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Review

Spinal Muscular Atrophy: An Overview

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Abstract

Spinal muscular atrophy, a leading cause of infant death, is a neurodegenerative disease classified categorically based on age of onset and achieved motor function. The standard method of diagnosis is through molecular genetic testing, ideally through the newborn screen to facilitate early diagnosis and treatment. There are 4 types of spinal muscular atrophy, each with varying degrees of symptoms based on the number of survival motor neurons. Current treatment options include gene therapy and supportive care. Future directions for treatment include complimentary non-gene targeted therapies and lifestyle changes.

Keywords

Genetics; SMN; nusinersen; risdiplam; onasemnogene abeparvovec

1. Introduction

Spinal muscular atrophy (SMA) is the leading genetic cause of infant deaths [1]. SMA is an autosomal recessive neurodegenerative disease that is classified into categories based on the age of onset and the level of motor function achieved. Recent advances in treatment options are aimed

at improving quality of life and extending life expectancy for the most severe cases of SMA, those classified as SMA type 1. Including SMA in the newborn screening program has helped facilitate early diagnosis and treatment which has had a profound impact of improving quality of life and prolonging the life expectancy [2].

2. Epidemiology

The exact prevalence of SMA in the United States is uncertain, however an overall prevalence of SMA is thought to be 1-2:100,000 people with a frequency of 1:11,000 births [3]. There are 4 types of SMA, with 1 being the most severe and 4 being the mildest. SMA type 1 is the most severe and most common, accounting for 50% of diagnoses [1]. SMA type 1 manifests before 6 months of age with death occurring in the first 2 years [1]. These individuals have the fewest copies of *SMN2* and therefore the most fatal type of SMA. SMA type 2 has onset between 6 and 18 months of age. Individuals with SMA type 2 may have a normal lifespan depending on the severity of their symptoms. SMA type 3 is a milder form with symptoms usually appearing around 18 months of age or in early childhood. Individuals with SMA type 3 are expected to have a normal life expectancy. SMA type 4 is very rare, affects 5% of the population of people with SMA, usually does not display symptoms until young adulthood and causes only mild motor impairment and no impact on life expectancy [4]. The estimated amount of people with SMA in the U.S. and Europe is thought to be between 30,000-35,000 cases with an overall incidence of 1:6,000-1:12,000 births [3]. Untreated individuals with SMA type 1 previously had a 50% survival probability at 8-10 months of age and an 8% survival probability at 20 months of age [3]. Overall survival is improving due to newborn screening, pre-symptomatic treatment, and gene therapies.

3. Genetics

SMA is an autosomal recessive disorder caused by homozygous deletion or mutation to the survival motor neuron 1 (*SMN1*) [1]. The disruption of the *SMN1* gene leads to the loss of production of the SMN protein. Humans have a gene that acts similarly to *SMN1* known as *SMN2* (survival motor neuron 2). Fewer copies of *SMN2* are associated with more severe disease. Individuals with 2 copies of *SMN2* option have the most severe type of SMA, SMA type 1, whereas individuals having 4 or more copies of *SMN2* have the less severe types [2].

Individuals with SMA have variants in both copies of *SMN1* to cause symptomology associated with the condition (Figures 1-4). Those who have only one copy altered are considered asymptomatic carriers (Figure 2). SMA is usually due to a deletion in exon 7 which can be detected by deletion/duplication analysis (95%-98%), however, approximately 2%-5% of individuals with SMA have the exon deletion as well as a sequencing variant (compound heterozygosity) [5, 6]. The number of copies of *SMN2* ranges from 0-8 [7]. Two methods that are used to determine SMN dosage are Quantitative PCR and MLPA. *SMN2* dosage can also be detected and help understand the potential phenotypic presentation of the individual [8-11].

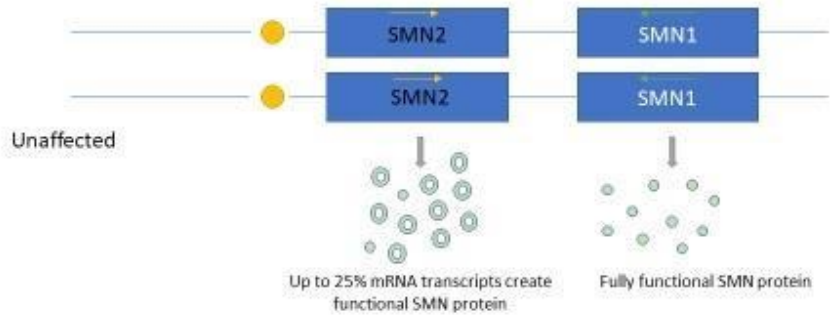

Figure 1 Unaffected.

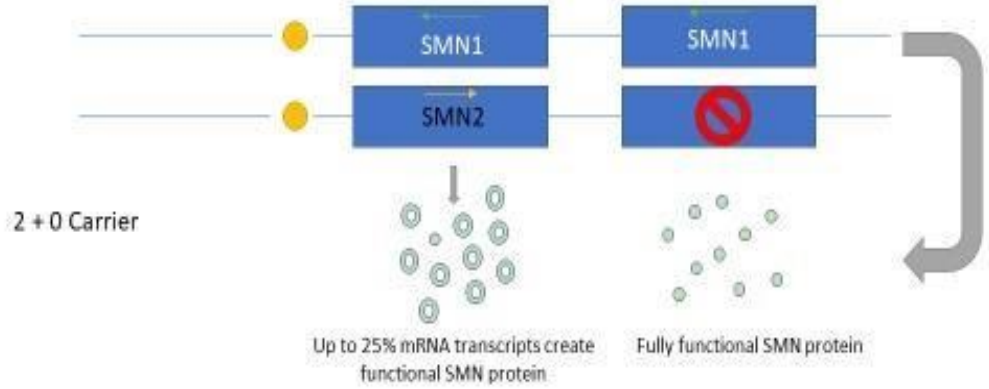

Figure 2 Asymptomatic carrier.

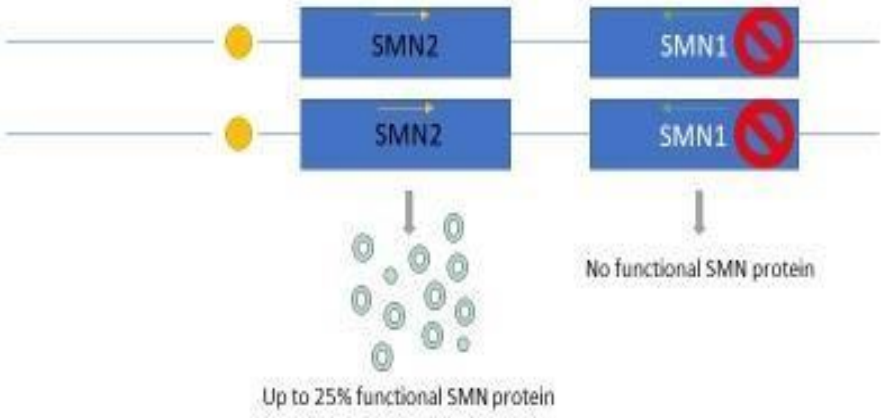

Figure 3 Loss of *SMN1* with 2 copies of *SMN2.*

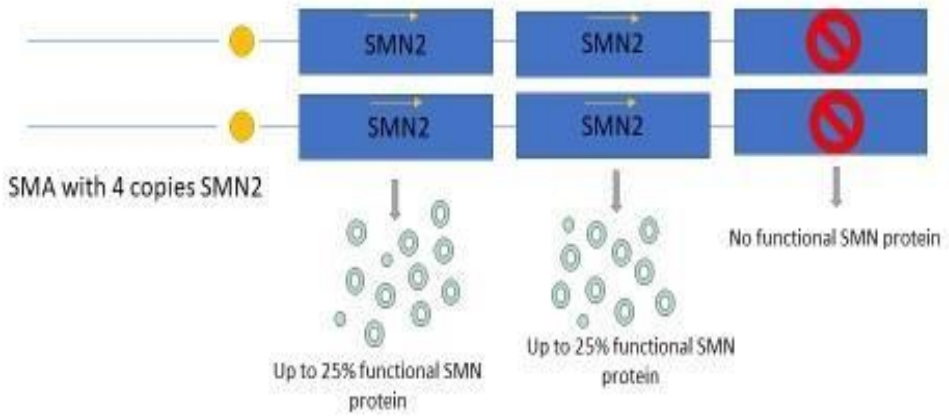

Figure 4 Loss of *SMN1* with 4 copies of *SMN2* have more SMN transcripts made than those with fewer *SMN2* copies and a modified presentation compared to those with fewer *SMN2* copies.

The severity of the presentation of SMA can be contributed to the amount of *SMN2*, which is very similar to *SMN1 (*Figures 3 and 4). *SMN2* homology to *SMN1* allows for some functional SMN proteins to be created. For parents that are both carriers for SMA there is a known autosomal recessive recurrence risk of 25% of having a child with SMA and a 50% chance of having asymptomatic carriers (Figure 2). There are still risks to parents with one known carrier and the other not known to be a carrier [8].

Approximately 6% of children with the traditional exon 7 deletion causing SMA have parents who will have normal carrier screening. False negatives may occur with *SMN1* dosage carrier screening because about 5%-8% [12, 13] of the individuals have both copies of *SMN1* on one 2 + 0 haplotype. This is when there are two copies of *SMN1* on one chromosome causing a normal dosage of SMN, but the second chromosome does not have a copy and those with subSaharan African ancestry are more likely to have this configuration [12, 14]. These individuals do not present with complications or symptoms and are missed by dosage testing. A specific single nucleotide variant (SNP) has been found in the Ashkenazi Jewish population and in black individuals and this SNP has been found to be more closely associated with 2 + 0 carrier status [12, 14]. This allows providers to help provide a more accurate carrier risk to families with these ancestries. 2% of individuals with SMA there was only one carrier parent and the second copy of *SMN1* had a de novo deletion at exon 7. A pan-ethnic carrier frequency within the United States is estimated at 1/54 with a detection rate of 91.2% by dosage testing. This creates an approximate 1/500 residual risk for individuals who have normal dosage testing [15].

SMN1 is located closer to the telomeres whereas *SMN2* is centromeric. *SMN1* and *SMN2* differ by only 11 sequencing changes [16]. The exons of the two genes differ by two nucleotides but create the same amino acid sequence. One is found in exon 7 and the other is in the non-coding exon 8. The rest are found within the intronic regions: insertion of 5 nucleotides in intron 6, 6 SNPs in intron 6, and 2 SNPs in intron 7 [16]. The functional difference between them is the C > T change inside exon 7. This variant in *SMN2* affects the splicing pattern allowing it to splice out exon 7, suggesting that the exon 7 nucleotide change affects an exon splice enhancer causing the non-functional protein to be created [16]. Patients with loss of *SMN1* who have multiple copies of *SMN2* can have

more SMN transcripts made than those with fewer *SMN2* copies, therefore, modifying the amount of useable SMN is transcribed.

Recurrence risks are slightly different from traditional autosomal recessive inheritance because of the de novo exon 7 deletion within *SMN1*. Having one carrier of an *SMN1*, there is not an increased risk for SMA, if the child had the de novo deletion on the second allele [17].

However, if both parents are carriers the recurrence risk remains 25% each pregnancy.

3.1 Genotype-Phenotype Correlation

The deletion of exon 7 of *SMN1* is seen at approximately the same rate in the different types of SMA (with only supportive therapies). Currently, there is no genotype-phenotype correlation within *SMN1*. The deletion and sequencing variants have been seen in all types of SMA. One of the modifiers for SMA is the number of *SMN2* copies which can range from 0-8. Up to ¼ of the mRNA that is generated from *SMN2* will create functional SMN proteins resulting in a milder phenotype such as SMA type 2 or SMA type 3. Individuals with two copies of *SMN2* have an 80% prediction of having an SMA type 1 phenotype. When there are four or more copies there is an 88% chance these individuals have SMA type 3 or SMA type 4 [18]. This phenotypic modification is without treatments and supportive care only.

4. Pathophysiology

Survival motor neuron (SMN) reduction impacts multiple cellular pathways that maintain neuronal homeostasis. SMN has a role in regulating the biogenesis of ribonucleoprotein (RNP) complexes, which in turn form small nuclear RNPs (snRNPs) [7, 19]. With the absence or reduction in the SMN protein, there is a decrease in SMN-profilin interaction.

This creates an increased amount of profilin–Rho-associated protein kinase (ROCK) complexes. This then leads to the activation of member A (RhoA). Rho A functions as a negative regulator of axon outgrowth [19, 20]. This leads to the progressive degeneration of the anterior horn cells in the spinal cord and brain stem nuclei. This loss is currently irreversible [21].

5. SMA Clinical Presentation

The clinical features of SMA vary based on the type (Table 1). Individuals with SMA type 1 appear normal at birth but develop hypotonia, delayed gross and fine motor milestones, and feeding difficulties within 6 months of life [4]. Their physical examination reveals axillary and appendicular hypotonia, flaccid paralysis, proximal muscle weakness, loss of reflexes, and tongue fasciculations. They also all have respiratory muscle involvement and require mechanical ventilation and never sit independently [4]. They develop weakened intercostal muscles and paradoxical breathing. Bulbar denervation causes tongue fasciculations and weakness with poor feeding and swallow reflex, and decreases airway protection [22]. This decreased airway protection increases the risk for aspiration pneumonia which is an important cause of morbidity and mortality [1]. Individuals with SMA type 2 usually have onset of symptoms between 6-18 months old, with the vast majority able to sit independently but never walk [4]. Some individuals with type 2 are able to stand with leg braces [1]. They usually develop kyphosis and fine tremors of their hands, and also experience weak swallowing which can lead to weight loss [1]. They also have bulbar denervation and weakened intercostal

muscles which increases the risk for aspiration pneumonia and respiratory insufficiency [1]. Individuals with SMA type 3 have a wide range of diverse symptoms. Most individuals with type 3 reach the major developmental milestones including walking independently [1]. Some require wheelchairs for assistance in childhood and others walk without difficulty [1]. Most experience minor muscle weakness in adolescence and young adulthood and often develop scoliosis along with symptoms of joint overuse due to weakness [1]. They do not tend to have the respiratory complications typical of types 1 and 2 [1]. SMA type 4 has adulthood onset, oftentimes over the age of 30 years.

Table 1 Clinical spectrum of SMA phenotypes without initiation of treatment-supportive care only.

SMA Subtype	Age of Onset	Milestones	Symptoms	Morbidity/Mortality
I	<6 months	Delayed gross & fine motor milestones "never sit independently"	Normal appearing at birth Develop hypotonia Feeding difficulties Mechanical ventilation	Respiratory insufficiency Aspiration pneumonia
II	6-18 months	Sit independently Stand with leg braces "never walk independently"	Kyphosis Hand fine tremor Weight loss	Respiratory insufficiency Aspiration pneumonia
III	Adolescence-young adulthood	Walk w/or w/o wheelchair assistance	Muscle weakness Scoliosis Symptoms of joint over use	Normal
IV	Adulthood >30 years	Meets all milestones on time	Mild motor impairment Walking into adult years	Normal

Individuals with type 4 usually demonstrate only mild motor impairment with no respiratory or nutritional problems and are able to walk into the adult years [1].

Supportive care is critical to prevent complications of SMA. The major complication of SMA is respiratory failure, which is the main cause of mortality in SMA [4]. Mechanical ventilation is the main respiratory treatment, including noninvasive ventilation for the milder forms of SMA and tracheostomy and invasive ventilation for the most severe cases [4]. Bracing to prevent and treat contractures and stretching exercises are important to maintain and improve function [4]. Scoliosis

is very prevalent among individuals with SMA types 1 and 2, therefore thoracic bracing is considered first line intervention in the treatment of scoliosis [4]. In more severe cases of scoliosis, surgical intervention is warranted to improve comfort and preserve pulmonary function [4]. Because bulbar weakness is very common and can lead to gastric dysmotility, close monitoring of nutritional status is warranted [4]. Monitoring of bone health via vitamin D levels and measuring bone mineral density is important to maintain bone health [4].

6. SMA Diagnosis

The standard tool for the diagnosis of SMA is molecular genetic testing. Molecular diagnostic methods include single strand conformation polymorphism (SSCP), restriction fragment length polymorphism (RFLP), competitive PCR, and realtime PCR [22]. Genetic testing should be considered in any infant with weakness or hypotonia [23]. Prior to the availability of genetic testing, muscle biopsy and EMG were the standard diagnostic tests, however molecular testing is readily available [23]. Individuals with SMA have homozygous loss of function of both *SMN1* copies; genetic testing for homozygous deletion will therefore confirm the diagnosis in 95% of the cases regardless of disease severity [23]. If an individual suspected of having SMA does not have evidence of *SMN1* gene deletion then *SMN1* dose analysis can be performed to look for the deletion of only 1 copy, and sequencing of the remainder *SMN1* gene to look for mutations [24]. The homozygous deletion of *SMN1* is specific to the diagnosis of SMA, and disease severity is predicted by the number of *SMN2* copies [23]. Muscle biopsy is no longer indicated because loss of nerve supply can be more readily demonstrated via EMG, which is less invasive than biopsy. Similarly, EMG should not be routinely performed for suspected cases of SMA and should be reserved for atypical cases. EMG shows features of motor neuron or motor axonal loss by active denervation and compensatory changes or the action potential enlargement [23]. Muscle biopsy cannot distinguish between SMA types. However, it can show some associations with disease severity [23]. While EMG and muscle biopsy were once the diagnostic mainstays for SMA, molecular genetic testing is less invasive and more readily available and is considered the first line diagnostic tool.

7. Treatments

By discovering the genes that cause SMA and the role of *SMN2* in the severity of disease, researchers were able to focus on therapies that increase the normal transcription of the *SMN2* gene and on therapies that deliver *SMN1* to the motor neurons [2]. Current FDA approved therapies include the *SMN2* modulators nusinersen and risdiplam, and the *SMN1* gene transfer therapy onasemnogene abeparvovec. Studies have shown positive outcomes regarding disease progression and have changed our knowledge of the natural history of SMA when treatment is implemented early on in the disease course or before symptoms begin [24-28].

The recommended uniform screening panel added SMA (exon 7 deletion specifically) due to these positive outcomes of earlier initiation of treatment. A key point in successful treatment of SMA is SMA newborn screening. Statewide screening for SMA began in New York in 2018, and over the first 3 years of implementation screened 650,000 infants and identified SMA in 34, the majority of which received gene replacement therapy prior to 6 weeks of age which resulted in improved outcomes [29]. Early diagnosis and treatment lead to improved motor outcomes.

The decision on when to initiate treatment after a positive newborn screening depends on the

symptomology of the patient (or absence of symptoms) and the underlying molecular cause with the dosage of *SMN2*. When SMA is confirmed, targeted therapies are recommended for all individuals with 2-3 copies of *SMN2*, even if symptoms are not present. For those with one copy of *SMN2*, treatment, and management plans are left to medical discretion. The decision needs to take into account the symptom presentation, how severe the complications are, and if the presentation starts prenatally or after birth. For individuals with 4 or more *SMN2* copies, targeted therapy can wait until symptom onset with careful monitoring (preferably by a neuromuscular expert) for the development of associated symptoms [28].

Nusinersen was the first FDA approved gene therapy for the treatment of SMA in 2016 [30, 31]. The therapy underwent randomized blinded clinical trials that showed efficacy in both early onset and later onset SMA [30]. Nusinersen is approved for all patients with SMA and is delivered intrathecally with 4 loading doses in 2 months and maintenance doses every 4 months. While it was initially approved for SMA type 1 and SMA type 2, it has shown benefit across all types of SMA. Nusinersen (Spinraza®) is an antisense oligonucleotide designed to manipulate gene expression by deleting or masking the splice site of exon 7 of SMN2 [31]. This mechanism allows increased inclusion of exon 7 in *SMN2*. Nusinersen binds to the ISS-N1 regulatory motif that is downstream of exon 7, increasing functional SMN mRNA being produced from *SMN2* [31, 32].

Onasemnogene abeparvovec was approved by the FDA in May 2019 for individuals under the age of 2 years with SMA. It is traditional gene therapy and is designed to deliver *SMN1* to motor neurons using an adeno-associated virus vector [2]. In a single center open label trial involving 15 infants with SMA type 1, none of the treated infants required permanent ventilation during the study and the overall cohort reached developmental milestones better than historical cohorts and had longer survival [2]. Onasemnogene abeparvovec can be administered intrathecally or intravenously [27]. In a 2 year follow-up study, infants required lower respiratory support and nutrition support, had improved motor function, and lower hospitalization rates than historical cohorts [2]. Onasemnogene abeparvovec-xioi (Zolgensma®) adeno-associated viral serotype 9 (scAAV9) is self-complementary. This aspect of the scAAV9 allows for the coding region of this recombinant virus to form a DNA template that is double-stranded [33] This allows the body to produce SMN transcripts from a functional *SMN1* gene.

Risdiplam is an oral *SMN2* modulator that was approved by the FDA in August 2020 for patients with SMA over the age of 2 months [2]. In an open label study, 21 infants with SMA type 1 were treated with risdiplam for 12 months and showed increased expression of the SMN protein [2]. Data from two clinical trials suggests improvement in motor function and survival without permanent ventilation in infants with SMA type 1 [2]. The favorability of orally administered risdiplam avoids the frequent lumbar punctures necessary for nusinersen [2].

8. Treatment Outcomes and Future Directions

Interest in including SMA as part of the newborn screening became more widespread after nusinersen was approved and evidence revealed better outcomes for patients treated earlier in the disease course. Recent data estimates that by screening all newborns for SMA in the US, 364 cases would be found and 68 deaths would be prevented annually [3]. SMA was recommended to be included as part of the newborn screening in 2018, after which time several states began implementing screening for SMA, and as of June 2022 97% of infants born in the US are screened

for SMA as part of the newborn screening [2]. Screening uses DNA extracted from dried blood spots with PCR targeting *SMN1* exon 7 which can be differentiated from *SMN2* exon 7 [3]. SMA screening has a 100% positive predictive value [3].

Despite the advances of treatment for SMA, treatment response and long-term outcomes are uncertain. Two thirds of the overall SMA population is comprised of older children and adults who may not have been treated early in their disease due to lack of availability of treatments [3]. The intrathecal route of administration of nusinersen is problematic for patients with scoliosis, spinal fusions, and contractures [3]. Onasemnogene abeparvovec and risdiplam are limited by a certain age population [3]. Finding targeted therapy that is approved across all SMA types is next on the horizon. Additional treatment to complement the current target-based therapy is necessary to improve outcomes [27]. Several studies are looking at targeting non-SMN genes neuroprotective therapies and muscle-enhancing therapies to complement the current SMN gene therapies [26]. CK-107 is thought to help increase muscle strength in individuals with SMA type 2 through 4 because it is a troponin complex activator [34] and is currently being researched as a drug in a clinical trial (NCT02644668) [35]. Another option is to prevent the degradation of SMN exon 7 which thereby stabilizes the SMN protein [33]. There are currently many different drug compounds in research that have shown progress in preventing the degradation of exon 7 [35]. Another area being researched is protection of motor neurons to prevent degradation in the first place [33]. The major tissue types affected by SMA are neurons and muscle tissue, and these have high energy demands. Research is ongoing that focuses on the targeting of energy pathways as neuroprotection for neurons and muscle tissue. The glycolytic enzyme phosphoglycerate kinase 1 (PGK1) was found to be dysregulated in SMA mouse models, and increasing its activity pharmacologically with terazosin or its expression genetically could ameliorate motor axon phenotypes in SMA zebrafish models [33]. A mitochondria targeted therapy, olesoxime, promotes cell survival and could therefore be applicable across a variety of neurodegenerative diseases including SMA [33].

Clinicians and researchers should continue to work together to find both gene targeted therapies and complimentary non-SMN therapies to improve clinical outcomes. Therapies that target the muscle are also thought to affect SMA through preserving proprioceptive synapses onto the motor neurons that are normally lost in SMA [33]. Myostatin is a negative regulator of muscle growth, and inhibition of the myostatin signaling pathway such as through the use of antibodies against myostatin has shown success in trials [33]. A myostatin inhibitor SRK-015 is in clinical trials (NCT03921528) for SMA II or III individuals to help improve muscle and bone deficiencies [36-38]. SMA is also associated with impairment at the neuromuscular junction, specifically with regard to development, maturation, and function [36]. Impairment of the neuromuscular junction leads to muscle weakness and fatigue, both common symptoms of SMA. There are a number of therapies targeting the neuromuscular junction such as overexpression of the agrin/MuSK signaling pathway which plays a key role in forming and maturing the neuromuscular junction [33]. Pyridostigmine is an acetylcholinesterase inhibitor that slows degradation of acetylcholine within the synaptic cleft and increase cholinergic transmission efficiency, and preliminary reports have shown that it also reduces fatigue in patients and therefore should be considered adjunctive therapy in SMA patients along with SMN targeted therapies [35]. Another complimentary approach to SMN targeted therapies includes lifestyle changes. It is well known that metabolic dysregulation is common in SMA, therefore lifestyle changes that enhance metabolism may have therapeutic benefits. Studies of mouse models with mild SMA have demonstrated that high intensity swimming and low intensity

running are beneficial for lipid and glucose metabolism [35]. Lifestyle changes are a relatively easy and cost-effective option to enhance SMN and non-SMN targeted therapies.

9. Conclusion

SMA is a leading cause of infant mortality, especially SMA type 1. Infants with SMA type 1 experience delayed gross and fine motor milestones and eventually develop respiratory distress requiring mechanical ventilation. Early diagnosis via SMA newborn screening is important because it allows for earlier treatment with gene targeted therapies which are crucial to improving clinical outcomes and improving quality of life.

Author Contributions

Mr. Ross and Dr. Kanungo were responsible for project development. Mr. Ross, Ms. Rudowski, and Dr. Kanungo were responsible for researching genetics. Mr. Ross was responsible for researching epidemiology, pathophysiology, clinical presentation, diagnosis, treatment, and future directions.

Competing Interests

The authors have declared that no competing interests exist.

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