Spinal Muscular Atrophy: Survival Motor Neuron Protein

MED-MI--00006-US 07/22

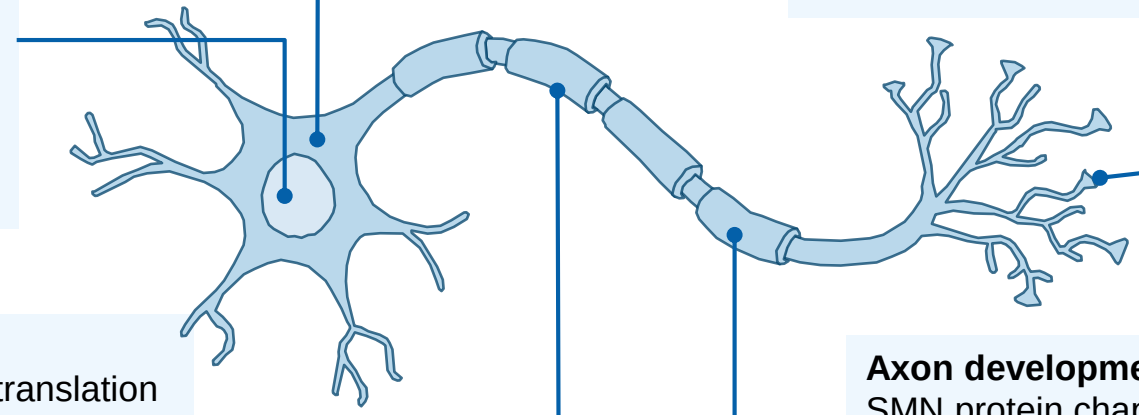
Bannockburn, IL 60015

SMN Protein in Healthy Neurons and Neuronal Development

SMN protein is ubiquitously expressed and has several functions in developing and mature neurons[1]; *SMN* expression is the highest during neuronal development,[2] and SMN protein localization switches from nuclear to cytoplasmic/axonal in mature neurons[3]

Nucleus
SMN protein is a subunit of the SMN complex, which facilitates spliceosome assembly by chaperoning snRNP biogenesis in the cytoplasm and subsequent trafficking to the nucleus[3]

Protein and mitochondrial homeostasis
SMN protein is linked to cellular homeostasis processes such as autophagy, ubiquitination, and mitochondrial bioenergetics[3]



Endocytosis and the NMJ
SMN protein is required for neurotransmitter release and maturation of the NMJ and the subsequent survival of motor neurons[4]

Protein translation
SMN protein regulates local translation of proteins via axonal mRNA transport, ribosomal association, and mTOR pathway regulation[3]

Axon development
SMN protein chaperones the binding of the RBP and mRNA transcripts at neurite growth cones, modulates cytoskeletal β-actin localization, and is required for correct neurite formation[3]

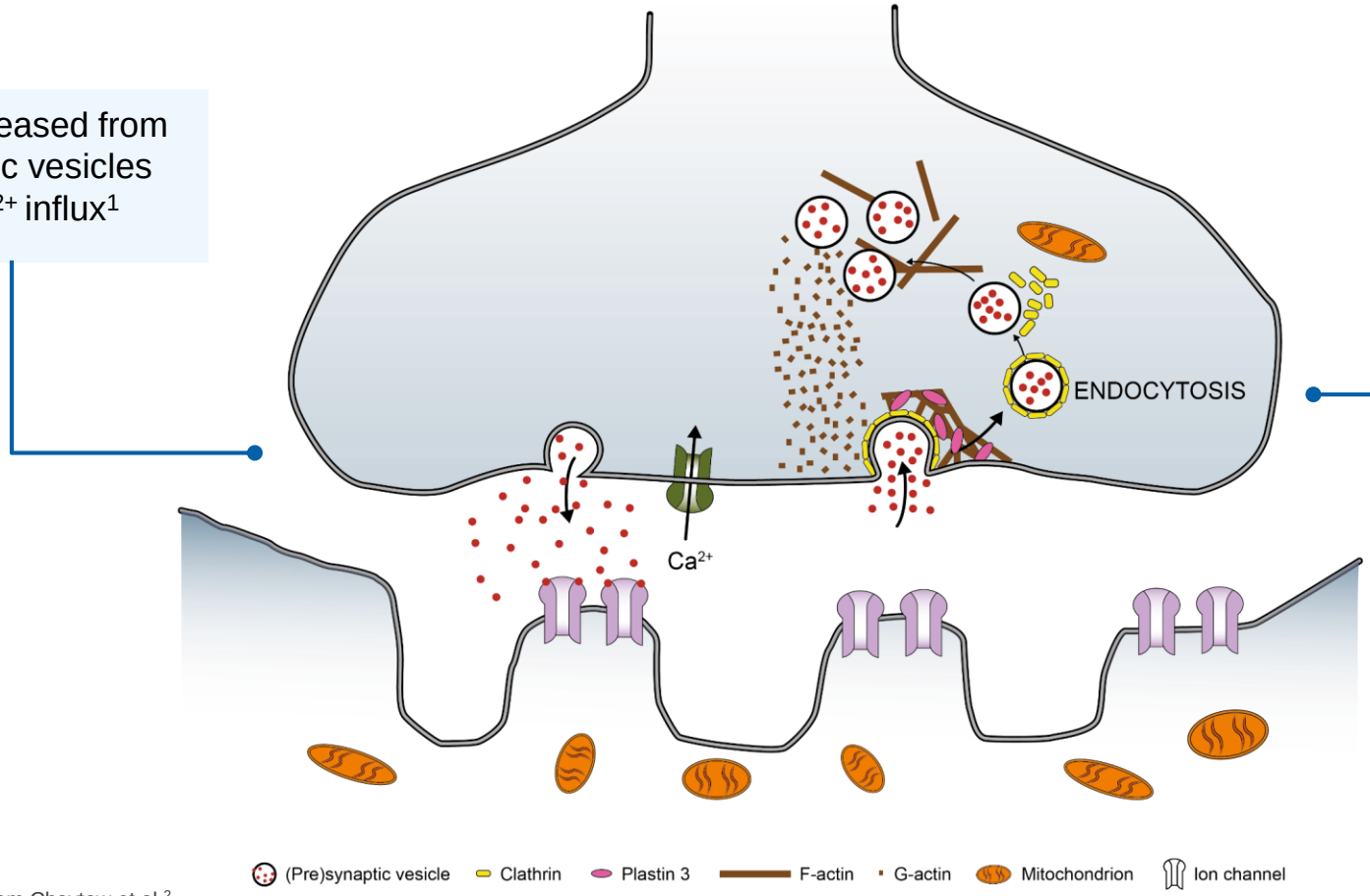
mRNA, messenger ribonucleic acid; mTOR, mammalian target of rapamycin; NMJ, neuromuscular junction; RBP, RNA binding protein; SMN, survival motor neuron; snRNP, small nuclear ribonucleoproteins.

1. Bowerman M et al. *Dis Model Mech.* 2017;10(8):943-954. **2.** Ramos DM et al. *J Clin Invest*. 2019;129(11):4817-4831.
3. Chaytow H et al. *Cell Mol Life Sci.* 2018;75(21):3877-3894. **4.** Vrbová G, Sławińska U. *Neuromuscul Disord*. 2018;28(5):385-393.

Healthy NMJ Signal Transmission

1. ACh released from presynaptic vesicles due to Ca^{2+} influx[1]

2. Presynaptic vesicles recycled by endocytosis via the actin cytoskeleton[2]



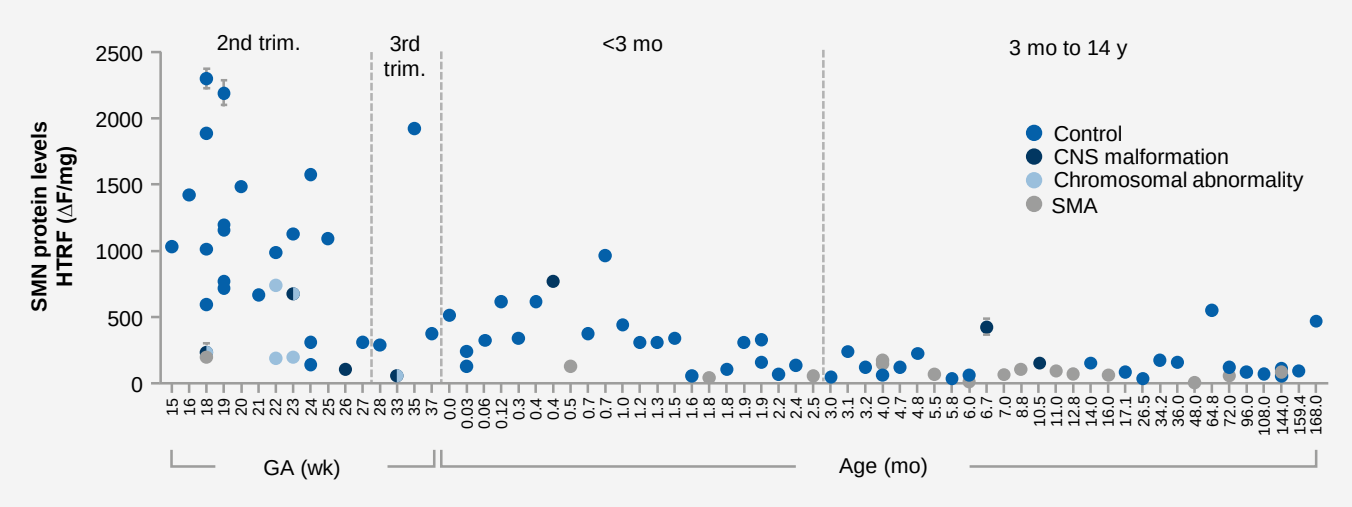
Adapted with permission from Chaytow et al.[2]

ACh, acetylcholine; NMJ, neuromuscular junction.

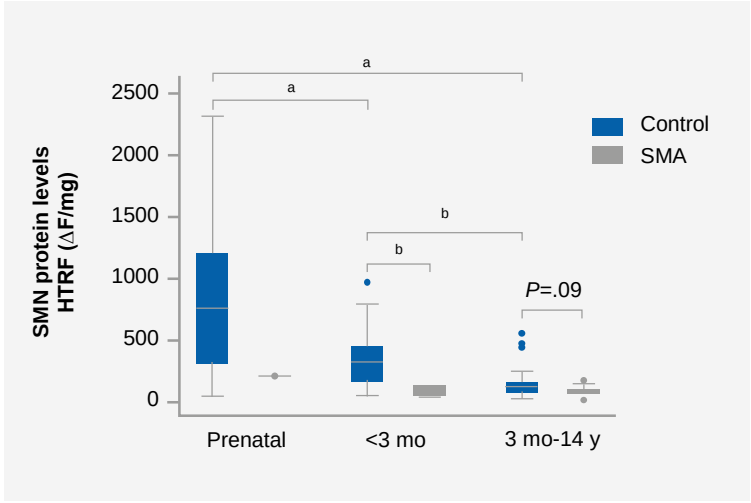
1. Vrbová G, Sławińska U. *Neuromuscul Disord*. 2018;28(5):385-393. **2.** Chaytow H et al. *Cell Mol Life Sci.* 2018;75(21):3877-3894.

SMN Protein Levels During Neuronal Development

Elevated SMN protein levels during early development suggest that SMN protein is particularly important in the CNS during the gestational and neonatal stages of motor neuron development



Full-length SMN protein expression in the spinal cord decreases by ~6.5-fold between fetal and postnatal stages as tissues mature



The greatest differences in SMN protein levels between those with and without SMA occur during the third trimester and 3 months postnatally

Adapted with permission from Ramos et al.

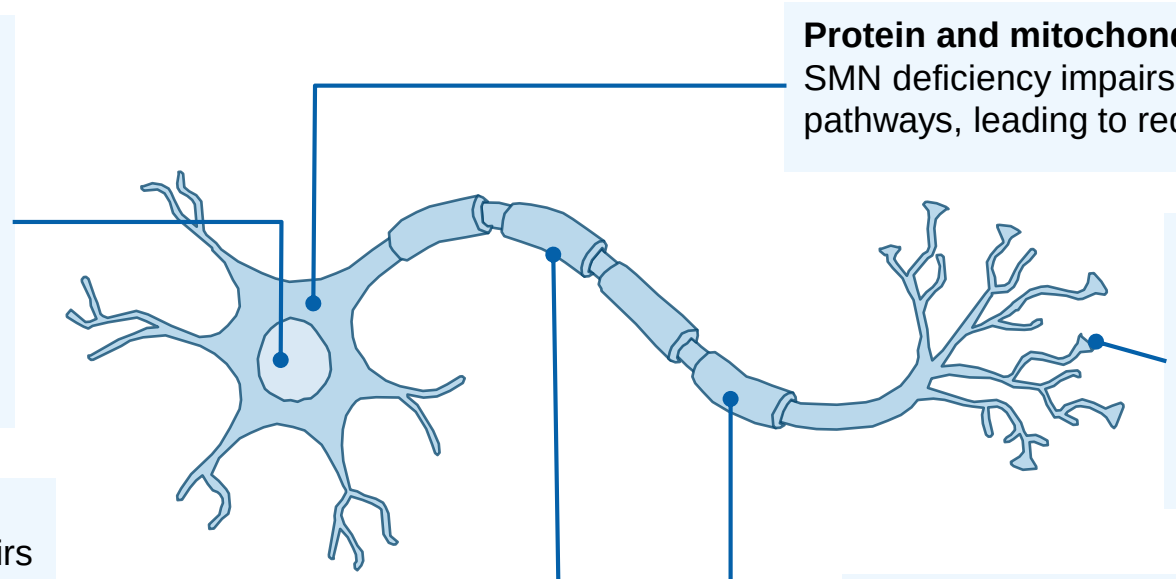
F, fluorescence intensity; CNS, central nervous system; GA, gestational age; HTRF, homogenous time-resolved fluorescence; SMA, spinal muscular atrophy; SMN, survival motor neuron.
[a]$P < 0.001$. [b]$P < 0.05$.
Ramos DM et al. *J Clin Invest*. 2019;129(11):4817-4831.

Effects of Insufficient SMN Protein in Neurons and Neuronal Development

Insufficient levels of SMN protein affect multiple cellular pathways, none of which solely underlie SMA pathophysiology[1]

Nucleus
- Reduction in snRNP biogenesis correlates with the clinical severity of SMA[2]
- Loss of SMN-dependent regulation of alternative splicing can lead to motor neuron death[3]

Protein translation
SMN protein deficiency impairs local axonal protein translation and ribosomal biogenesis[2]



Protein and mitochondrial homeostasis
SMN deficiency impairs mitochondria and bioenergetic pathways, leading to reduced ATP levels[2]

Endocytosis and the NMJ
SMN depletion impairs the recycling and release of the synaptic vesicle at the NMJ, which is required for motor neuron development in the early postnatal period[2,4]

Axon development
SMN protein deficiency results in neurite extension deficits and reduced axonal growth cone size[2]

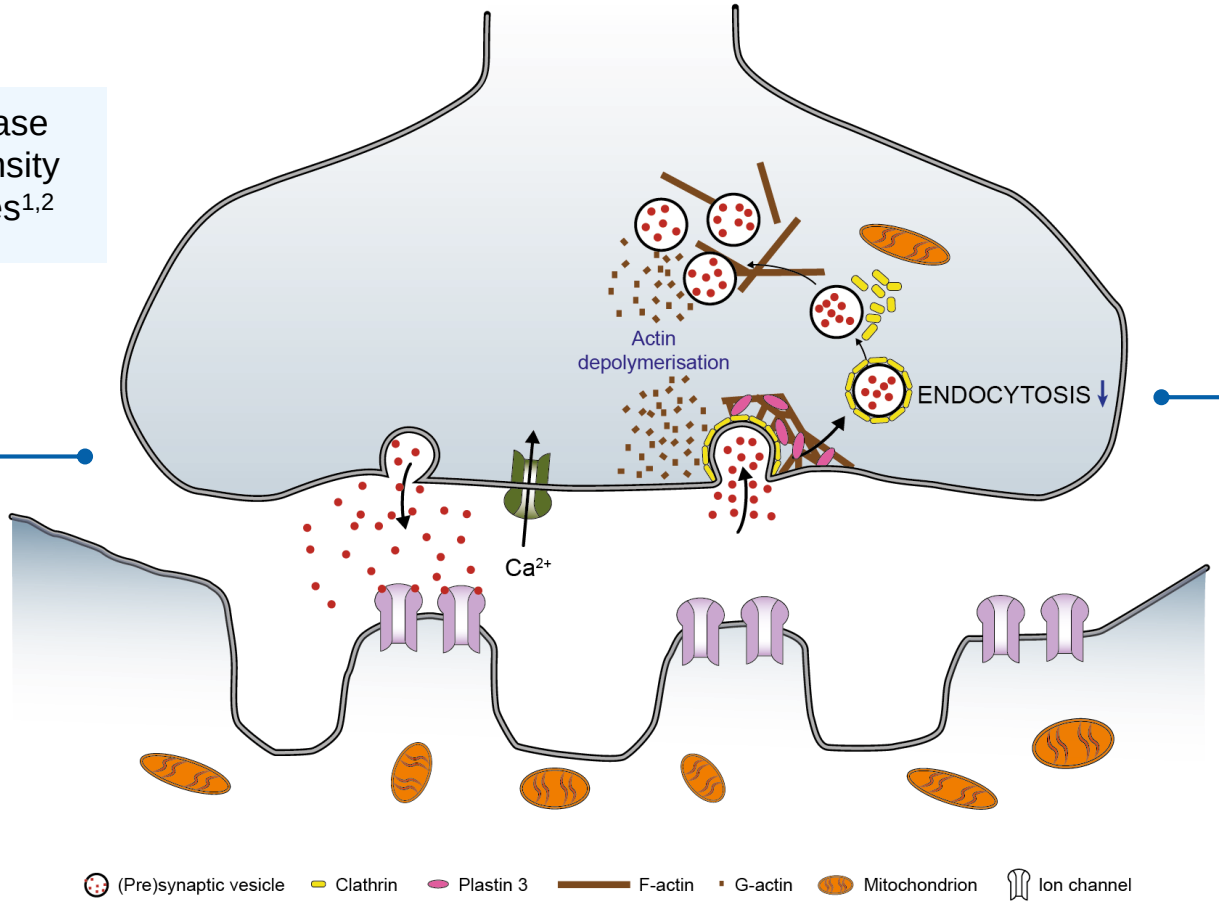
ATP, adenosine triphosphate; NMJ, neuromuscular junction; SMA, spinal muscular atrophy; SMN, survival motor neuron; snRNP, small nuclear ribonucleoproteins.

1. Bowerman M et al. *Dis Model Mech.* 2017;10(8):943-954. **2.** Chaytow H et al. *Cell Mol Life Sci.* 2018;75(21):3877-3894. **3.** Van Alstyne M et al. *Genes Dev*. 2018;32(15-16):1045-1059. **4.** Kong L et al. *J Neurosci.* 2009;29(3):842-851.

SMN-Deficient NMJ Signal Transmission

1. Reduced ACh release due to decreased density of presynaptic vesicles[1,2]

2. Reduced endocytosis and recycling of presynaptic vesicles, likely owing to disruption of actin cytoskeleton[3]



Disruption to the NMJ and decreased NMJ activity[3]

Adapted with permission from Chaytow et al.[3]

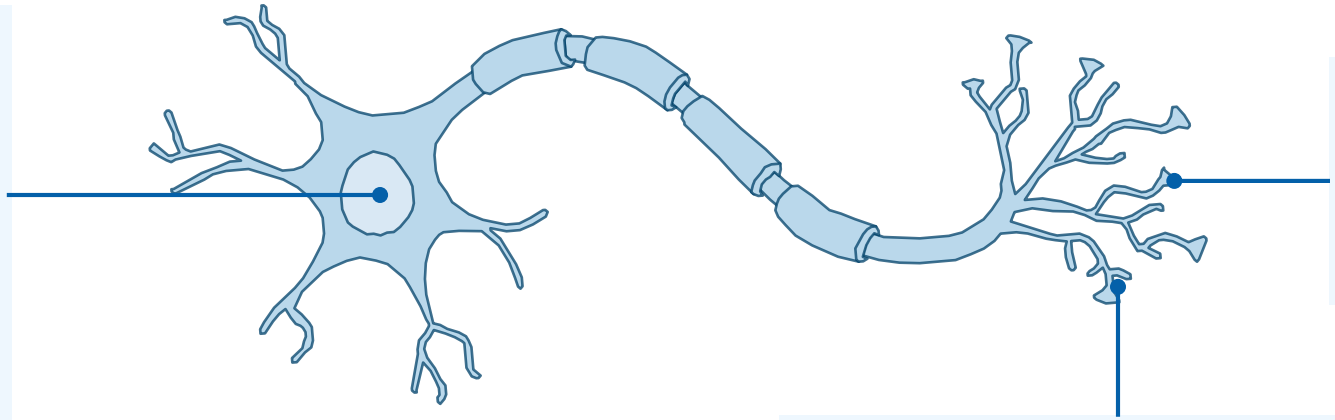
ACh, acetylcholine; NMJ, neuromuscular junction; SMN, survival motor neuron.

1. Vrbová G, Sławińska U. *Neuromuscul Disord*. 2018;28(5):385-393. **2.** Kong L et al. *J Neurosci.* 2009;29(3):842-851. **3.** Chaytow H et al. *Cell Mol Life Sci.* 2018;75(21):3877-3894.

Intermediate SMN Deficiency Results in Distinct Pathology

Intermediate loss of SMN protein is associated with a distinct pathology, potentially due to compensatory mechanisms and differences in SMN's role beyond neuronal development

- SMN protein localization shifts from predominantly nuclear to predominantly cytoplasmic and axonal in mature neurons[1]
- In $SMN^{+/-}$ mice, cytoplasmic SMN appears diminished, whereas SMN-dependent splicing is not affected[2]



NMJ
NMJ pathology differs between severe and intermediate SMA mouse models[4]

Axonal function
$SMN^{+/-}$ mice display compensatory CNTF-dependent axonal sprouting and a 2-fold increase in the amplitude of single motor unit action potentials[5]

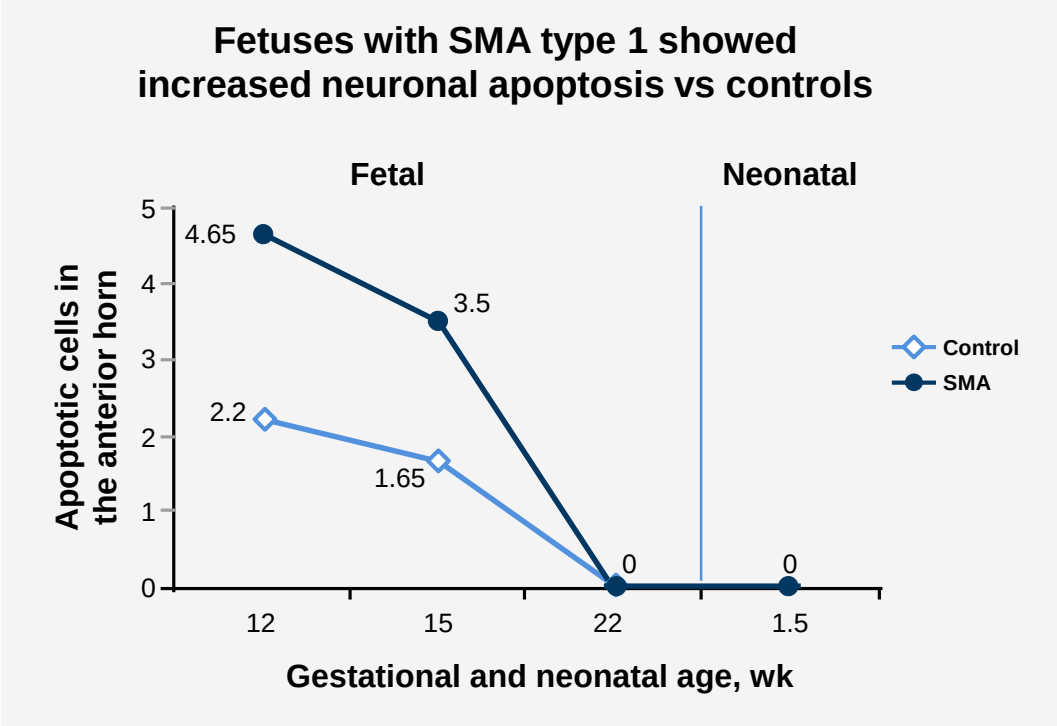
- In mouse models of SMA, a loss of ~85% of SMN protein is needed before an SMA phenotype is evident[3]
- However, a reduction of SMN protein below 50% of healthy levels is associated with later-onset motor neuron loss[2,4,5] and may serve as a model for SMA type 2 or 3

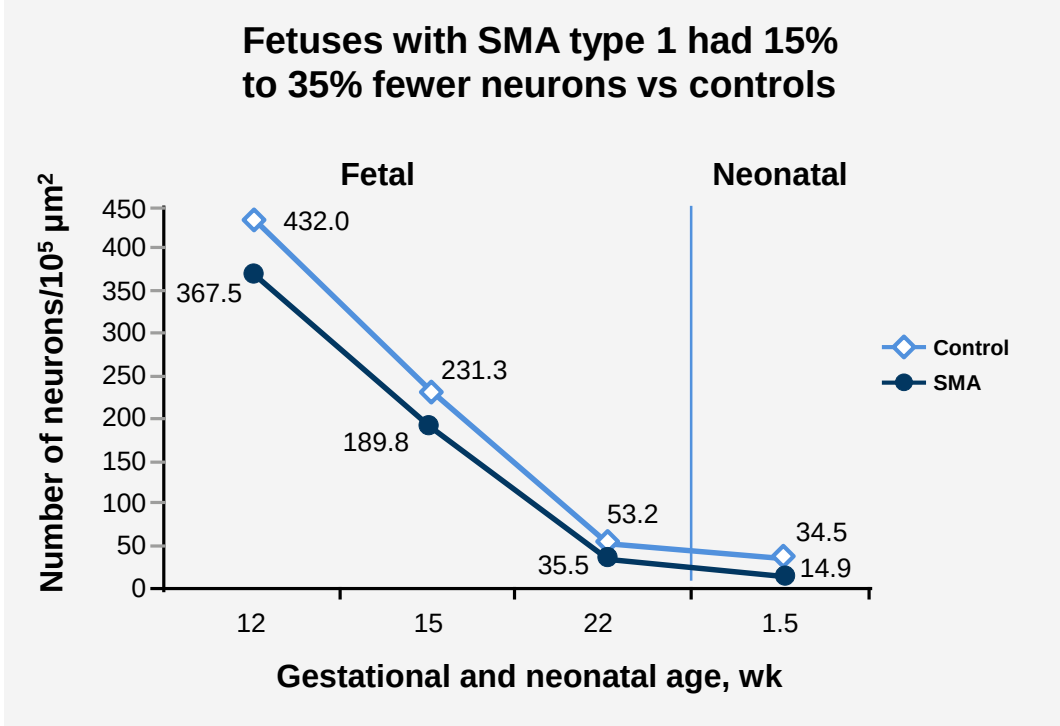
CNTF, ciliary neurotrophic factor; NMJ, neuromuscular junction; SMA, spinal muscular atrophy; SMN, survival motor neuron.

1. Chaytow H et al. *Cell Mol Life Sci.* 2018;75(21):3877-3894. **2.** Jablonka S et al. *Hum Mol Genet.* 2000;9(3):341-346. **3.** Bowerman M et al. *Dis Model Mech.* 2017;10(8):943-954. **4.** Bowerman M et al. *Neuromuscul Disord.* 2012;22(3):263-276. **5.** Simon CM et al. *Hum Mol Genet.* 2010;19(6):973-986.

Fetal Motor Neuron Loss

Diminished SMN protein production in patients with SMA impacts neurons early in gestation





Adapted with permission from Soler-Botija et al.

SMA, spinal muscular atrophy; SMN, survival motor neuron.
Soler-Botija C et al. *Brain.* 2002;125(Pt 7):1624-1634.