The genetics of spinal muscular atrophies
Claribel D. Wee, Lingling Kong and Charlotte J. Sumner

Introduction
Since the 19th century, the term spinal muscular atrophy (SMA) has been used to describe diseases characterized by anterior horn cell (AHC) loss and progressive muscle weakness and atrophy without corticospinal or sensory neuron involvement. Today, SMA often refers to the most common form of disease, proximal SMA, now known to be caused by mutation of the survival motor neuron 1 (SMN1) gene. Nevertheless, there are multiple forms of SMA that can be distinguished based on age of onset, pattern of muscle involvement, and inheritance pattern. The SMAs can be divided into those that have proximal greater than distal muscle weakness and those with distal predominant weakness. These distal forms include the ‘distal’ SMAs and the hereditary motor neuropathies (HMNs). In this review, only SMAs with known gene abnormalities will be discussed. They are summarized in Table 1.

Clinical presentations and genetics
Proximal SMA is the most common inherited cause of infant mortality with an incidence of approximately 1 in 10 000 live births. It is an autosomal recessive disease caused by mutations of the SMN1 gene [1]. These are most often large deletion mutations that include the exon 7 and 8 regions. All patients retain between 1–4 copies of the centromeric copy of the gene, SMN2, which predominantly encodes a truncated, unstable form of the SMN protein. SMA disease severity is inversely correlated with SMN2 gene copy number [20,21]. SMA is divided into four types based on clinical severity. SMA type I, or Werdnig–Hoffman disease, usually presents before 6 months of age. Patients are unable to sit unaided and death often occurs before 2 years without respiratory support. Patients with SMA type II become symptomatic before 18 months, can sit but not stand unaided, and survival is variable. SMA type III, also known as Kugelberg–Welander disease, presents in adolescence. Patients can walk or run and survive to adulthood. Type IV SMA denotes adult-onset cases. The pattern of weakness in SMA is symmetrical with proximal muscles more affected than distal muscles, legs more affected than arms, and little involvement of the facial muscles. Truncal muscles are prominently affected in severe forms with involvement of the intercostal muscles, but relative sparing of the diaphragm (Fig. 1a and b).

Purpose of review
This article reviews clinical, genetic, and therapeutic advances in spinal muscular atrophies (SMAs), inherited disorders characterized by motor neuron loss and muscle weakness.

Recent findings
There has been progress in defining the clinical and genetic features of at least 16 distinct forms of SMA. The genes associated with 14 of these disorders have been identified in the last decade, including four within the last year: TRPV4, ATP7A, VRK1, and HSPB3. Genetic testing is now available for many SMAs, providing important diagnostic and prognostic information. Cell and animal models of SMAs have been used to further understand how mutations in SMA-associated genes, which code for proteins involved in diverse functions such as transcriptional regulation, RNA processing, and cytoskeletal dynamics, lead to motor neuron dysfunction and loss. In the last year, there has also been remarkable progress in preclinical therapeutics development for proximal SMA using gene therapy, antisense oligonucleotides, and small molecules.

Summary
The advances in the clinical and genetic characterization of different forms of SMAs have important implications for clinical evaluation and management of patients. The identification of multiple, novel SMA-causing genes will lead to an improved understanding of motor neuron disease biology and may provide novel targets for therapeutics development.

Keywords
anterior horn cell, hereditary motor neuropathy, spinal and bulbar muscular atrophy, spinal muscular atrophy
Table 1 Spinal muscular atrophies with known gene abnormalities

<table>
<thead>
<tr>
<th>Type of SMA</th>
<th>Inheritance</th>
<th>Locus</th>
<th>Causative gene</th>
<th>Type of mutation</th>
<th>Distinguishing clinical features</th>
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<tr>
<td>Proximal SMAs</td>
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<tr>
<td>Proximal spinal muscular atrophy (SMA)</td>
<td>Autosomal recessive</td>
<td>5q12.2–q13.</td>
<td>Survival motor neuron 1 (SMN1) [1]</td>
<td>Large deletions including exons 7 and 8</td>
<td>Most often infantile/childhood onset. Proximal greater than distal limb weakness. Intercostal and truncal weakness in severe forms. Daphragm and facial muscles relatively spared.</td>
</tr>
<tr>
<td>SMA with pontocerebellar hypoplasia</td>
<td>Autosomal recessive</td>
<td>14q32</td>
<td>Vaccinia related kinase1 (VRK1) [3]**</td>
<td>Nonsense: R358X</td>
<td>Congenital or infantile onset. Diffuse weakness. Microcephaly and upper-limb ataxia. Arthrogryposis in severe cases.</td>
</tr>
<tr>
<td>Distal SMAs or hereditary motor neuropathies (HMNs)</td>
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<tr>
<td>HMN 2C</td>
<td>Autosomal dominant</td>
<td>5q11.2</td>
<td>HSPB3 (HSP27, protein 3) [9***]</td>
<td>Missense: R7S</td>
<td>Missense: F647S</td>
</tr>
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</table>
Other SMAs that present in infancy include infantile SMA with arthrogryposis (XL-SMA), SMA with pontocerebellar hypoplasia (SMA-PCH/PCH1), SMA with mitochondrial dysfunction, and SMA with respiratory distress 1 (SMARD1/HMN6). XL-SMA presents with proximal weakness and congenital joint contractures particularly of the hands [22–24]. SMA/PCH1 presents with diffuse weakness, microcephaly, and arm ataxia [3**]. SMA with mitochondrial dysfunction causes proximal weakness, cardiomyopathy, cytochrome c oxidase (COX) deficiency, and lactic acidosis [4,25]. SMARD1 causes predominantly distal limb and early diaphragm weakness [24].

Spinal and bulbar muscular atrophy (SBMA), also known as Kennedy’s disease, affects approximately 1 in 40,000 men. It is an adult-onset (beginning age 30–60 years), slowly progressive SMA inherited in an X-linked fashion [26]. It is caused by an expansion of a CAG trinucleotide repeat (coding for polyglutamine) in exon 1 of the androgen receptor (AR) gene [5]. CAG-repeats greater than 40 are associated with disease and larger repeat sizes are correlated with earlier disease onset and increased disease severity [27]. Proximal limb muscles are somewhat more affected than distal muscles and there is particular involvement of the tongue and lower facial muscles with distinct perioral muscle twitching (Fig. 1c and d). Some patients experience pharyngeal and respiratory muscle weakness late in the disease course. Patients may have signs of mild androgen insensitivity including gynecomastia, testicular atrophy, and erectile dysfunction (Fig. 1e) [26].

Distal SMAs or HMNs present with distal limb weakness. Many of these disorders are allelic with subtypes of Charcot Marie Tooth 2 (CMT2) disease (Table 1) with the principle distinction being mild to moderate sensory nerve involvement in CMT2. The autosomal dominant HMN types 2A–C are associated with missense mutations in members of the small heat-shock protein family, HSPB8, HSPB1, and HSPB3, respectively [7,8,9**]. They present with distal leg and subsequent distal arm weakness. In contrast, HMNSA presents with weakness beginning in the hand muscles, specifically the thenar and first dorsal interosseus muscles, with subsequent weakness of distal legs. Causative mutations are found in the gene encoding glycyl-tRNA synthase (GARS) [11]. HMN7B is caused by a G59S missense mutation in the DCTN1 gene and causes vocal fold paresis and hand atrophy, and subsequent distal leg weakness (Fig. 1h and i) [13].

Figure 1 (continued)
fold paresis. This causes a failure of vocal fold opening as seen in (g, left image) compared to an unaffected individual (g, right) which can cause stridor and airway obstruction. Patients with HMN7B present with early weakness and atrophy in the hands (h and i). SMBA, spinal and bulbar muscular atrophy.
Recently, mutations in the transient receptor potential cation channel, subfamily V, member 4 (TRPV4) have been associated with 3 different disease phenotypes: congenital distal SMA, scapuloperoneal SMA (SPSMA), and CMT2C [14**,16**,17**]. These diseases have an autosomal dominant pattern of inheritance and share the clinical features of distal limb and vocal fold weakness. SPSMA and CMT2C are phenotypically distinct, however, with SPSMA being characterized by scapuloperoneal distribution weakness and atrophy, whereas CMT2C is characterized by distal motor and sensory involvement, diaphragm, intercostal weakness, and hearing loss (Fig. 1f and g).

**Clinical diagnosis and management**

Nerve conduction studies and electromyography are indispensable tools for confirming neurogenic findings in a patient with suspected SMA. Motor nerve conduction studies may show reduced amplitudes and electromyography will show increased size of motor units consistent with chronic denervation. Muscle biopsy is no longer necessary in most SMA cases. Genetic testing is available for most of the genes listed in Table 1. Please consult GeneTests (http://www.ncbi.nlm.nih.gov/sites/GeneTests) for details.

Current disease management of the SMAs centers on supportive care. Patients with infantile and childhood-onset SMA can develop severe respiratory impairment and contractures including severe scoliosis. These patients may require respiratory support and spinal fusion surgeries. In HMN7B or SPSMA particular attention must be paid to vocal fold paresis, which causes stridor and eventually airway obstruction. Vocal fold tie back procedures or tracheostomy may be indicated. Patients with SBMA can develop pharyngeal weakness that can result in aspiration. Patients with distal SMAs may benefit from walking aids and/or ankle foot orthoses for distal weakness.

**Pathogenic mechanisms**

Patients with proximal SMA lack a functional SMN1 gene, but retain one or more copies of the SMN2 gene. Although SMN1 and SMN2 are highly related in sequence, SMN2 contains a translationally silent C→T transition located in exon 7 that alters an exonic splice enhancer motif. This motif normally recruits the SR protein, SF2/ASF, that directs exon 7 inclusion [28]. In SMN2-derived transcripts, this motif is disrupted and an exonic splicing silencer (ESS) motif is created favoring the recruitment of hnRNPA1 [29] and RNA-binding protein (RBP) Sam68 [30] resulting in frequent exon 7 exclusion. The resulting SMN transcripts lack exon 7 and code for SMN protein that is highly unstable and degraded by the ubiquitin–proteasome system (UPS) [31**]. A minority of SMN2-derived transcripts are spliced to include exon 7 which codes for a full-length SMN transcript and protein. SMA thus results from reduced expression levels of full-length SMN protein.

The SMN protein is widely expressed and together with the Gemins forms a complex that is important in the assembly, recycling, and maintenance of small nuclear ribonucleoproteins (snRNPs), which are components of the spliceosome [32]. Reduced SMN protein levels in cultured cells and SMA tissues has been associated with reduced fidelity of splice-site pairing causing a general splicing defect of multi-intron-containing genes [33,34]. These data suggest that SMA could directly result from abnormalities of RNA splicing. Nonetheless, it has also been suggested that SMN has alternative functions in motor neurons that might explain the particular susceptibility of this cell type to SMN protein deficiency. Axonal transport and translation of β-actin mRNA play an important role in axonal growth and presynaptic differentiation. SMN has been shown to traffic in motor nerve axons [35–37] and, together with binding partner hnRNP-R, to regulate β-actin mRNA translocation into the growth cone of cultured motor neurons [38]. Further, SMN-deficient motor neurons show shortened axons and small growth cones lacking β-actin mRNA [39]. Thus, SMN protein could play important roles in β-actin mRNA trafficking and local translation that are critical to normal motor neuron cytoskeletal integrity and synapse stability. Importantly, plastin 3 (PLS3), which codes for an actin modifying protein, has been recently identified as a genetic modifier of SMA in female patients [40].

**Spinal and bulbar muscular atrophy**

Spinal and bulbar muscular atrophy is caused by expansion of the polymorphic CAG repeat sequence in the AR gene [5]. This CAG repeat is translated into an expanded polyglutamine (poly Q) stretch in the N-terminal domain of the AR protein. AR is a ligand-activated transcription factor with a DNA binding domain and transactivation domain. When AR binds testosterone or dihydrotestosterone, it translocates to the nucleus. Ligand binding and nuclear translocation are critical steps in SBMA disease pathogenesis as absence of ligand prevents disease in females or castrated male mice [41]. Mutant AR forms nuclear inclusions of aggregated protein [42]. Transcriptional co-activators such as cAMP-response element binding protein-binding protein (CBP) have been shown to be sequestered in these nuclear inclusions, which may result in generalized transcriptional dysregulation [43]. In addition, specific transcriptional dysregulation of DCN7I coding for a microtubule motor protein (see also HMN7B below) was shown in a mouse model of SBMA, highlighting the potential importance of disrupted axonal transport [44].
A failure to clear misfolded, aggregated AR is central to SBMA disease pathogenesis. Recently AR has been shown to undergo SUMOylation, during which small ubiquitin-like modifier proteins are conjugated to the protein. This post-translational modification reduces aggregation of mutant AR and could serve as a future therapeutic target in SBMA [45]. Heat shock proteins (HSPs) assist in the refolding of misfolded proteins and maintain correct folding, assembly, and intracellular transport of newly synthesized proteins. Impaired HSP induction has also been implicated in SBMA pathogenesis [46].

**Distal spinal muscular atrophies**

Some distal SMAs are associated with mutations in genes implicated in RNA processing and protein synthesis. SMARD1 is caused by mutations in IGHMBP2 [47], which is an ATP-dependent 5′→3′ helicase involved in DNA replication, pre-mRNA splicing, and transcription. It co-localizes with the translation factor eIF4G2 and ribosomal RNA in the cytoplasm and regulates mRNA metabolism [48]. Mutations of GARS cause HMSN5A [11], whereas mutations in another tRNA synthetase, tryosyl-tRNA synthetase (YARS), cause dominant-intermediate CMT [49]. The mutant GlyRS enzyme retains aminoacylation activity, suggesting that the HMN phenotype is not caused by reduced canonical GlyRS activity or insufficient protein synthesis [50].

Other forms of distal SMA have been associated with genes encoding proteins involved in organelle movement in cells. The dynein/dynactin microtubule motor complex mediates retrograde axonal transport and the intracellular movement of vesicles and protein complexes. A G59S missense mutation in the p150Glu subunit of dynactin causes HMSN7B [51,52]. This mutation occurs in the glycine rich cytoskeleton-associated protein (CAP-Gly) domain, inhibiting dynactin microtubule binding. The mutation also causes misfolding and aggregation of the mutant protein [53]. Recently, other mutations in the CAP Gly domain of p150Glu-dynactin have been shown to cause Perry syndrome, a neurodegenerative disorder characterized by Parkinsonism [54]. Mutations of VAPB cause amyotrophic lateral sclerosis (ALS) and late-onset SMA [6]. VAPB participates in the vesicle-binding and exocytosis functions of the endoplasmic reticulum-Golgi apparatus. Mutant VAPB proteins form aberrant aggregates, cause Golgi dispersion and decreased endoplasmic reticulum anchoring of lipid-binding proteins [55].

Mutations in TRPV4 cause congenital distal SMA, SPSMA, and CMT2C. TRPV4 is a member of the TRP channel superfamily and is the first ion channel shown to cause hereditary peripheral nerve disease [14**–16**]. TRPV4 is a nonselective cation channel modestly permeable to Ca^{2+}. The disease-causing mutations are localized to the intracellular N-terminal domain of the channel within the ankyrin repeats domain, which mediates protein–protein interactions. In cultured cells, mutant TRPV4 channels show increased channel currents and calcium influx resulting in cellular toxicity. However, much work needs to be done to understand the biology of TRP channels in motor neurons because ion channels expressed at the cell surface membrane could be accessible therapeutic targets.

**Synaptic dysfunction in spinal muscular atrophies**

Although SMA disease-causing genes encode a range of proteins with diverse functions (Fig. 2), animal models of many of these disorders show early abnormalities of synaptic structure and transmission. In SMA mice, abnormalities of the neuromuscular junction (NMJ) may be evident prior to motor axonal degeneration or AHC death [56,57]. SMA NMJs demonstrate reduced synaptic vesicle release with evoked stimulation due to reduced synaptic vesicle density in presynaptic terminals [58]. In addition, anomalous augmentation of intraterminal Ca^{2+} levels during repetitive stimulation suggests altered homeostasis of Ca^{2+} levels in SMA NMJ synapses [59]. A mouse model of HMSN5A with G1AR mutation also shows abnormal morphology and impaired synaptic transmission at NMJ synapses [50] and a fly model of a VAPB mutation shows structural and functional alternations at the NMJ [60]. Further characterization of the nature and timing of these synaptic abnormalities is particularly relevant for therapeutics development as improving connected but dysfunctional motor units will be more feasible than rescuing degenerating and/or dead motor neurons.

**Therapeutic approaches to spinal muscular atrophy**

Different therapeutic strategies are currently being pursued for proximal SMA (Fig. 3). Recently, two gene therapy studies have shown remarkable results in SMA mice treated with SMN2 packaged in adeno-associated viral vectors. In one study, scAAV9 was injected intravascularly, with long term survival of SMA mice that usually die at 2 weeks [61**]. In the other, scAAV8 was injected directly to the CNS and extended survival to a median of 157 days [62**]. Another approach to SMA therapeutics is to increase expression of the SMN2 genes present in all patients. Histone deacetylase (HDAC) inhibitors and quinazoline compounds increase survival and motor function in SMA mouse models [63–66]. Promotion of exon 7 inclusion during splicing of SMN2-derived transcripts is another strategy for increasing full-length SMN protein expression. Intracerebroventricular (ICV) delivery of antisense oligonucleotides, which block an intronic splice suppressor element,
Several spinal muscular atrophy (SMA)-associated proteins participate in steps of RNA processing and protein translation: SMN regulates snRNP assembly, IQHMBP2 is an ATP-dependent 6’–3’ helicase, and GARS esterifies glycyl to its cognate tRNA. Others play roles in protein folding and degradation, including HSP22, HSP27, and UBE1. Some SMA-associated proteins are involved in key aspects of organelle movement. Dynactin p150Glued is a subunit of the dynein/dynactin complex that mediates retrograde axonal transport of vesicles and organelles, and VAPB mediates vesicle exocytosis from the ER-Golgi apparatus. Seipin, the protein product of BSCL2, localizes to the ER, whereas SCO2 localizes to mitochondria. Misfolding and aggregation of mutant protein with resulting cytotoxicity has been implicated in the pathogenesis of SMAs caused by mutant AR, p150Glued dynactin, seipin, HSP22, and VAPB. In contrast, deficient expression levels of the SMN protein result in loss of normal function causing abnormalities of RNA splicing and/or a failure of mRNA trafficking and local RNA processing in motor nerve axons. Mutations in TRPV4 likely cause an increase in normal ion channel function that could result in increased calcium influx and cytotoxicity. Despite the diversity of causative mutant proteins, an early, common pathological feature of many SMAs may be abnormalities of NMJ synapse structure and function including cytoskeletal disruption within the presynaptic terminal and reduced synaptic vesicle release. AChR, acetylcholine receptor; AR, androgen receptor; AZ, active zone; ER, endoplasmic reticulum; MT, mitochondria; NI, neuronal inclusion; NMJ, neuromuscular junction; SV, synaptic vesicles; UPS, ubiquitin–proteasome system.

Spinal and bulbar muscular atrophy therapy

Given the ligand-dependency of pathogenic AR accumulation and toxicity, antiandrogen therapies have been examined in SBMA mice and humans. The luteinizing hormone-releasing hormone analogue, leuprolin, prevents nuclear translocation of aberrant AR proteins, resulting in a significant improvement of the disease phenotype in SBMA mice [71]. Leuprolin was recently studied in a clinical trial in SBMA patients and did not show significant efficacy [72*]. HDAC inhibitors have been used to correct transcriptional dysregulation in SBMA. Oral administration of sodium butyrate ameliorates the symptomatic and histopathological phenotypes of a mouse model of SBMA through upregulation of histone acetylation in neural tissues [73]. Prevention of mutant AR protein aggregation has been another goal of SBMA therapeutics development. The Hsp90/Hsp70-based chaperone machinery regulates the activity and degradation of many signaling proteins. Hsp90 stabilizes client proteins, whereas Hsp70 interacts with chaperone dependent E3 ubiquitin ligases to promote protein degradation. Inhibition of Hsp90 with 17-allylamino geldanamycin (17-AAG) arrested neurodegeneration by activating the ubiquitin–proteasome system in SBMA mice [74]. On the other hand, inhibition of Hsp70 by methylene blue impaired polyglutamine protein degradation and enhanced ligand-dependent AR aggregation in vitro [75]. Overexpression of insulin-like growth factor 1 (IGF-1) in muscle of SBMA mice has been shown to promote AR phosphorylation, reduce mutant AR protein aggregation, and attenuate disease pathology [76*].

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**Conclusion**

In the last several years, many new genes have been identified that cause different forms of SMA. It is likely that several more genes will be identified in the coming years. Although the genes identified to date encode proteins with diverse, seemingly unrelated, functions they provide new opportunities to understand the biology of normal and diseased motor neurons and the possibility of new therapeutic targets. In those disorders for which the causative gene has been known for some years, such as SMA and SBMA, there has been remarkable progress in preclinical therapeutics development. Further work is needed to identify which of these potential therapies offers real promise for clinical efficacy.

**Acknowledgements**

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**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 542–543).

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31 Burnett BG, Munoz E, Tordon A, et al. Regulation of SMN protein stability. Mol Cell Biol 2009; 29:1107–1115. This study shows that the SMN protein is stabilized by oligomerization and degraded by the ubiquitin proteasome system.


33 Fox-Walsh KL, Hertel KJ. Splice-site pairing is an intrinsically high fidelity mechanism in SMN2 exon 7 splicing. Mol Biol Cell 2007; 18:761–770.


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Neuromuscular diseases: muscle


59 SMA mice show severe muscle weakness prior to loss of motor neurons. In this study, the authors show that there is synaptic dysfunction at the neuromuscular junction synapse that may lead to impaired development of the NMJ and muscle weakness.


61 This study also reports an impairment in SMA NMJ function as well as increased intraneuronal calcium concentrations that lead to an increase in asynchronous release of neurotransmitter in SMA mice.


63 In this study, treatment of SMA mice by intravenous injection with SMN packaged in self-complementary adeno-associated virus 9 resulted in marked behavioral improvement and long-term survival.

64 In a mouse model by early postnatal delivery of SMN. Nat Neurosci 2010; 13:465–481.

65 In this study, SMA mice were treated with primed neural stem cells derived from embryonic stem cells. Treatment resulted in improved survival, weight gain, and increased neuromuscular junction and muscle size.


67 In this study, the authors administered a single intracerebroventricular injection of a trans-splicing vector to SMA mice. This resulted in SMN2 exon 7 inclusion, improved SMN, and improved survival.


69 Leuprolin was shown to suppress the accumulation of mutant AR and slow disease progression modestly in a phase 2 clinical trial of SBMA patients.


72 In this study, IGF-1 overexpression in muscle was shown to reduce accumulation of mutant AR aggregates, decrease motor neuron loss, and significantly increase survival of SBMA mice.