Neurotherapeutics (2015) 12:290–302
DOI 10.1007/s13311-014-0314-x

REVIEW

The Genetics of Spinal Muscular Atrophy: Progress and Challenges

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Published online: 21 November 2014

Abstract Spinal muscular atrophies (SMAs) are a group of inherited disorders characterized by motor neuron loss in the spinal cord and lower brainstem, muscle weakness, and atrophy. The clinical and genetic phenotypes incorporate a wide spectrum that is differentiated based on age of onset, pattern of muscle involvement, and inheritance pattern. Over the past several years, rapid advances in genetic technology have accelerated the identification of causative genes and provided important advances in understanding the molecular and biological basis of SMA and insights into the selective vulnerability of the motor neuron. Common pathophysiological themes include defects in RNA metabolism and splicing, axonal transport, and motor neuron development and connectivity. Together these have revealed potential novel treatment strategies, and extensive efforts are being undertaken towards expedited therapeutics. While a number of promising therapies for SMA are emerging, defining therapeutic windows and developing sensitive and relevant biomarkers are critical to facilitate potential success in clinical trials. This review incorporates an overview of the clinical manifestations and genetics of SMA, and describes recent advances in the understanding of mechanisms of disease pathogenesis and development of novel treatment strategies.

Keywords Spinal muscular atrophy · Hereditary motor neuropathy · Motor neuron · Gene · Survival motor neuron (*SMN*) · Biomarker

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Introduction

The spinal muscular atrophies (SMAs) are a group of inherited disorders characterized by motor neuron loss in the spinal cord and lower brainstem, muscle weakness, and atrophy. The clinical phenotype incorporates a wide spectrum that is differentiated based on age of onset, pattern of muscle involvement, and inheritance pattern. Broad categories include proximal SMA and distal SMA (DSMA, also known as hereditary motor neuropathy or dHMN), demonstrating considerable genetic and clinical heterogeneity. SMA often refers to the most common form, caused by mutations of *SMN1* [1], termed SMA5q or survival of motor neuron (SMN)-related SMA. SMA remains the leading genetic cause of infant death, and without a disease-modifying treatment.

Recently, there have been important advances in understanding the genetic and molecular basis of SMA. Next-generation sequencing technology has accelerated gene discovery, with 13 SMA genes identified since 2011. In total, 33 causative genes have been identified to date. Common pathophysiological themes include defects in RNA metabolism and splicing, axonal transport, and motor neuron development and connectivity.

There is currently great promise to develop a successful, disease-modifying treatment for SMN-related SMA, and extensive efforts are being undertaken towards this aim. Promising therapeutic strategies in development include small-molecule SMN enhancers, antisense oligonucleotides

to correct *SMN2* splicing, neuroprotectants, stem cell and gene therapies, and regulators of muscle function. This review will focus on recent genetic discoveries in SMA, cellular mechanisms underlying motor neuron degeneration, understanding disease progression, and initiatives to address "clinical trial readiness" and the development of novel treatment strategies.

Clinical Presentations and Genetics

Proximal SMA

SMN1-related SMA

The most common form of SMA is caused by homozygous disruption of *SMN1* on chromosome 5q and results in insufficient levels of SMN protein in motor neurons. It is one of the most common autosomal recessive diseases, with an incidence of 1 in 6000–10,000 live births and a carrier frequency of 1 in 40–60 adults [2]. The disease typically presents in infancy or childhood, leading to severe physical disability. The weakness is usually symmetrical, more proximal than distal, the legs are more affected than the arms, and there is relative sparing of the diaphragm, and extraocular and facial muscles. Despite relative sparing of the diaphragm respiratory insufficiency is an important complication of SMA5q. Deep tendon reflexes are generally absent or diminished. There is a broad spectrum of clinical severity, with phenotypes divided into types 1–4 [3], determined principally by maximal motor milestone attained and age of onset. Infants with SMA type 1, or Werdnig–Hoffman disease, do not achieve independent sitting, with onset before 6 months of age, and respiratory failure usually leads to death within the first 2 years without respiratory support. SMA type 2 displays weakness before the age of 18 months. Patients achieve independent sitting, but are not able to stand or walk independently, and life expectancy is often into adulthood. Individuals with SMA type III (also known as Kugelberg–Welander disease) attain the ability to walk unaided and usually manifest after 18 months of age. There is marked variability in the clinical course, with some patients requiring wheelchair assistance in childhood and others walking in adulthood. Life expectancy is normal. SMA type 4 has onset in adulthood.

Non-SMN1-related SMA

Less than 5 % of infantile SMA is non-SMN-related, termed infantile SMA variants or SMA "plus" syndromes, in which additional clinical features may be evident, including arthrogryposis, abnormalities of extraocular movements, brainstem signs, or cardiomyopathy. These are characterized by congenital hypotonia, progressive postnatal weakness and areflexia with anterior horn cell degeneration. The differential diagnosis includes X-linked infantile SMA with arthrogryposis (XL-SMA) [4, 5], SMA due to mitochondrial dysfunction [6–8], SMA with pontocerebellar hypoplasia (SMA-PCH/PCH1) [9–13], and SMA with respiratory distress (SMARD) (Table 1) [14–16]. SMARD1 (or HMN type VI) is probably the second most commonly encountered pediatric from of SMA due to mutations in *IGHMBP2* [14]. SMARD1 typically presents with very early respiratory failure due to diaphragmatic paralysis and weakness, which may be diffuse or predominantly upper limb and distal muscles. The phenotype of SMARD1 has recently broadened and includes mild weakness without severe signs of respiratory involvement [17].

While the majority of proximal SMA cases are related to recessive *SMN1* mutations, the genetic heterogeneity of proximal SMA beyond infancy has also been recognized for several decades [2, 18, 19], with autosomal dominant SMA accounting for <2 % of cases. This includes lower extremity-predominant SMA types 1 and 2, caused by heterozygous mutations in *DYNC1H1* and *BICD2*, respectively [20, 21]. Muscle weakness and atrophy predominantly affect the proximal lower limbs, although upper limb and distal lower limb involvement may occur [22, 23]. Some patients may demonstrate mild upper motor signs, foot deformities, or lower limb contractures [23, 24]. Sensation, bulbar, and cognitive functions are preserved. Lower extremity-predominant SMA may be static or have very slow progression throughout life. Late-onset autosomal dominant proximal SMA may also be associated with dominant mutations in vesicle-associated membrane-associated protein, protein B, allelic with amyotrophic lateral sclerosis (ALS) type 8 [25]. Although *LMNA* mutations are more commonly associated with muscle disease (particularly Emery–Dreifuss muscular dystrophy), the phenotypic spectrum also includes adult-onset autosomal dominant SMA followed by cardiomyopathy [26]. Significantly, the phenotype of tauopathies has recently broadened to include lower motor neuron disease, with autosomal dominant mutations in *MAPT* producing proximal weakness of the upper limbs, and respiratory insufficiency without dementia, pyramidal, or bulbar involvement [27].

Proximal SMA may also be associated with recessive mutations in *PLEKHG5* [28]. Although classified as DSMA4, the clinical characteristics are proximal muscle weakness resulting in difficulty walking and climbing stairs with onset by the age of 3 years.

Importantly, the most common adult-onset SMA is bulbospinal muscular atrophy, also known as Kennedy's disease, related to increased CAG repeats in the androgen receptor [29]. This X-linked recessive neurodegenerative disorder is characterized by widespread and prominent fasciculations, muscle weakness and atrophy, dysarthria, and dysphagia. In addition, patients may have endocrine manifestations,

Table 1 Spinal muscular atrophies (SMAs) with known gene abnormalities/loci

Type of SMA	Inheritance	Age of onset	Clinical phenotype	Gene/locus	Year gene identified
Proximal SMA					
SMA5q or SMN-related SMA	Autosomal recessive		Proximal greater than distal limb weakness; diaphragm and facial muscles relatively spared	*SMN1*	1995
SMA type 1 (infantile SMA, Werdnig–Hoffman disease)		By 6 months			
SMA type 2		6–18 months			
SMA type 3 (Kugelberg–Welander disease)		After 18 months			
SMA type 4		Adult			
X-linked infantile SMA type 2	X-linked	Infantile	Similar to SMA type 1. Severe congenital hypotonia, arthrogryposis	*UBE1*	2008
SMA phenotype due to mitochondrial dysfunction	Autosomal recessive	Infantile	Similar to SMA type 1 with dilated cardiomyopathy, ptosis, impaired extraocular movements	*SCO2*	1999
SMA with pontocerebellar hypoplasia type 1	Autosomal recessive	Congenital or infantile	Diffuse weakness, microcephaly±arthrogryposis	*EXOSC3*	2012
				TSEN54	2011
				RARS2	2011
				VRK1	2009
SMALED1	Autosomal dominant	Congenital to adult	Proximal greater than distal leg weakness, arms normal	*DYNC1H1*	2012
SMALED2	Autosomal dominant	Congenital to adult	Proximal and distal muscle weakness of the lower limbs±mild upper limb weakness, mild upper motor signs	*BICD2*	2013
DSMA4	Autosomal recessive	By 3 years	Proximal weakness, difficulty walking and climbing stairs, progressing to nonambulant and respiratory weakness	*PLEKHG5*	2007
Adult-onset proximal SMA	Autosomal dominant	Adult	Proximal greater than distal weakness	*VAPB*	2004
Adult-onset proximal SMA followed by cardiac involvement	Autosomal dominant	Adult	Proximal greater than distal weakness	*LMNA*	2007
Adult-onset proximal SMA with respiratory failure	Autosomal dominant	Adult	Proximal weakness of upper limbs followed by prominent respiratory failure	*MAPT*	2014
Spinal and bulbar muscular atrophy (Kennedy syndrome)	X-linked recessive	Adult	Widespread and prominent fasciculations, Progressive proximal and distal limb and bulbar muscle weakness and atrophy, dysphagia, gynaecomastia, and androgen resistance	*AR*	1991
Distal SMA/HMN*					
HMN1	Autosomal dominant	Child to young adult	Distal leg then arm weakness	*HSPB1*, *HSPB8*, *GARS*, *DYNC1H1*, *7q34*	
HMN2	Autosomal dominant	Adult	Distal leg then arm weakness		
HMN2A				*HSPB8*	2004
HMN2B				*HSPB1*	2004
HMN2C				*HSPB3*	2010
HMN2D				FBXO38	2013
HMN3 (DSMA3)	Autosomal recessive	Childhood	Mild distal leg then arm weakness	11q13	
HMN4 (DSMA3)	Autosomal recessive	Infancy to young adult	Severe proximal and distal weakness, diaphragmatic palsy	11q13	

Table 1 (continued)

Type of SMA	Inheritance	Age of onset	Clinical phenotype	Gene/locus	Year gene identified
HMN5	Autosomal dominant	Child to adult	Upper limb predominance with onset in thenar and first dorsal interosseus muscles and subsequent weakness of legs		
HMN5A				*GARS*	2003
HMN5B				*REEP1*	2012
HMN5C				*BSCL2*	2004
HMN6 (DSMA1 or SMARD1)	Autosomal recessive	Infancy	Early diaphragm weakness, distal greater than proximal limb weakness	*IGHMBP2*	2001
HMN7	Autosomal dominant	Juvenile/young adult	Vocal cord paresis, hand weakness and subsequent distal leg weakness		
HMN7A				*SLC5A7*; *CHT*	2012
HMN7B				*DCTN1*	2003
DSMA5	Autosomal recessive	Young adult	Progressive distal greater than proximal lower limb muscle weakness and atrophy	*DNAJB2*; *HSJ1*	2012
HMN with upper motor neuron signs	Autosomal dominant	Juvenile	Distal leg then arm weakness with pyramidal signs	*SETX*	2004
HMNJ (DSMA2)	Autosomal recessive	Juvenile	Distal leg then arm weakness with pyramidal signs originating from the Jerash region of Jordan	9p21	2000
X-linked dHMN (SMAX3)	X-linked recessive	Child-to-adult	Distal leg then arm weakness	*ATP7A*	2010
SMARD2	X-linked recessive	Neonatal	Distal weakness , early onset diaphragmatic weakness and respiratory failure	*LASIL*	2014
Scapuloperoneal SMA	Autosomal dominant	Young adult	Distal and scapuloperoneal weakness±congenital absence of muscles or laryngeal palsy	*TRPV4*	2010
Congenital DSMA	Autosomal dominant	Congenital	Proximal and distal nonprogressive lower limb weakness±vocal cord paralysis or arthrogryposis	*TRPV4*	2010
DSMA with mitochondrial dysfunction	Mitochondrial	Childhood-to-adult	Episodic weakness associated with a later-onset distal lower limb weakness and atrophy	*mtATP6*	2012
	Mitochondrial	Childhood-to-adult	Episodic weakness associated with a later-onset distal motor neuropathy	*mtATP8*	2013

*Distal HMN is based on the original classification by Harding

SMN survival motor neuron, *SMALED* spinal muscular atrophy with lower extremity predominance, *DSMA* distal spinal muscular atrophy, *HMN* distal hereditary motor neuropathy, *SMARD* spinal muscular atrophy with respiratory distress, *HMNJ* distal hereditary motor neuropathy, Jerash type, *dHMN* distal HMN

including gynecomastia, reduced fertility, and erectile dysfunction, related to androgen insensitivity, and diabetes mellitus.

DSMA or HMN

In contrast to proximal SMA, DSMA also known as dHMN or HMN, is characterized by a slowly progressive symmetrical and predominantly distal limb weakness and atrophy. Since 2001, mutations in 19 genes for dHMN have been identified, and it has emerged that they are clinically and genetically heterogeneous, with various phenotypes related to individual genes (Table 1). Harding originally classified dHMN into 7 categories, based on inheritance pattern, age of onset, severity, and distinguishing clinical features (Fig. 1) [30]. Autosomal dominant dHMN types I and II present with distal leg and subsequent distal arm weakness in childhood and adulthood, respectively, and are associated with mutations in *HSPB1* and *HSPB8* [31–33], *HSPB3* [34], *GARS* [35], *FBXO38* [36], and *DYNC1H1* [37]. Linkage to 11q13 has been demonstrated in the autosomal recessive types III and IV (synonymous with DSMA type 3). HMN type V is distinguished by onset of weakness in the hand muscles, and may be associated with dominant mutations in *BSCL2* [38], *GARS* [39], or *REEP1* [40]. HMN type VII is distinguished by vocal cord paresis and may be due to dominant mutations in *SLC5A7* (*CHT*) [41], or *DCTN1* [42]. The genetic heterogeneity of HMN is further highlighted with the identification of recessive mutations in *DNAJB2* (*HSJ1*) causing a nonspecific presentation of lower limb predominant slowly progressive weakness with young adult onset, known as DSMA type 5 [43].

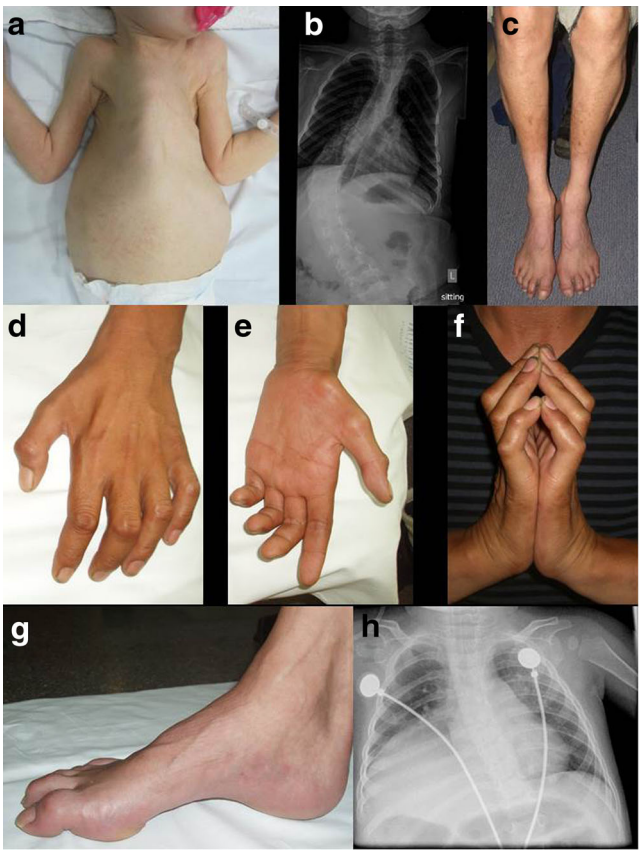

Fig. 1 Clinical features of spinal muscular atrophies (SMAs). (**a**) Infant with SMA type 1 with severe weakness, bell-shaped chest, and respiratory insufficiency. (**b**) Thoracolumbar radiograph of patient with SMA type 2 demonstrating thoracolumbar scoliosis. (**c**–**g**) A 36-year-old patient with distal hereditary motor neuropathy type 5C and mutation in *BSCL2* demonstrating (**c**) distal wasting of the legs, (**d**–f) early and marked weakness and atrophy in the hands with finger contractures, and (**g**) pes cavus. (**h**) Chest radiograph of infant with SMA with respiratory distress type 1 demonstrating eventration of the right hemidiaphragm

The clinical spectrum of dHMN/SMA continues to expand and may also include congenital onset and X-linked or mitochondrial inheritance. There may be minor sensory involvement and/or pyramidal signs, and many of these disorders are allelic with axonal Charcot-Marie-Tooth disease (*HSPB1*, *HSPB8*, *BSCL2*, *GARS*, *TRPV4*), juvenile forms of ALS (*SETX*) [44, 45], and hereditary spastic paraplegia (*BSCL2*, *HSPB1*). In addition, autosomal recessive dHMN with pyramidal signs, linked to 9p21.1-p12, has been described originating from the Jerash region of Jordan (dHMN-J) [46]. X-linked recessive dHMN/DSMA may be associated with mutations in *ATP7A* (X-linked dHMN, allelic with Menkes disease) [47, 48] and *LAS1L* (SMARD2) [16]. Recently, mutations in *TRPV4* have been associated with congenital distal SMA and scapuloperoneal SMA [49, 50]. Associated distinguishing clinical features may include vocal cord paralysis, scoliosis, contractures, or proximal upper limb weakness. Challenging the dogma of varied and multisystem involvement in mitochondrial disease, mutations in *MT-ATP6* and *MT-ATP8* have recently been shown to cause less severe phenotypes, including recurrent attacks of symmetrical limb paralysis and a later-onset distal motor neuropathy, mimicking periodic paralysis due to channelopathies [51, 52].

Diagnostic Evaluation and Management

For any patient presenting with clinical symptoms consistent with proximal SMA, testing for homozygous deletion of *SMN1* should be undertaken. This test has 95 % sensitivity and nearly 100 % specificity in confirming the diagnosis of SMN-associated SMA or SMA5q [1, 53]. A negative *SMN1* prompts review of clinical features, measurement of creatinine kinase, and neurophysiological studies with repetitive stimulation to help distinguish between motor neuron disease, myopathy, and neuromuscular junction disorders. Compound muscle action potentials (CMAPs) are typically reduced in SMA while motor conduction velocities and distal latencies

are normal, or only modestly reduced when the CMAP amplitude is substantially reduced. The identification of sensory involvement is not expected in SMA and suggests Charcot-Marie-Tooth disease, wherein patients may have minimal sensory symptoms or signs. Electromyography shows chronic denervation with motor units of increased amplitude and duration, though in infants with severe SMA there may be normal-sized residual voluntary motor units.

In patients without homozygous *SMN1* deletions and proximal SMA, measurement of *SMN1* copy number will guide further investigations. A single *SMN1* copy may suggest compound heterozygozity, with a deletion on 1 allele and a point mutation on the other, and *SMN1* sequencing is indicated [53, 54]. When 2 *SMN1* copies are demonstrated, then other motor neuron disorders such as SMARD, Kennedy's disease, distal SMA, and ALS should be considered. Additional investigations including magnetic resonance imaging of brain and spinal cord, and metabolic and genetic studies may be undertaken. Diagnostic algorithms based on phenotype guide genetic testing in DSMA, although the yield currently remains low [55]. If neurophysiological studies reveal characteristic patterns associated with diseases in muscle, nerve, or neuromuscular junction then muscle or nerve biopsy and edrophonium test may be undertaken.

Practice guidelines for patients with SMN-related SMA have been established, with consensus on pulmonary, gastrointestinal, and orthopedics/rehabilitation to provide consistent management [56]. These incorporate a multidisciplinary and supportive approach. The medical practice and goals of therapy vary according to the patient's level of function and philosophy of the patient and family. The appropriate level of interventional support to prolong life, particularly in SMA type 1, is controversial, and discussions with the family to explore and define potential quality of life and palliative care issues are important. Patients with SMA may have impaired cough and poor clearance of lower airway secretions, hypoventilation, and recurrent infections related to weakness. Respiratory management includes administration of routine immunizations, employing airway clearance techniques and cough assistance as necessary. Additionally, nocturnal noninvasive ventilation has been routinely introduced for sleep-disordered breathing in patients with SMA types 2 and 3. Inadequate oral intake and malnutrition are managed proactively to avoid potential complications. Treatment strategies may include nutritional supplementation, modifying food consistency, optimizing oral intake, positioning, and seating alterations. Contractures and significant scoliosis from muscle weakness are universal in SMA types 1 and 2, and may also occur in SMA type 3. Provision of equipment to assist with mobility, self-care and function, orthotics, and scoliosis surgery are important interventions.

Insights into SMN1-related SMA Pathogenesis

Almost 2 decades after the identification of *SMN1* as a SMA-determining gene, substantial progress has been made in unraveling the molecular, cellular, and physiological processes of disease. Common pathophysiological themes underlying the various forms of SMA include defects in RNA metabolism and splicing, axonal transport, and motor neuron development and connectivity. Taken together, these themes resonate more generally amongst the motor neuron disease. Of further relevance, SMN is a genetic risk factor for ALS [57–59].

The development of the most common type of SMA relates to insufficient levels of SMN protein expression in motor neurons by homozygous deletion/mutation of *SMN1* [1]. Humans possess a variable copy number of *SMN2* (0–8 copies) from which SMN is solely derived in patients with SMA [53, 60]. *SMN2* is almost identical to *SMN1*, except that a single translationally silent C to T nucleotide transition causes exon 7 skipping in the splicing of the majority of SMN2 transcripts, producing a truncated and unstable form of the SMN protein [61]. This nucleotide change disrupts an exon splice enhancer sequence and creates an exonic splicing silencer element that binds the splicing repressor heterogenous ribonuclear protein (hnRNP) A1 [62, 63]. A small fraction of *SMN2* transcripts are spliced to include exon 7 and produce full-length SMN. The number of *SMN2* copies and resultant amount of full-length SMN protein produced in patients with SMA (10–40 % of normal SMN protein levels) correlates with SMA disease severity. Consequently, *SMN2* copy number broadly predicts SMA phenotype with the majority of SMA type 1 patients having 1 or 2 copies, type 2 patients usually have 3 copies, and most type 3 patients have 3 or 4 copies. Zero copies of *SMN2* is embryonically lethal or associated with SMA type 0 [64–66].

The ubiquitous SMN protein has numerous and diverse functions in cells. The best characterized "housekeeping" function of SMN is in the nucleus and cytoplasm as part of a large macromolecular complex together with other proteins known as gemins [67]. The SMN complex is important in the production, recycling, and maintenance of small nuclear ribonucleoproteins of the Sm class, which are involved in the splicing of pre-mRNA into mRNA [68–70]. Recent studies have provided insights into the selective vulnerability of lower motor neurons to degeneration in SMA, including defective splicing of a subset of lower motor neuron-specific genes [71–73]. In addition, a negative feedback loop specific to motor neurons has been demonstrated, in which SMN depletion decreases exon 7 inclusion, further decreasing the splicing of its own mRNA [74]. SMN also has a role in axonal transport through its ability to regulate actin dynamics, forming a complex with β-actin [75, 76]. SMN also interacts with profilin (ALS18) to influence indirectly actin filament stability [77, 78]. Animal models with SMN depletion are

deficient in β-actin mRNA and protein, and numerous disturbances of motor neuron axonal growth and development have been identified [75, 79, 80]. Significantly, plastin 3 has recently been identified as a protective modifier of SMA in females, coding for an actin-modifying protein [81]. Abnormalities in the neuromuscular junction also contribute to SMA pathogenesis, with rapid and progressive dysfunction occurring around the time of clinical disease onset [82–86].

While an important research focus is the direct effect of low SMN on motor neurons, it is not the sole site of pathology. Spinal motoneuronal function is modified by direct and indirect sensory afferent input, such as the spinal reflex circuit [87], and recent SMA animal models indicate that these interactions are important in SMA pathogenesis, shifting the pathophysiological paradigm to one of motor circuit dysfunction. Reduced SMN produced early abnormalities in proprioceptive synaptic input onto motor neurons that paralleled clinical alterations in animal motor behavior [88, 89]. In this model, these abnormalities occurred while motor neuron function was relatively maintained and this suggested deafferentation of motor neurons may be an early event in SMA pathogenesis. In turn, alterations in synaptic inputs induced functional changes in spinal motor neurons, with a compensatory hyperexcitability that may be related to alterations in the activity of ion channels at the cell membrane. Neurophysiological findings in patients with SMA provide support to these observations, with alterations in spinal H reflexes, spinal circuitry ,and ion channel function in motor nerves identified [90–92]. Of therapeutic relevance, increasing the excitability of motor circuits through the pharmacological inhibition of K^+ channels ameliorated SMA in animal models [93]. Further evidence of the wider impact of SMN deficiency in disease pathogenesis comes from abnormalities in Schwann cells, skeletal muscle, heart, bone, pancreas, liver, hippocampus, thalamus, and the vascular system [94–101]. Controversy remains over the relative contribution of organ systems other than the motor neuron in SMN1-related SMA pathogenesis. It may be debated that the motor neuron is the only significant tissue, as specific elimination of *SMN1* in motor neurons recapitulates all features of SMA and neuronal SMN restoration is necessary and sufficient for therapy [84, 102–104]. In spite of this, SMN upregulation within both the central and peripheral nervous systems may be necessary for optimal therapeutic outcomes [84, 94]. The detrimental effects of low SMN on neuromuscular circuitry raises a significant challenge of solving where SMN targeted therapy will be required and what will be best delivery mode in patients with SMA.

The concept that the pathogenesis of SMA is associated with ubiquitously expressed proteins involved in diverse cellular pathways is reinforced by mechanisms underlying neurodegeneration in non-SMN SMA. Mutations in SMA-related genes are associated with defects in DNA/RNA metabolism and protein synthesis, axonal guidance and transport, protein misfolding and degradation pathways, ion channel function, and energy production (Fig. 2, Table 2). These diverse functional pathways suggest that motor neuron degeneration may be a final common outcome with a number of upstream causes. Future studies incorporating next-generation sequencing and functional models will provide further insights into pathomechanisms underlying motor neuropathy. Furthermore, the overlap among various motor neuron diseases may make similar treatment strategies between these disorders possible. A recent mouse model of ALS demonstrated improvement of neuromuscular function and motor neuron survival with upregulation of SMN overexpression, encouraging further investigation of the potential SMN as a modifier of ALS [105].

Challenges is SMN1-related SMA Therapeutics

These pathophysiological insights have revealed potential novel treatment strategies and extensive efforts are being made towards expedited therapeutics, with clinical trials already in progress. Promising therapeutic strategies in development include small-molecule SMN enhancers, antisense oligonucleotides to correct *SMN2* splicing, neuroprotectants, stem cell and gene therapies, and regulators of muscle function (Sumner et al, this issue). To date, the 26 clinical trials that have investigated the effect of 12 potential treatments in patients with SMA have failed to show benefit owing to a number of factors, yet have enabled expertise in trial design to be developed [106, 107]. Experience has suggested that younger patients might be more responsive to treatment [107, 108]. Older patients may be refractive to treatment because they have been living with their disease for longer periods of time or because critical tissues have been irreversibly damaged. Defining therapeutic windows and developing sensitive and relevant biomarkers are critical to facilitating potential success in clinical trials.

There remains a lack of consensus over the extent to which SMA is progressively degenerative and over the rate of clinical progression in SMA. Natural history studies and understanding pathophysiology reveal part of the problem and suggest SMA manifests in 2 phases, with an initial rapid decline followed by the development of a relative plateau phase such that the rate of motor neuron loss in SMA is nonlinear [109, 110]. Some studies suggest gradually progressive degeneration throughout life [111–114], while others suggest that gradual declines are due to the effects of physical growth placing greater demands on the motor system [115]. In further contrast, others describe a static course over a period of up to 2.5 years [116, 117]. The slow rate of progression poses a major challenge to clinical trials in SMA because most trials need to be completed within 1–2 years. Even within SMA

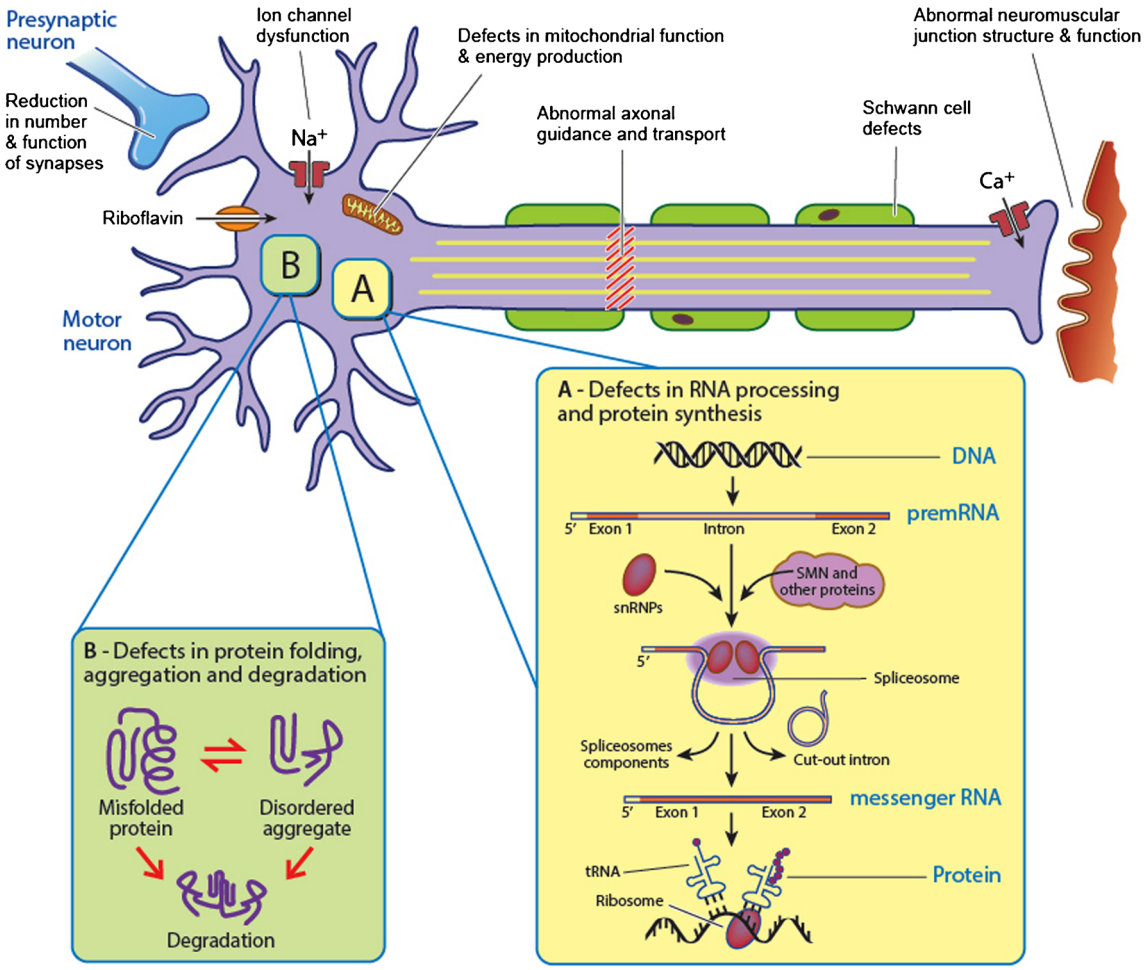

Fig. 2 Proposed mechanisms underlying spinal muscular atrophies (SMAs). Most of these genes associated with SMA encode for ubiquitously expressed proteins with diverse cellular functions: protein translation and synthesis (glycyl-tRNA synthetase, Bernardinelli-Seip congenital lipodystrophy 2), RNA/DNA metabolism (senataxin, immunoglobulin μ-binding protein 2), axonal guidance and trafficking [heat shock protein (HSP)27, dynactin 1, pleckstrin homology domain containing, family G (with RhoGef domain) member 5], cellular protection (HSP22, HSP27), and apoptosis (HSP27). snRNP=small nuclear ribonucleic particles; SMN=survival motor neuron

subtypes there is significant variation in severity and progression rate throughout the lifespan. Such heterogeneity among clinical trial participants may potentially obscure treatment effects. Further, the combination of clinical decline and plateau phases with simultaneous motor development, myelination, and physical growth complicates the assessment of motor function. In addition, secondary complications such as scoliosis and contractures further obscure the reliability of clinical observations.

Neurophysiological studies have provided further insights into the timing of SMA pathogenesis, with motor unit number estimation (MUNE) and CMAP used to track disease

Table 2 Proposed mechanisms underlying spinal muscular atrophies

Pathogenic mechanism	Implicated genes
RNA splicing and metabolism and protein synthesis	*SMN*, *SETX*, *IGHMBP2*, *DCNT1*, *GARS*, *BSCL2*, *EXOSC3*, *TSEN54*, *RARS2*, *REEP1*, *LAS1L*
Protein folding, aggregation and degradation pathways (tau, ubiquitin)	*HSPB1*, *HSPB8*, *BSCL2*, *UBE1*, *AR*, *VAPB*, *DCNT1*, *MAPT*
Axonal guidance and transport	*DCNT1*, *DYNC1H1*, *PLEKHG5*, *HSPB1*, *SMN*, *BICD2*, *FBX034*
Ion channel function	*TRPV4*
Mitochondrial function and neuronal energy production	*SCO2*, *mtATP6*, *mtATP8*, *?GARS*

progression [109, 111, 118]. An age-dependent decline in MUNE and CMAP amplitude occurs in both SMA 1 and 2, and significant progressive denervation may be present before the onset of symptoms [111, 119]. The capacity for prolonged motor neuron survival has also been demonstrated by establishing relative stability of CMAP and MUNE values in cross-sectional and longitudinal studies [112, 120]; however, this may not always correlate with decrements in motor function over the same period. In further contrast, while CMAP may remain stable over time, MUNE has been shown to increase, suggesting new motor unit development as a compensatory process [118]. Axonal excitability studies also support a mixed pathology comprising features of axonal degeneration and regeneration [121].

While caution in translating data from preclinical studies into humans remains a challenge, these provide further support for an initial rapid onset of disease, or "up-front course", coinciding with fetal and early postnatal neurodevelopment. Disruption of neuronal growth, axon branching, and neuromuscular connectivity was observed in zebrafish [122]. While developmental processes were maintained in mouse models, denervation was evident during embryogenesis and early in the postnatal course, around the time of disease onset [86, 123]. In SMA mouse models the temporal requirement for SMN protein encompasses the early postnatal course, with depletion of SMN in adults having minimal effect, coinciding with relative maturity of the neuromuscular junction [82, 124].

Taken together these studies suggest a spectrum in the rate of motor neuron denervation, survival, and potential compensation in SMA that remains a challenge in defining therapeutic windows that may prevent, stabilize, or reverse motor neuron degeneration in humans. It may be expected that early or presymptomatic therapies will provide optimal benefit, particularly in SMA type 1, such that advancing early diagnosis (newborn screening) will be essential. In milder types of SMA, a more extended period for intervention may also possible. National and international SMA registries are expected to accelerate the recruitment process of patients with SMA into new clinical trials, thereby facilitating potential early interventions. Clinical trial design (defining who, when, and where to treat, and expected outcomes) will be critical in developing future treatments. Furthermore, success depends on meeting the efficacy requirements of regulatory agencies, such that outcomes must be both realistic and meaningful.

The search for biomarkers in SMA is one of several research priorities. A number of clinical functional outcome measures have been used in SMA trials; however, current outcome measures may be unable to identify sensitively subtle changes to demonstrate a significant effect. In addition, while all of the scales demonstrate good reliability, the validity of measurement of motor performance in children with different severities of SMA is in question and the relationship to pathophysiology unclear. Ongoing Rasch analyses of multiple motor function scales are expected to create more robust scales [125]. MUNE, CMAP, electrical impedance myography, and axonal excitability have potential as alternative outcome measures that may also enable individual characterization of disease severity and reflect underlying pathophysiology. Aligned with therapeutic approaches designed to increase SMN levels, efforts to develop candidate biomarkers have also concentrated on measurement of SMN protein expression. While this can reliably be measured in peripheral blood and relates to SMA type, SMN protein levels do not predict severity of motor function, and it remains to be determined if this reflects what is happening in motor systems [126, 127]. Development of non-SMN molecular biomarkers using proteomic, metabolomics, and transcriptomic approaches holds promise for SMA, even though these measures may not be able to distinguish primary (initiating) and secondary (responsive) changes in gene expression. The recent BforSMA study identified a new set of 27 validated plasma protein SMA biomarkers significantly associated with motor function and other measures of SMA disease activity, and a commercial SMA-MAP biomarker panel was generated [128, 129]. Further studies will be required to investigate sensitivity to change with disease progression, and assess potential impact on clinical trial design.

Conclusion

SMA is a devastating genetic neuromuscular disorder, leading to significant infant and childhood mortality and morbidity. The most common mutation is homozygous disruption of *SMN1* and causative genes implicate altered RNA processing, axonal transport, and protein degradation. Significant advances in patient care and knowledge of the genetics and biology of SMA over the last 2 decades have revealed promising strategies for therapeutics development, with clinical trials already in progress. Extensive efforts are being undertaken towards translating these to reach the ultimate goal of finding an effective treatment in the clinic. If there is hope of identifying a treatment for this disease, a further understanding of the site and timing of disease progression, and potential adaptations, in humans, as well as developing very sensitive and relevant biomarkers over this period of time, is required.

Acknowledgments This work was supported by funding to Forefront, a collaborative research group dedicated to the study of motor neuron disease, from the National Health and Medical Research Council of Australia program grant (#1037746), and grant support from the Thyne Reid Foundation.

Required Author Forms Disclosure forms provided by the authors are available with the online version of this article.

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