primary neural stem cells injected into the spinal canal, improving motor function and life expectancy (Corti et al., 2008b, 2010).

3.5.3.2 Neuroprotection

Treatments that are neuroprotective attempt to maintain the function of, and ensure the survival of motor neurons, cells that are most severely impacted by SMA.

- **Aminoglycosides:** Aminoglycosides appear to promote read-through of the exon 8 stop codon, stabilizing the SMN protein and offering a different way to raise SMN protein levels in patient fibroblasts. A lot of methods exist to accomplish this. The aminoglycoside geneticin (G418) was administered to animals with SMA, and this medication boosted the levels of SMN protein and improved motor function; nevertheless, it was extremely toxic to mice (Darras et al., 2015a).

- **Olesoxime:** Olesoxime (TRO19622) is a substance from the trophos cholesterol-oxime family that aids in protecting nerve cells. Pre-clinical studies show that it helps neurons work better and stay alive. At the end of 24 months of treatment, SMA type 2 and type 3 individuals displayed stable muscular function. However, a follow-up trial conducted 18 months later failed to demonstrate a significant therapeutic benefit (Bertini et al., 2017; T.-H. Chen, 2020).
- **Riluzole and Gabapentin:** Researchers have looked into how riluzole and gabapentin might help treat SMA. Riluzole has successfully completed a phase 2/3 multicenter, randomised, double-blind study in young patients with SMA types 2 and 3. (Haddad et al., 2003). This study looked at how well it worked and how safe it was. Most of the results were not good, or the tests weren't good enough to show that they worked (T.-H. Chen, 2020; Darras et al., 2015b).

3.5.4 Combination therapies

Combination therapy is combining different types of therapy which could make SMA treatment more effective. Even though it may not be possible to use expensive drugs in combination therapies at some point, there is evidence that such combinations work on humans more effectively than single drug (T.-H. Chen, 2020). These approaches have produced successful SMA treatments, but they are costly and their long-term safety is unknown (Poletti & Fischbeck, 2020).

- An SMA animal model showed that combination of ASO-induced SMN2 exon inclusion that is SMN-dependent and SMN-independent myostatin suppression give beneficial outcomes (Zhou et al., 2020).
- Zolgensma and Nusinersen work in different ways, so it is less likely that they will combine with each other or give drug-drug interaction event. Nusinersen works by focusing on a sequence of introns to improve the inclusion of exon 7. But a relocated zolgensma gene doesn't have any introns, therefore nusinersen translation is unlikely to be hampered by it. (Sumner & Crawford, 2018).
- Replacing SMN1 with a gene delivered by AAV9 (Zolgensma), adeno-associated virus serotype 9 (Poletti & Fischbeck, 2020).
- Fixing the incorrect way of SMN2 by splicing with one small molecule (risdiplam, nusinersin), an antisense oligonucleotide (Poletti & Fischbeck, 2020).

3.6 Management

A multidisciplinary approach is used to treat Spinal Muscular Atrophy (SMA) and improve quality of life and function. The kind and severity of SMA determine management techniques.

i. **Medical Care and Monitoring:**

- Regular neurologist or neuromuscular specialist visits.
- Monitoring motor, muscular, respiratory, and other characteristics.

ii. **Management of Orthopedic Conditions:**

Bracing, surgical intervention, and physical therapy are among treatment and management options for orthopedic issues such as scoliosis and joint contractures. In the severe spectrum of SMA individual need orthopedic monitoring to detect scoliosis and contractures. Scoliosis often needs surgical intervention and coordinating support for breathing and eating during surgery can help keep problems to a minimum. Fractures and hip subluxation frequently occur in individuals with milder forms of SMA (II and III). The distal femur is the most commonly affected bone, followed by the lower leg, ankle, and upper arm. Fortunately, these fractures can be treated without the need of any surgical intervention (Darras et al., 2015a; Dominguez et al., 2011).

iii. **Clinical Tests and Currently Emerging Treatments:**

- Participating in clinical tests for possible new treatments and therapies.
- Maintaining awareness of the most recent developments in SMA research and treatment is essential.

iv. **Psychosocial Support:**

- Offering individuals diagnosed with SMA and their family emotional and psychological support is essential.
- It is important to make genetic counselling available to families, particularly those with a family history of spinal muscular atrophy (SMA) or those who are thinking about starting a family.

v. **Gastrointestinal care:**

Patients with type I SMA are weak and uncomfortable while eating, which causes stunting, aspiration, and recurrent respiratory infections. In SMA patients, a significant rate of silent gastric reflux may be related to aspiration. according to small retrospective research early laparoscopic Nissen fundoplication and gastrostomy in type I SMA patients, led to improved nutritional status and possibly fewer long-term aspiration incidents (Durkin et al., 2008).

Positive outcomes are seen in patients undergoing Laparoscopic Nissen fundoplication and gastrostomy tube insertion with postoperative noninvasive ventilation. The procedure resulted in no adverse events and fewer pneumonias in some patients.

Finally, patients with SMA may experience constipation, which can increase reflux or respiratory symptoms, particularly in young type I individuals (Darras et al., 2015a; Yuan et al., 2007).

vi. Nutrition Management:

Babies with type I SMA and some persons with type II SMA who are very ill, frequently fail to develop or grow. Nevertheless, despite the fact that many type II patients plot as having a "normal" BMI (sometimes as low as the 3% for a healthy child their age), they may actually have too much fat mass relative to their muscle mass. Patients with non-ambulatory SMA who had clinically high functioning were more likely to be overweight than low functioning non-ambulatory and ambulatory patients. This is likely because they were given more calories than they needed, even though they burned less energy at rest (Sproule et al., 2009, 2010). Because of this, people with all types of SMA need to pay close attention to their nutrition, and they need to consult to a nutritionist who is knowledgeable with these particular difficulties (Darras et al., 2015a).

3.7 Pre-clinical Development Therapies

The collection of adequate evidence of safety and efficacy in order to justify moving the medicine into human clinical trials is the primary goal of the preclinical development phase of the research and development process. The data that are generated during preclinical investigations are sent to regulatory agencies for the purposes of review and approval before clinical testing may begin. The drugs that are used in preclinical development therapies are:

- **Celecoxib**: This medication is an FDA-approved nonsteroidal COX-2 inhibitor with the ability to penetrate the blood-brain barrier. Celecoxib operates by activating the p38 pathway, which regulates the stability of SMN transcripts. In a mouse model of severe SMA, treatment with Celecoxib resulted in increased SMN protein levels, improved motor function, and enhanced survival. Currently, it is under investigation in a preliminary, open-label, dose-response study involving individuals diagnosed with SMA2 or 3. (Servais et al., 2021).

- **Moxifloxacin:** Synthetic fluoroquinolone antibiotic moxifloxacin inhibits topoisomerase II, altering gene splicing, including SMN2. Moxifloxacin increases SMN protein dose-dependently through boosting SMN2 exon 7 inclusion. In HeLa cells, SMN overexpression enhanced the amount and size of Cajal bodies, which were almost doubled after moxifloxacin therapy. Cajal bodies increased in number and size due to increasing SMN protein levels and snRNP abundance. Splicing factor expression changes with moxifloxacin. Thus, this chemical may alter splicing of additional transcripts besides SMN2 pre-mRNA (Konieczny & Artero, 2020).
- **Salbutamol**: It is a β2-adrenoreceptor agonist. It promotes exon 7 by modulating SMN2 gene splicing. Salbutamol, which affects NMJs, is a common congenital myasthenic syndrome treatment (McMacken et al., 2019). A preliminary open-label research on patients with SMA2 and 3 demonstrated that the drug enhances motor function and is well tolerated. 45 adult SMA patients participated in a 1-year randomized double-blind, placebo-controlled salbutamol study. (37 completed) demonstrated a significant and gradual rise in peripheral blood full-length SMN2. Most individuals treated with salbutamol improved in exploratory motor evaluations (Servais et al., 2021; Tiziano et al., 2019).
- **Securinine:** Through encouraging exon 7 inclusion, it promotes the expression of full-length SMN2 mRNA and protein in lymphoid cells generated from SMA patients. Securinine affects splicing factor protein levels. For instance, it decreases hnRNP A1 and increases Tra2-β1. In a SMA animal model, securinine administered intraperitoneally, caused increased in brain and spinal cord. SMN2 exon 7 inclusion and protein expression (Y. C. Chen et al., 2017; Servais et al., 2021).

Chapter 4

Discussion

Spinal Muscular Atrophy, also known as SMA, is a neuromuscular condition that is inherited genetically and is characterized by a complicated interaction of molecular components. The pathogenesis of the disorder begins with a deficiency of the survival motor neuron (SMN) protein due to mutations in the SMN1 gene. This shortage is what causes the disorder. This results in progressive the spinal cord's motor neurons are degenerating, which results in turn leads to are reducing muscle tissue and a loss of strength hence resulting in atrophy. SMA presents itself with a spectrum of symptoms, ranging from severe infantile onset (Type 1), which is characterized by respiratory distress and restricted motor ability, to later onset types (2, 3, and 4) with different degrees of muscle weakness. Depending on the type of SMA, the symptoms might vary in severity. Techniques for diagnosis, such as genetic analysis of the SMN1 and SMN2 genes, play crucial role in accurately classifying the disease and determining the patient's prognosis. Recent therapeutic advances have led to a paradigm shift in the landscape of SMA treatment, with nusinersen (Spinraza) emerging as the first medicine to be authorized by the FDA. Nusinersen is an antisense oligonucleotide (ASO), and it functions by modulating the RNA splicing of SMN2 gene. As a result, more functional SMN protein is formed. This revolutionary treatment has shown remarkable efficiency in enhancing both motor function and quality of life in patients with the condition. Also, research is being done on gene treatments with the goal of delivering functioning SMN1 genes to motor neurons directly. For the comprehensive management of SMA, a multidisciplinary approach is necessary. This approach includes nutritional support to address potential feeding difficulties, respiratory care to ensure lung health, physical and occupational therapy to maintain motor function, and orthopaedic interventions to manage associated musculoskeletal complications. In addition, the significance of ongoing clinical trials and research efforts cannot be overstated because these activities are directed towards the improvement of existing treatment strategies and the development of new therapeutic pathways. SMA's complex pathogenesis, diverse symptomatology, evolving diagnostic techniques, innovative treatments, and inclusive management strategies collectively highlight the dedication of medical professionals, researchers, and families in the pursuit of improving the lives of individuals impacted by this debilitating disorder.

The treatment for SMA is made more complicated by the availability of multiple therapy approaches that either depend on or do not require SMN. Combinatorial therapies are therefore undergoing the protocolization process after their efficacy has been established. All of these advancements should include SMN2 copies, variations, and structures in integrated patient characterization who are having treatment that is expensive and often lasts a lifetime. The determination of the SMN2 copy number involves a primary workflow (Costa-Roger et al., 2021). After the SMN2 copy number has been determined, there is a process of SMA patients who are dealing with different symptoms at the same time rather than the fact that they are receiving treatment. This is the case regardless of whether or not they are being treated. After SMN2 determination, we will be able to determine the sequencing of the SMN2 gene, which will eventually lead to the study of gene sequencing, and we will be able to prescribe medication that is more appropriate.

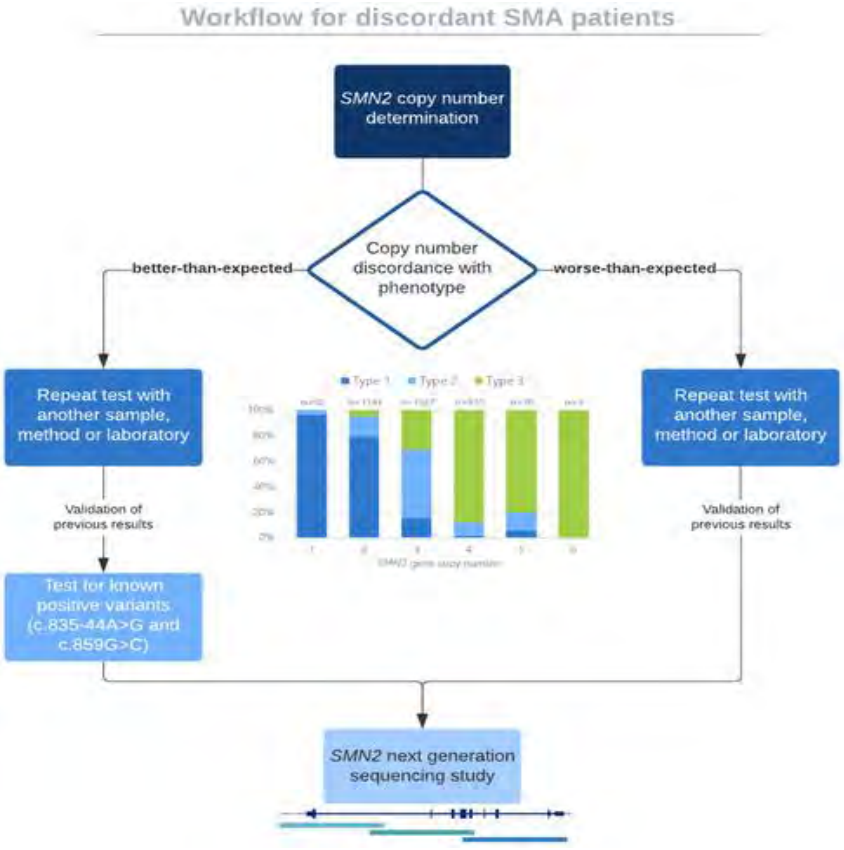

Figure 7: Workflow for SMA people with different symptoms.

Once SMN2 is discovered and a difference is discovered (according to the graphic in the middle of the procedure, which is based on a meta-analysis of 3459 instances), the process is complete.(Calucho et al., 2018). *To repeat the test, a new sample, a different procedure, or even another lab should be utilized. If the difference is confirmed, which occurs when the trait*

performs better than expected, we can look for known positive variations (c.859G>C and c.835-44A>G) that have previously been tested and found to be true. If the phenotype is worse than expected, an NGS test should be performed to check for negative modifiers, hybrid structures, or intragenic deletions that could explain the phenotype. (Cuscó et al., 2020).

Life Expectancy of SMA:

People with Spinal Muscular Atrophy (SMA) have a very different life expectancy based on the type of SMA they have and whether or not they have access to medical treatments and supported care. People with Type 1 SMA usually have the most serious form of the disease. Their motor neurons drop off quickly and they have trouble breathing. Many babies born with Type 1 SMA didn't live more than a couple of years (Emmady & Bodle, 2023). Type 2 SMA has milder symptoms than Type 1, although motor function and muscle weakness remain. Life expectancy for Type 2 SMA varies greatly. With proper medical treatment, some people can live near-normal lives. 70% live 25 years or longer, some live even more than that (Burr & Reddivari, 2023b). Most people with Type 3 SMA have a normal life expectancy, and many can live a full life with the right medical care and treatment of symptoms. SMA type 4 is the mildest form of the disease, and people with this form may only have mild problems moving around. A person with type 4 SMA has the same life expectancy as a person without SMA (Emmady & Bodle, 2023). While there have been significant advancements in the treatment of SMA in recent years, there are still limitations associated with the disease and its treatment:

- **Genetic nature:** SMA is caused by mutations in the SMN1 gene, and currently available treatments do not cure the underlying genetic defect. They aim to modify the course of the disease and manage symptoms but do not address the root cause.
- **Variable severity:** SMA presents with a wide range of severity, from very severe (Type 1) to milder forms (Type 4). Treatments are most effective when administered early in the disease course, and their efficacy may vary depending on the subtype and stage of the disease.
- **Limited accessibility:** Some of the most effective treatments for SMA, such as gene therapies like Zolgensma and disease-modifying drugs like Spinraza, can be extremely expensive and may not be accessible to all patients due to cost, insurance coverage, or healthcare system limitations.
- **Risks and side effects:** Treatments for SMA may come with risks and side effects. For example, gene therapies like Zolgensma carry the potential for immune reactions and

adverse effects, and Spinraza requires repeated spinal injections, which can be uncomfortable and may lead to complications.

- **Long-term effectiveness:** The long-term effectiveness of newer SMA treatments is still being studied, and their impact on patients as they age is not fully understood. Continued research is needed to determine their durability and any potential need for ongoing treatments.
- **Quality of life:** While SMA treatments can slow disease progression and improve motor function, they may not fully restore normal muscle strength and function. Quality of life issues, such as respiratory function, joint contractures, and pain management, still need to be addressed in many SMA patients.
- **Supportive care:** SMA management often involves a multidisciplinary approach that includes physical therapy, occupational therapy, respiratory support, and nutritional interventions. Access to these supportive care services may vary depending on the patient's location and healthcare resources.
- **Ethical and emotional challenges:** Decisions about SMA treatment can be ethically and emotionally challenging for families, especially in cases of severe disease where the prognosis is uncertain. These challenges can add stress and emotional burden to families already dealing with a difficult diagnosis.

Although there are limitations in our understanding of SMA as well as its treatment, continuous research and advances in genetic therapeutics offer those who are afflicted with the condition hope for improved outcomes and an enhanced quality of life. It is absolutely necessary to confer with medical experts who specialize on SMA in order to develop and implement the treatment strategy that is going to be most effective, given to the specifics of each individual patient's case.

Chapter 5

5.1 Future Prospect

The SMA community is on the edge of a very exciting period, which will come when it will be possible to translate the astounding success that has been achieved in treating SMA mice models into a therapy that can be helpful for humans who have SMA. In the past 20 years, researchers have made a lot of headway towards understanding the molecular pathogenesis of this disease. As a consequence of this, they are now in a position to test possible medicines in

vitro as well as in accurate animal models. They can then move promising agents on to human trials in people who are in early stage in the disease's progression or possibly in a pre-symptomatic stage (Kolb, 2011).

Newborn screening: Our observations, as well as those of other researchers, indicate that for the mouse model of the disease, regardless of the treatment delivery method, the most favorable outcomes are achieved when treatment is initiated early. Through newborn screening, babies with SMA can be found before they have any symptoms. This is an important step in treating SMA effectively. In summary, our intervention must occur proactively, preempting any harm. To achieve this, we must swiftly identify infants with SMA, as they represent the most suitable candidates for evaluating the potential efficacy of promising treatments in the coming years. If the disease is already developed in a child, the best combinational approach may still help, but the goal will be to slow the progression of the disease and keep the remaining tissues, including motor neurons working, not to completely reverse the disease (Farooq et al., 2013).

Creating clinical trial strategies for SMA: Over the past five years, substantial progress has been made in potential translational research using SMA animal models. This research is rapidly advancing toward the pre-clinical phase. But it's hard to make a great clinical trial for SMA because –

- the disease's symptoms change
- there aren't any molecular biomarkers
- It's hard to get to treatment centers
- No one agrees on the standard of care and how to treat the disease.

However, it is highly probable that these challenges will find solutions due to the remarkable level of teamwork and partnership among researchers, healthcare professionals, industry representatives, government agencies, and volunteer organizations in recent times. This concerted effort is aligning stakeholders to address these issues and reach a consensus on the formulation of universal human clinical trial protocols for SMA worldwide (Farooq et al., 2013).

Chapter 6

Conclusion

SMA, or Spinal Muscular Atrophy, is a condition affecting nerves and muscles, resulting from a complex interplay of genetic elements. It is a long-term disease of the motor neurons that is passed through the generations from parent to child and for which there is no known cure. Despite the challenges, there is cause for optimism as ongoing research continues to expand our understanding of the molecular genetics and underlying factors contributing to SMA. The primary culprit behind this condition is the deficiency of the survival motor neuron (SMN) protein, a consequence of mutations in the SMN1 gene. This leads to the gradual death of motor neurons, which makes muscles weak and atrophy. This chain of adverse events leads to a wide range of symptoms, from serious motor problems and breathing problems in Type 1 SMA to milder forms that show up later in Types 2, 3, and 4. To figure out the type and prognosis, the diagnostic process involves a number of steps, such as clinical evaluations and genetic research. Still, new medicines like Nusinersen (Spinraza), which use RNA modulation to increase SMN protein production and improve motor neuron function and quality of life, have brought about an opportunity in the treatment world. Gene therapies are still being researched, which gives people with SMA hope for the future. A holistic approach to managing SMA includes medical, rehabilitative, and supportive measures that are customized to meet the needs of each person. Medical workers, researchers, carers and families are all working together to change the course of SMA. Though spinal muscular atrophy (SMA) can't be completely cured right now, but study is still going on to find new treatments. This shows how hard people are working to improve the lives of those who have this complicated disorder.

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