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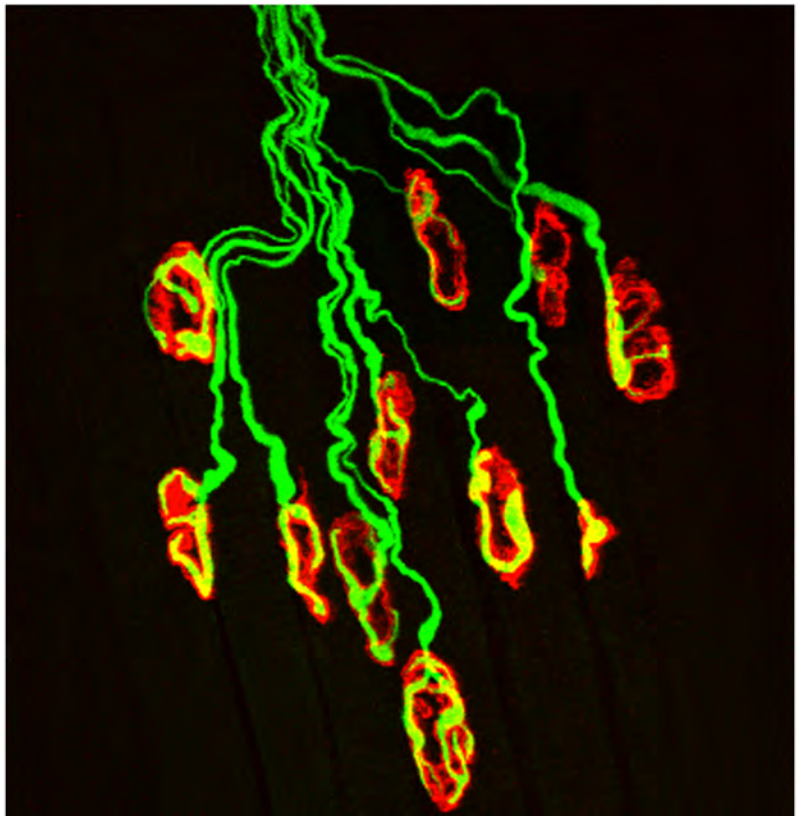

Systemic pathology in spinal muscular atrophy (SMA)

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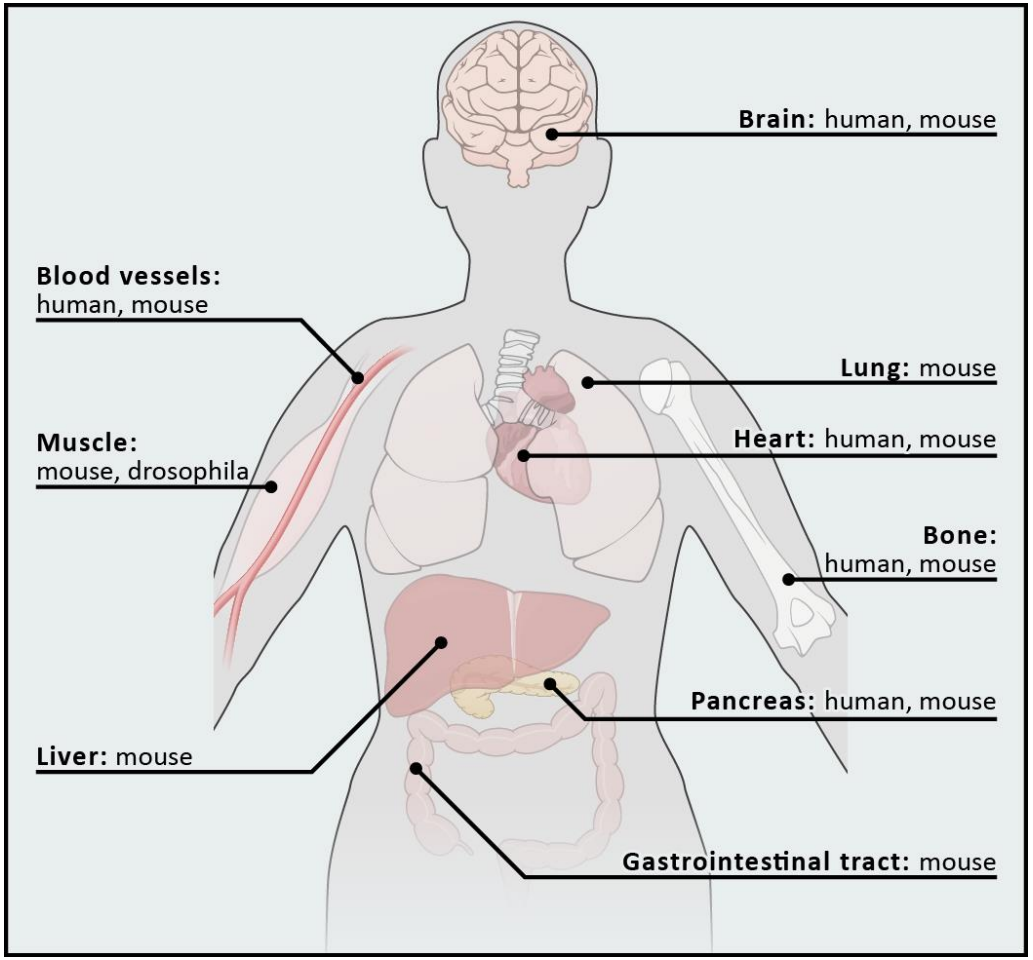
SMA as a multi-system disorder



LETTER

doi:10.1038/nature10485

Peripheral SMN restoration is essential for long-term rescue of a severe spinal muscular atrophy mouse model

Yimin Hua[1], Kentaro Sahashi[1], Frank Rigo[2], Gene Hung[2], Guy Horev[1], C. Frank Bennett[2] & Adrian R. Krainer[1]

Spinal muscular atrophy (SMA) is a motor neuron disease and the leading genetic cause of infant mortality; it results from loss-of-function mutations in the survival motor neuron 1 (*SMN1*) gene[1]. Humans have a paralogue, *SMN2*, whose exon 7 is predominantly skipped[2], but the limited amount of functional, full-length SMN protein expressed from *SMN2* cannot fully compensate for a lack of *SMN1*. SMN is important for the biogenesis of spliceosomal small nuclear ribonucleoprotein particles[3], but downstream splicing targets involved in pathogenesis remain elusive. There is no effective SMA treatment, but SMN restoration in spinal cord motor neurons is thought to be necessary and sufficient[4]. Non-central nervous system (CNS) pathologies, including cardiovascular defects, were recently reported in severe SMA mouse models and patients[5–8], reflecting autonomic dysfunction or direct effects in cardiac tissues. Here we compared systemic versus CNS restoration of SMN in a severe mouse model[9,10]. We used an antisense oligonucleotide (ASO), ASO-10-27, that effectively corrects *SMN2* splicing and restores SMN expression in motor neurons after intracerebroventricular injection[11,12]. Systemic administration of ASO-10-27 to neonates robustly rescued severe SMA mice, much more effectively than intracerebroventricular administration; subcutaneous injections extended the median lifespan by 25 fold. Furthermore, neonatal SMA mice had decreased hepatic *Igfals* expression, leading to a pronounced reduction in circulating insulin-like growth factor 1 (IGF1), and ASO-10-27 treatment restored IGF1 to normal levels. These results suggest that the liver is important in SMA pathogenesis, underscoring the importance of SMN in peripheral tissues, and demonstrate the efficacy of a promising drug candidate.

To compare the effectiveness of ASO-10-27 delivered centrally versus systemically, we administered an intracerebroventricular (ICV) injection of 20 μg ASO-10-27 on postnatal day 1 (P1) to increase SMN expression in CNS tissues, or we administered a subcutaneous (SC) injection of the ASO on two separate days at 50 μg per g of body weight (μg g^{-1}), between P0 and P3 (two doses). These doses were based on our previous studies with this ASO[11,12]. We also evaluated combined ICV and SC injections, as well as repeated SC injections (Supplementary Table 1). Control heterozygous mice ($Smn^{+/-}$ $SMN2^{+/0}$) that received ICV and/or SC ASO-10-27 injections had normal survival and behaviour. Severe SMA mice ($Smn^{-/-}$ $SMN2^{+/0}$) that received ICV and/or SC saline injections survived for 1–2 weeks, with a median survival time of ~10 days, similar to untreated mice (Fig. 1a, Supplementary Figs 1a and 2a, and Supplementary Movie 1). Delivery of the ASO only into the CNS efficiently corrected *SMN2* exon 7 splicing in the spinal cord and led to a striking increase in SMN protein levels, but modestly extended the median survival to 16 days, with a single pup surviving for 1 month (Fig. 1a–c and Supplementary Fig. 2b–d). In marked contrast, systemic treatment with two SC injections resulted in a median survival of 108 days (Fig. 1d). Combining ICV and SC injections of the ASO further increased the median survival to 173 days, and two additional SC injections on P5 and P7, after the initial SC injections at P0–P3, extended the median survival to 137 days (Fig. 1d).

Treated SMA mice varied in size from runts to comparable to their heterozygous littermates; their average weight was low, and their tails were much shorter than normal (Supplementary Figs 3 and 4). The surviving runts slowly gained weight, reaching ~18 g at ~3 months.

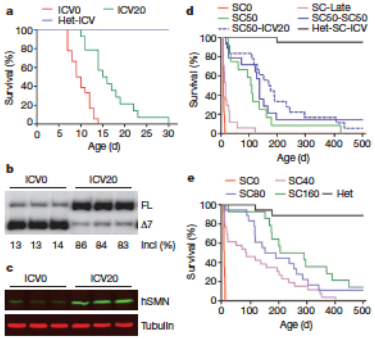


Figure 1 | Systemic versus ICV ASO-10-27 injections in SMA mice. **a**, Survival curves for mice after ICV administration of ASO-10-27 on P1. Administration of 20 μg ASO-10-27 (ICV20, $n = 14$) or saline (ICV0, $n = 18$) resulted in mean survival times of 17 and 10 days (d), respectively ($P < 0.001$). ASO-10-27-treated heterozygotes (Het-ICV, $n = 15$) served as controls. **b**, **c**, Spinal cord RNA and protein samples ($n = 3$) were analysed on P7 by using radioactive RT–PCR (**b**) or immunoblotting with a monoclonal antibody specific for human SMN (hSMN) (**c**). Δ7, exon-7-skipped mRNA; FL, full-length mRNA; in d, exon 7 inclusion; % in d = 100 × Δ7/(FL + Δ7). **d**, Survival curves after SC administration of saline (SC0, $n = 26$) or ASO-10-27 (SC50, $n = 12$) twice between P0 and P3. SC50-SC50 ($n = 14$) mice received two additional SC injections on P5 and P7. Het-SC-ICV ($n = 13$) and SC50-ICV20 ($n = 18$) were heterozygous and SMA mice, respectively, that received combined P1 ICV and P0–P3 SC injections. SC-Late ($n = 17$) were SMA mice that received only two SC injections, on P5 and P7. Each SC injection dose was 50 μg g^{-1} body weight. $P < 0.0001$ for all groups versus SC0 except for SC-Late, $P < 0.05$. **e**, Dose-dependent survival after two SC injections at P0–P3 with 40 (SC40, $n = 26$), 80 (SC80, $n = 18$) or 160 (SC160, $n = 14$) μg g^{-1} of ASO-10-27. Saline-treated SMA (SC0, $n = 23$) or heterozygous mice (Het, $n = 18$) served as controls. $P < 0.0001$ for all groups versus SC0.

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Hamilton & Gillingwater 2013. Trends Mol Med

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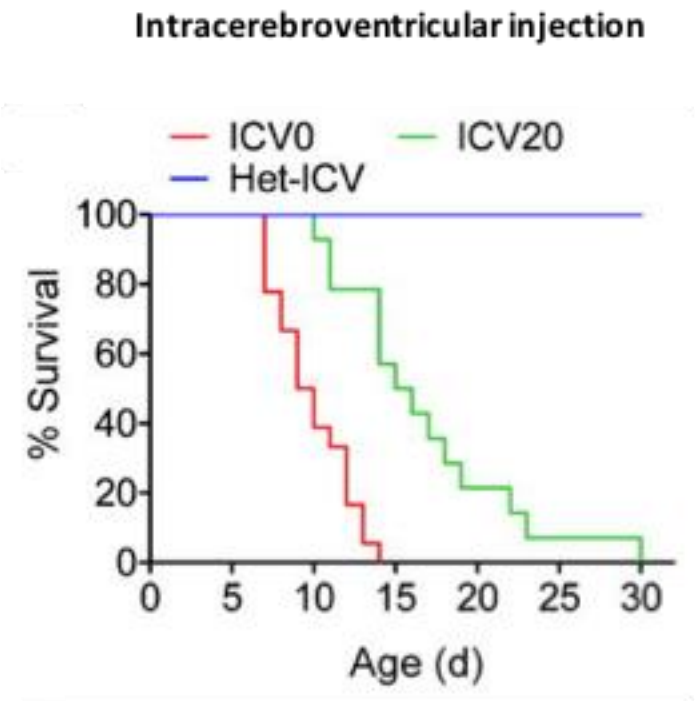
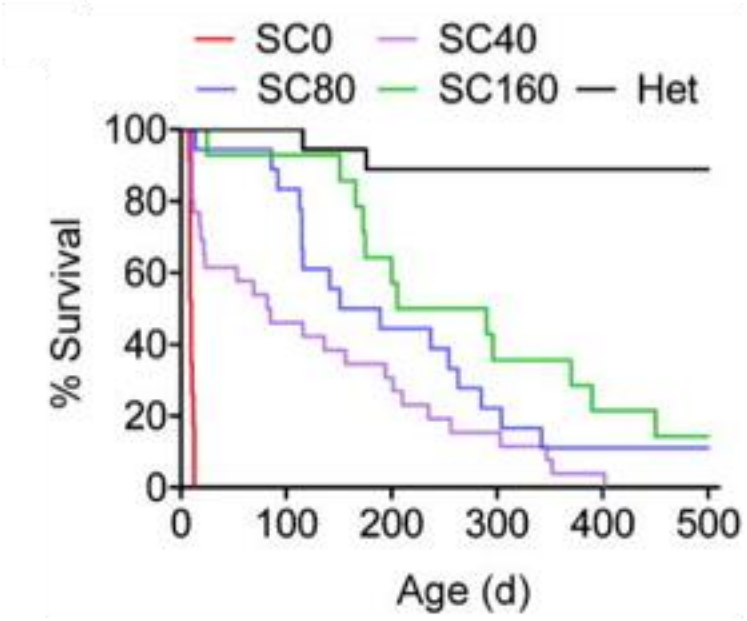
SMA as a multi-system disorder

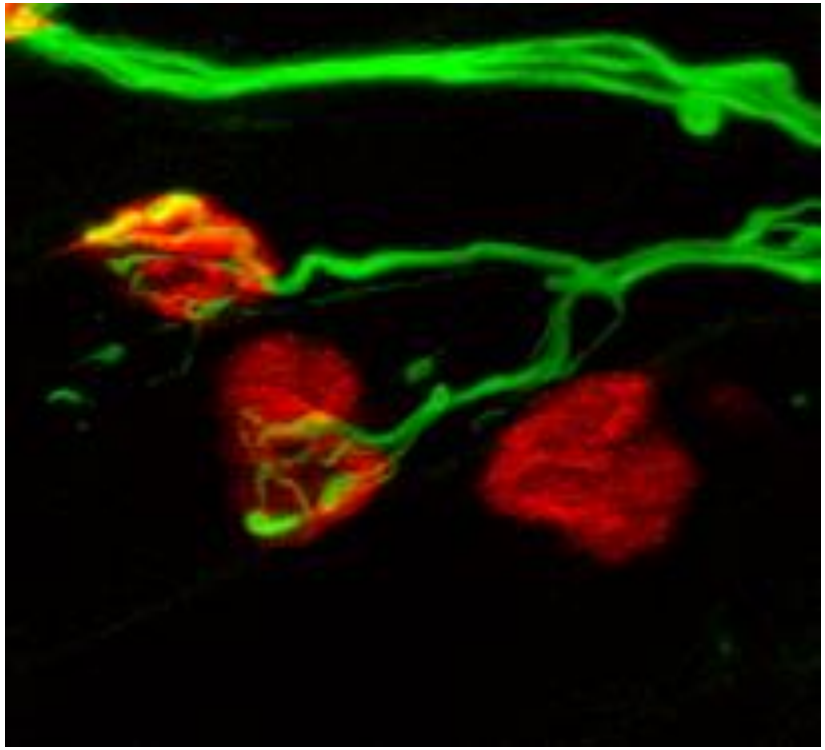


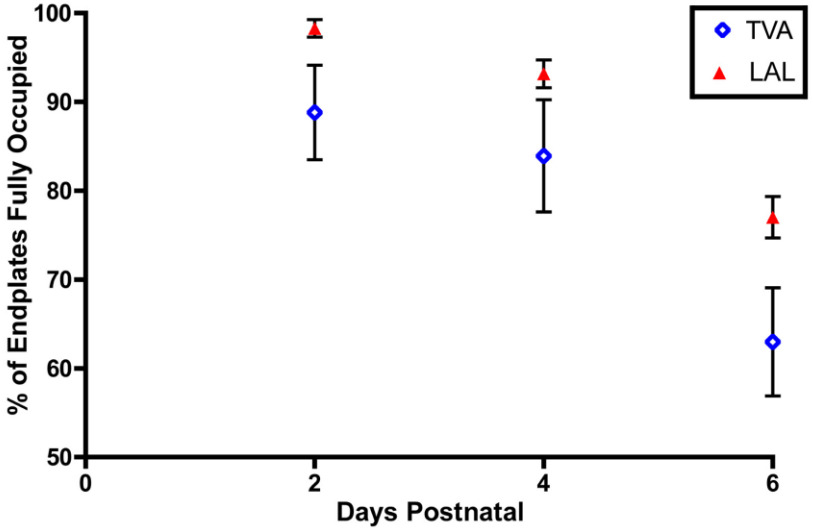


Subcutaneous injection increased median survival from 17 to 100+ days compared to ICV injection

Hua et al 2011. Nature

NMJ pathology in SMA mice





Murray et al 2008. Hum Mol Genet

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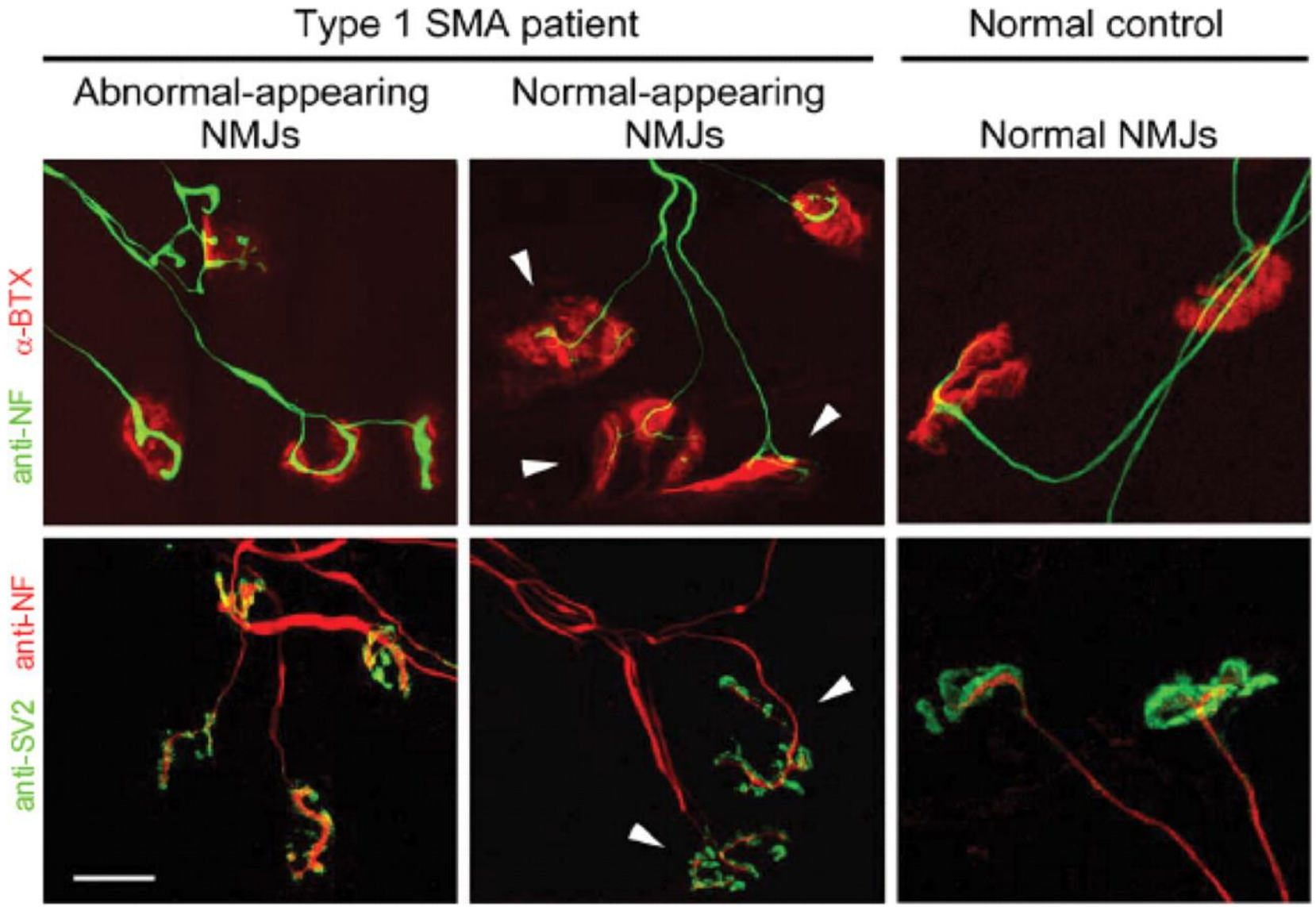
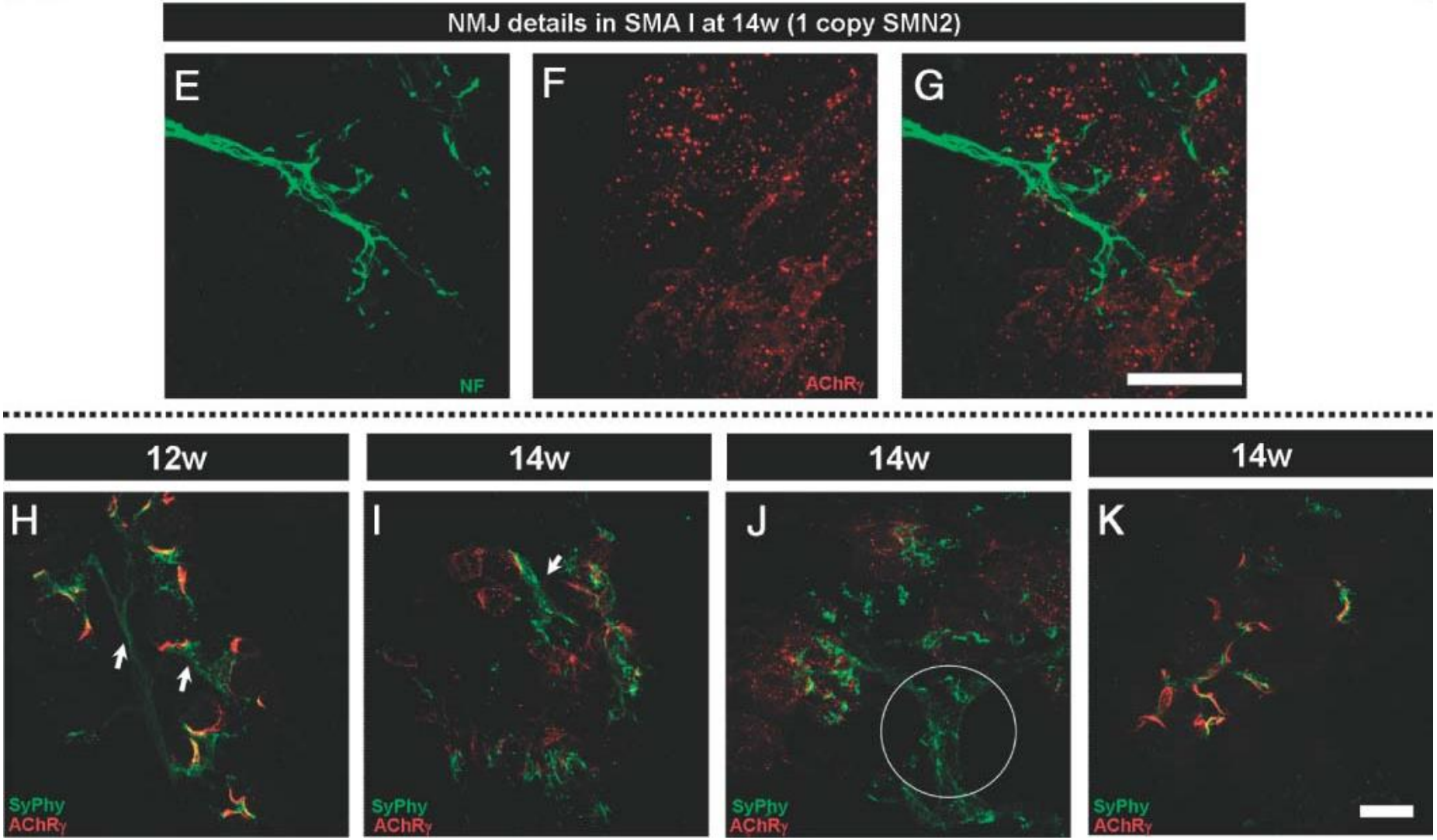
NMJ pathology in SMA patients



Kariya et al 2008. Hum Mol Genet

Pre-natal NMJ pathology in SMA patients



Martínez-Hernández et al 2013. J Pathol

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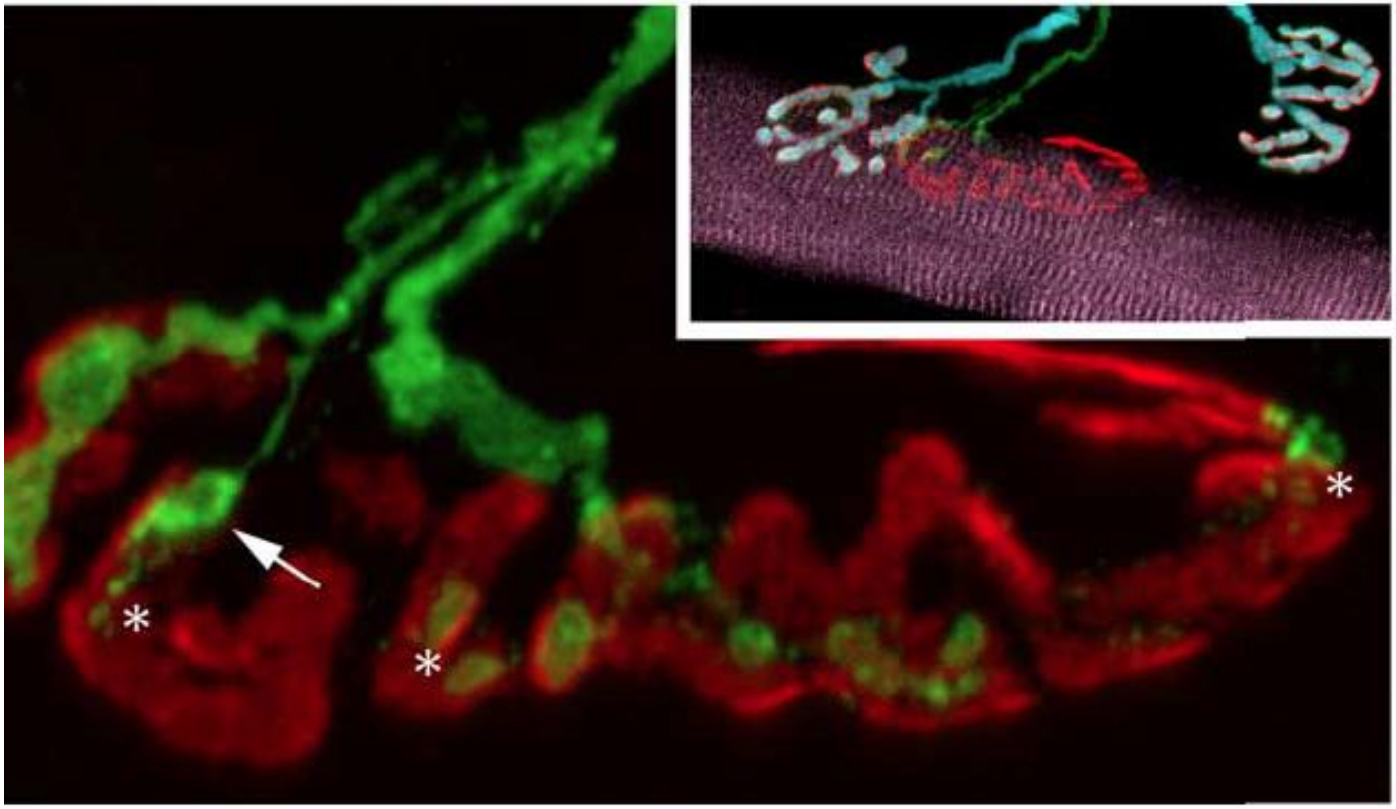
Could muscle defects cause NMJ pathology in SMA?

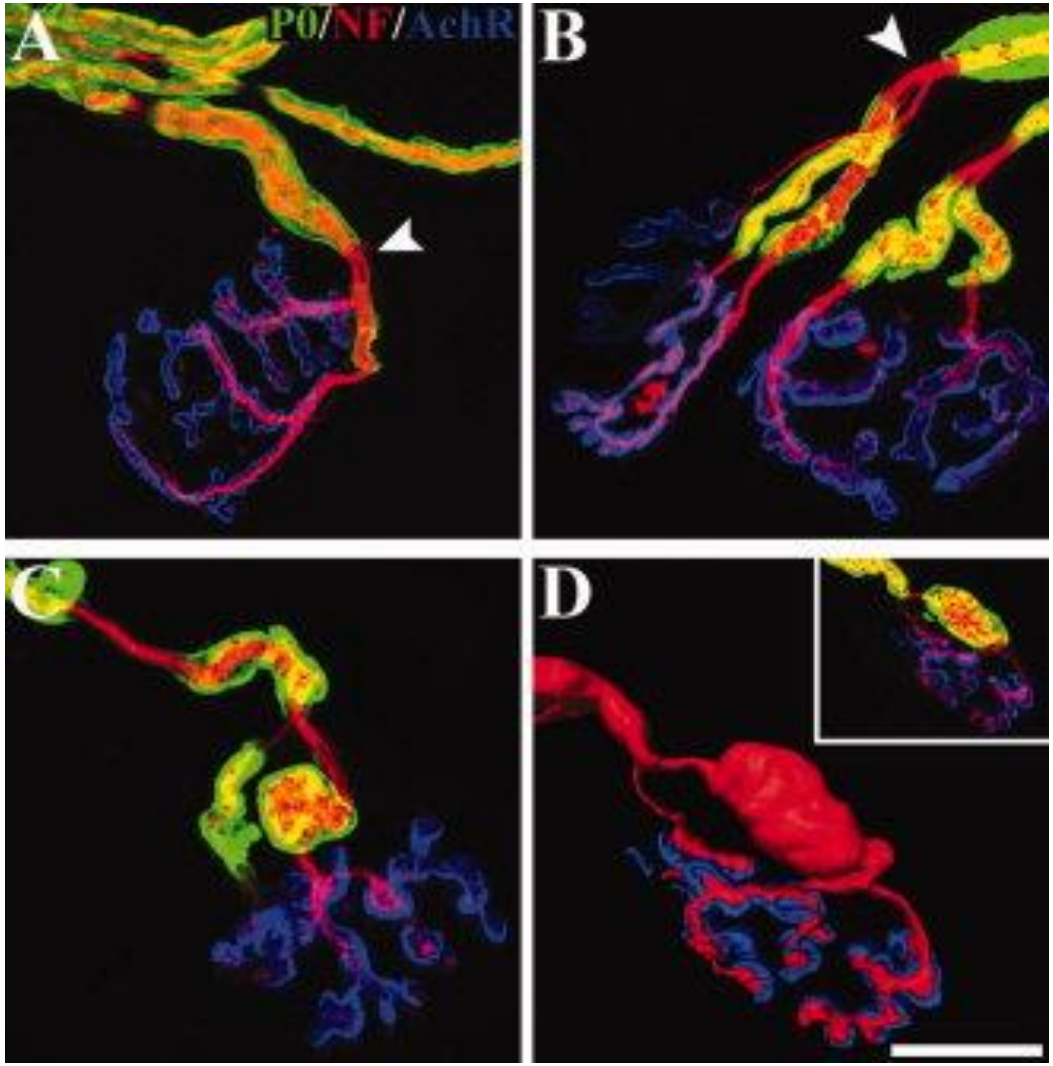


McCann et al 2007. J Neurosci

Could glial defects cause NMJ pathology in SMA?

WT mouse

Prx-/- mouse

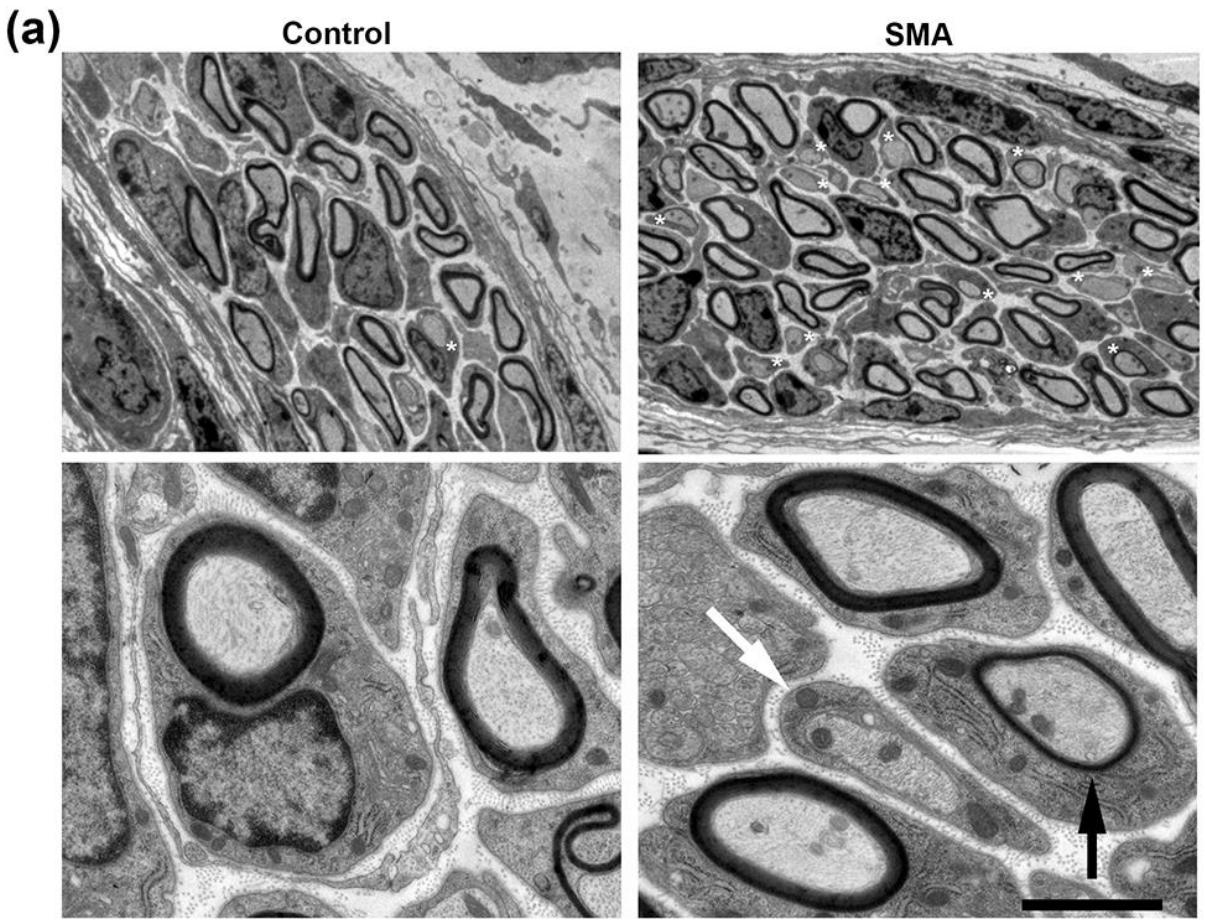


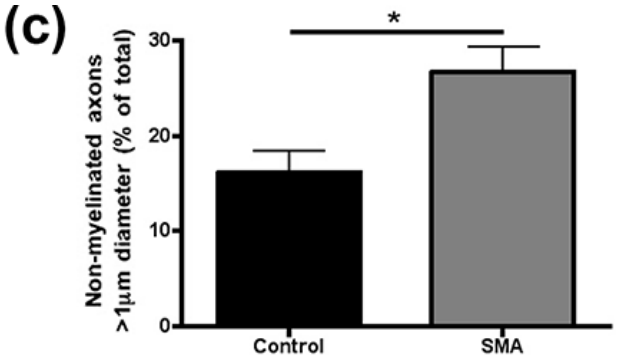
Prx-/- mouse

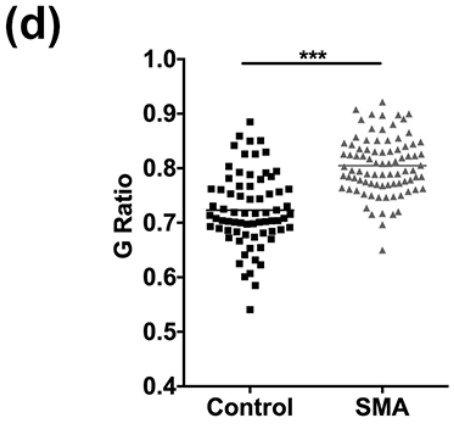
Prx-/- mouse

Court et al 2008. Glia

Schwann cell pathology in SMA mice







Hunter et al 2014. Hum Mol Genet

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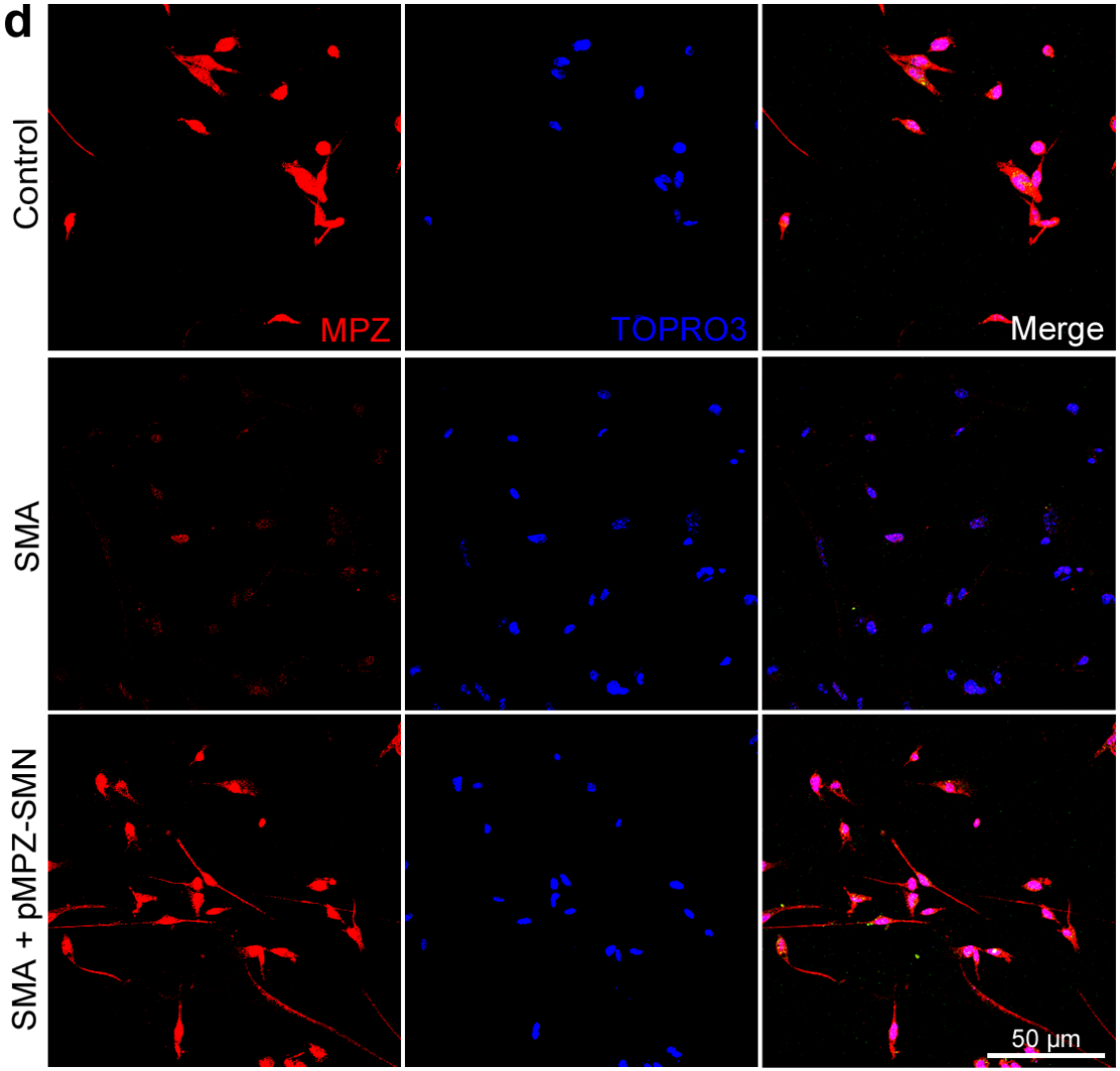
Schwann cell pathology in SMA patients

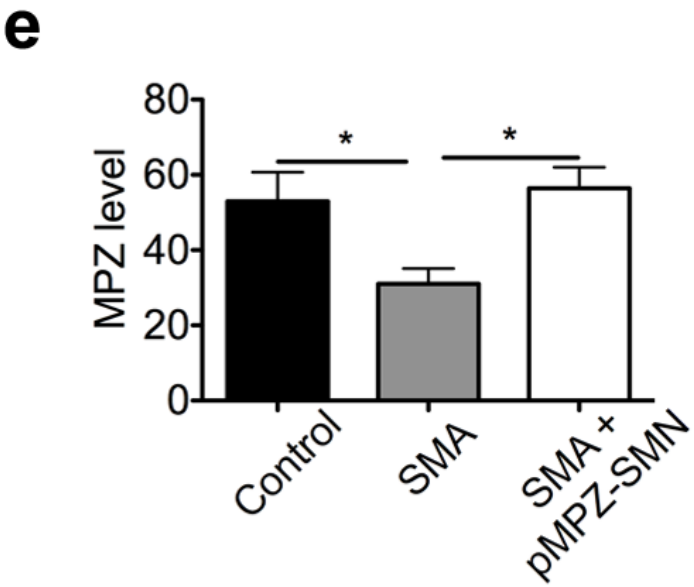
- MN loss correlates with a reduction in the number and diameter of large myelinated motor axons

- Increase in the proportion of small, immature, unmyelinated motor axons suggests an impairment of axon development in Type I SMA patients

Unpublished data courtesy of Charlotte Sumner (Johns Hopkins)

Reversible/SMN-dependent Schwann cell pathology





Hunter et al 2014. Hum Mol Genet

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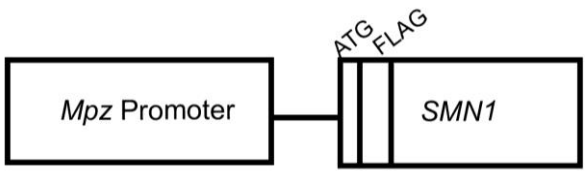
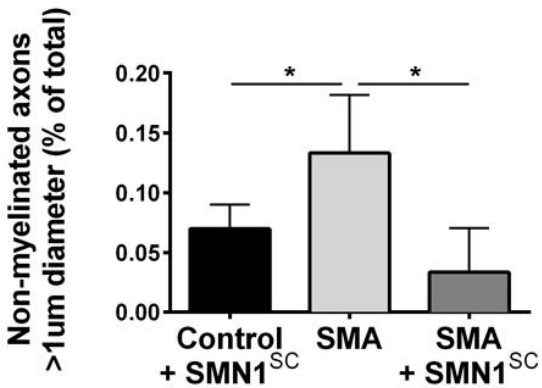
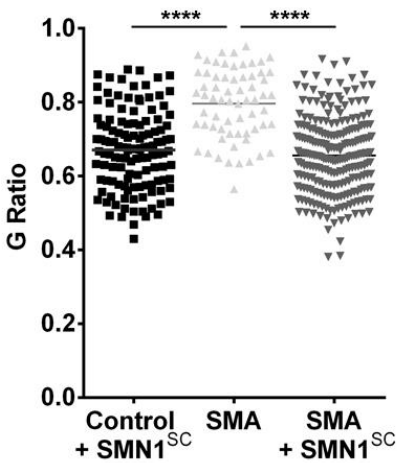
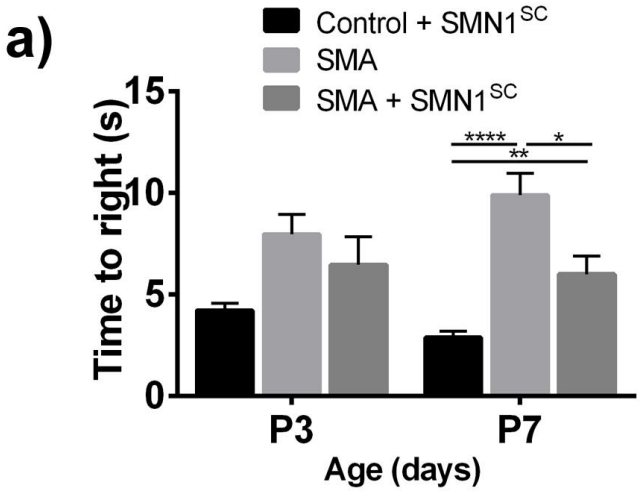
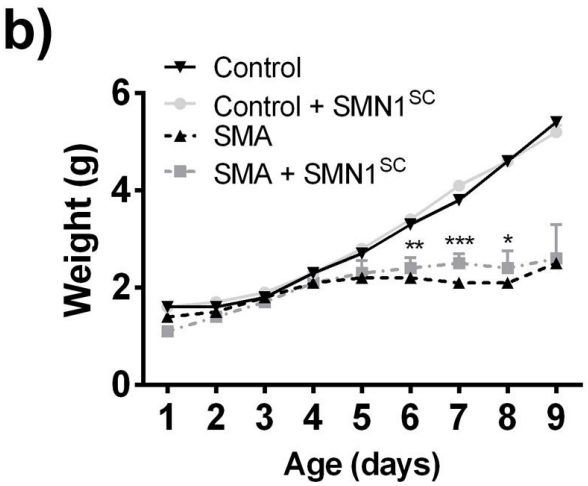
Restoring SMN in Schwann cells in vivo











Hunter et al 2016. Hum Mol Genet

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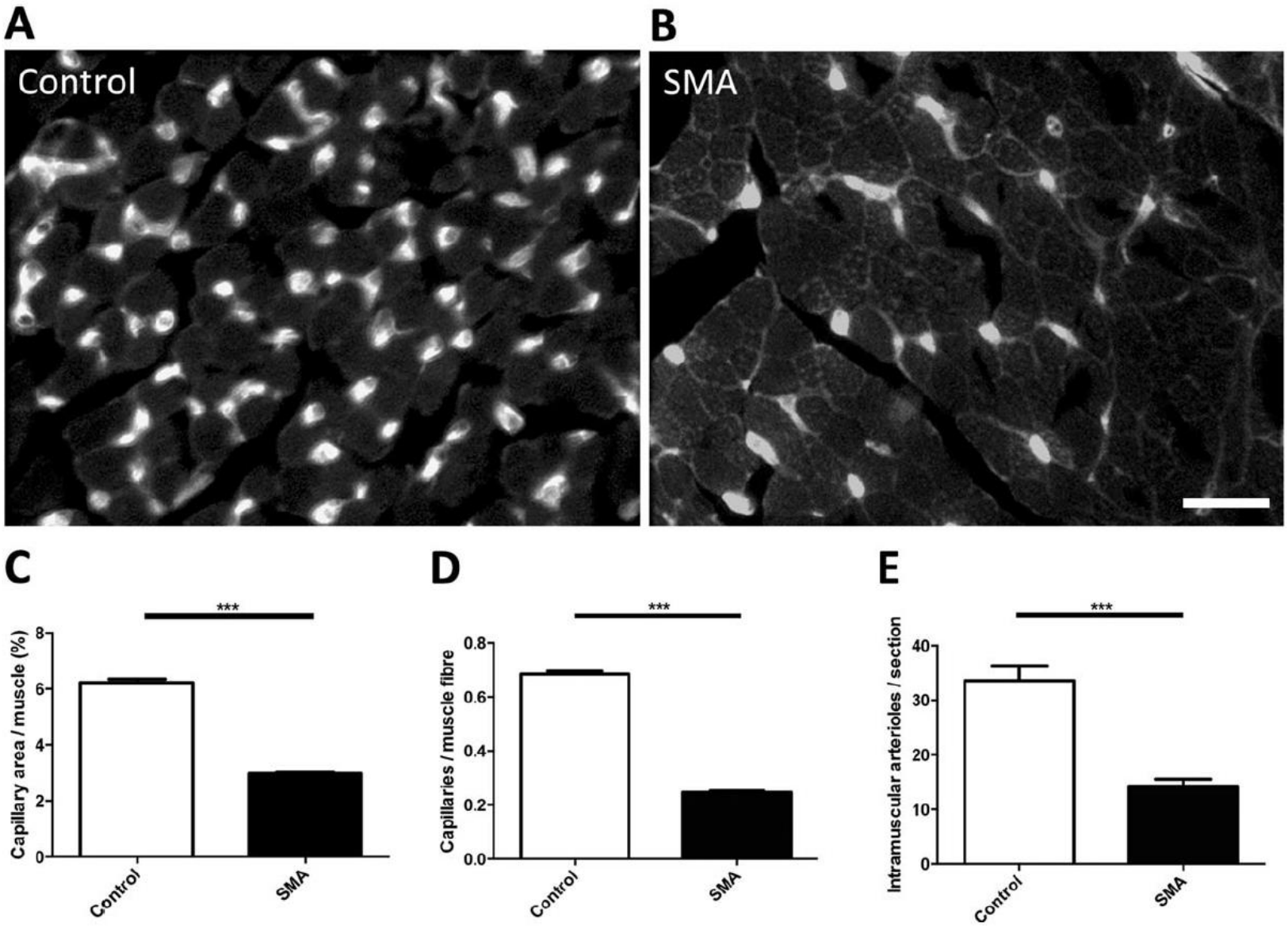
Vascular defects in skeletal muscle: SMA mice



Somers et al 2016. Ann. Neurol.

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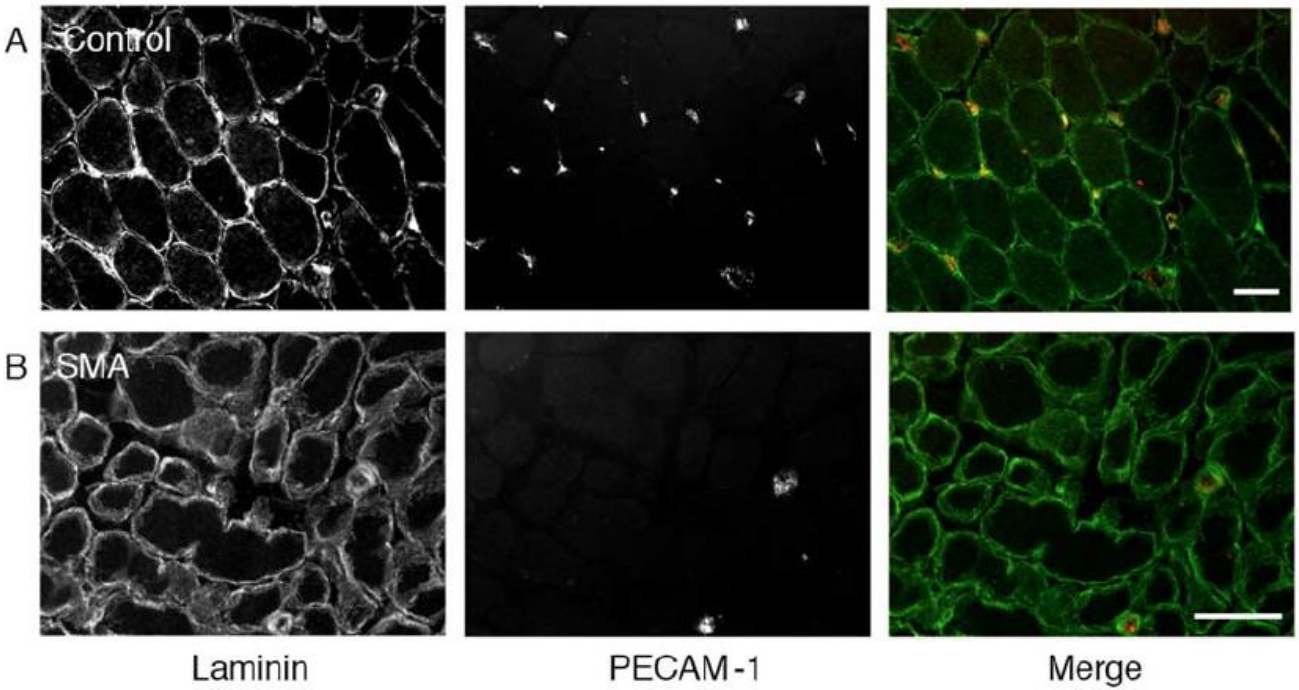
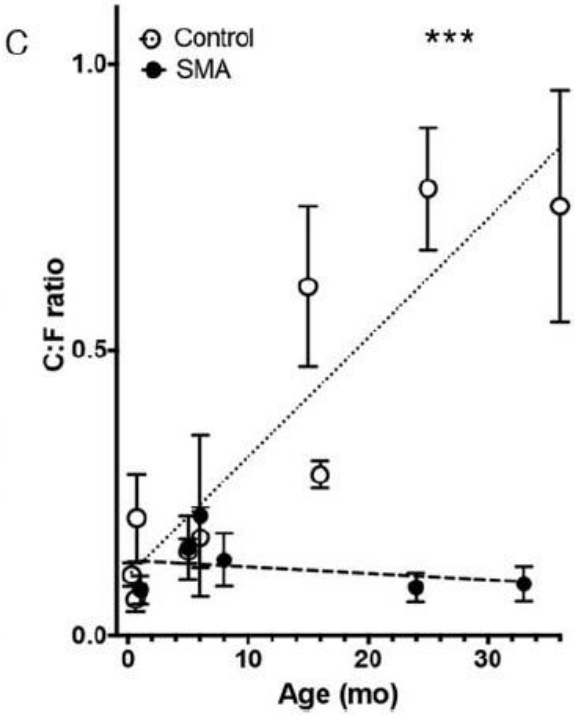
Vascular defects in skeletal muscle: SMA patients





Somers et al 2016. Ann. Neurol.

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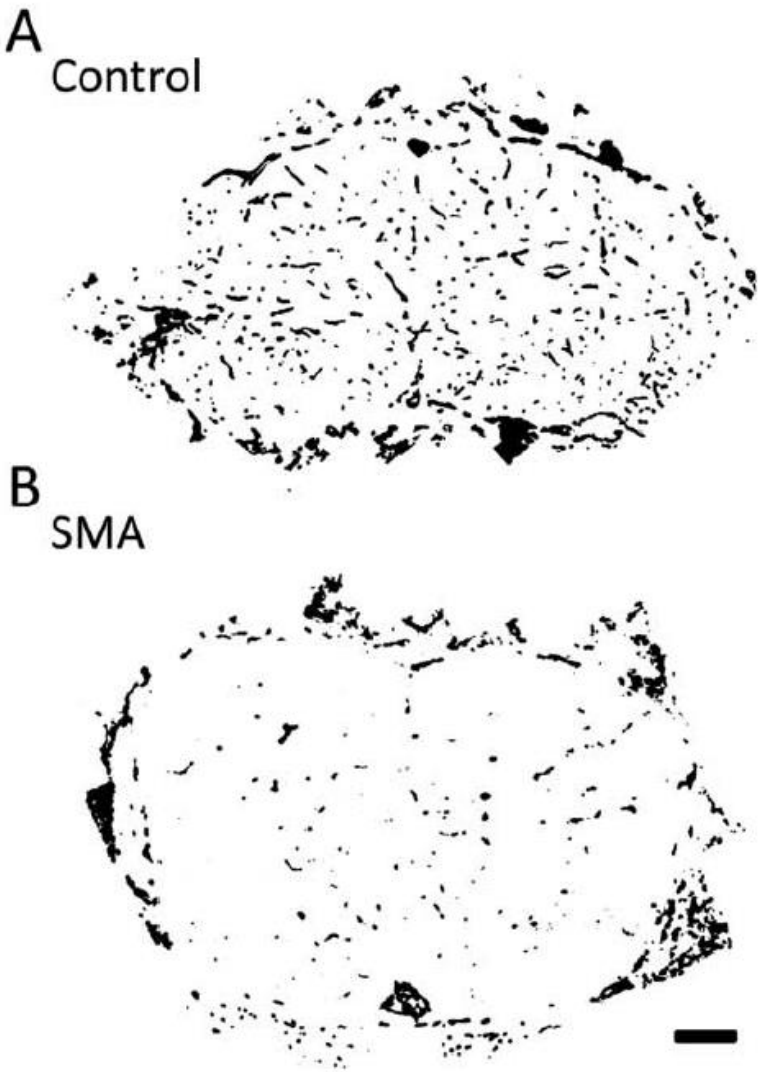
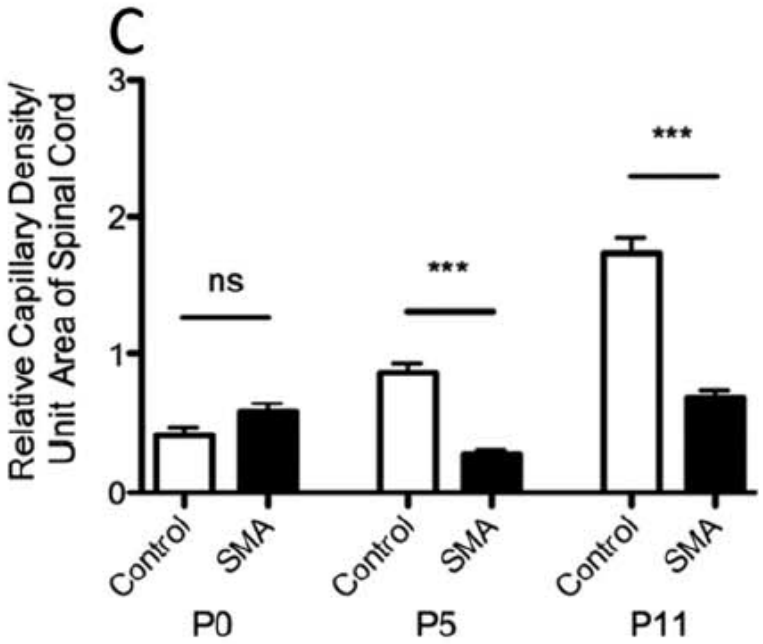
Vascular defects in spinal cord: SMA mice





Somers et al 2016. Ann. Neurol.

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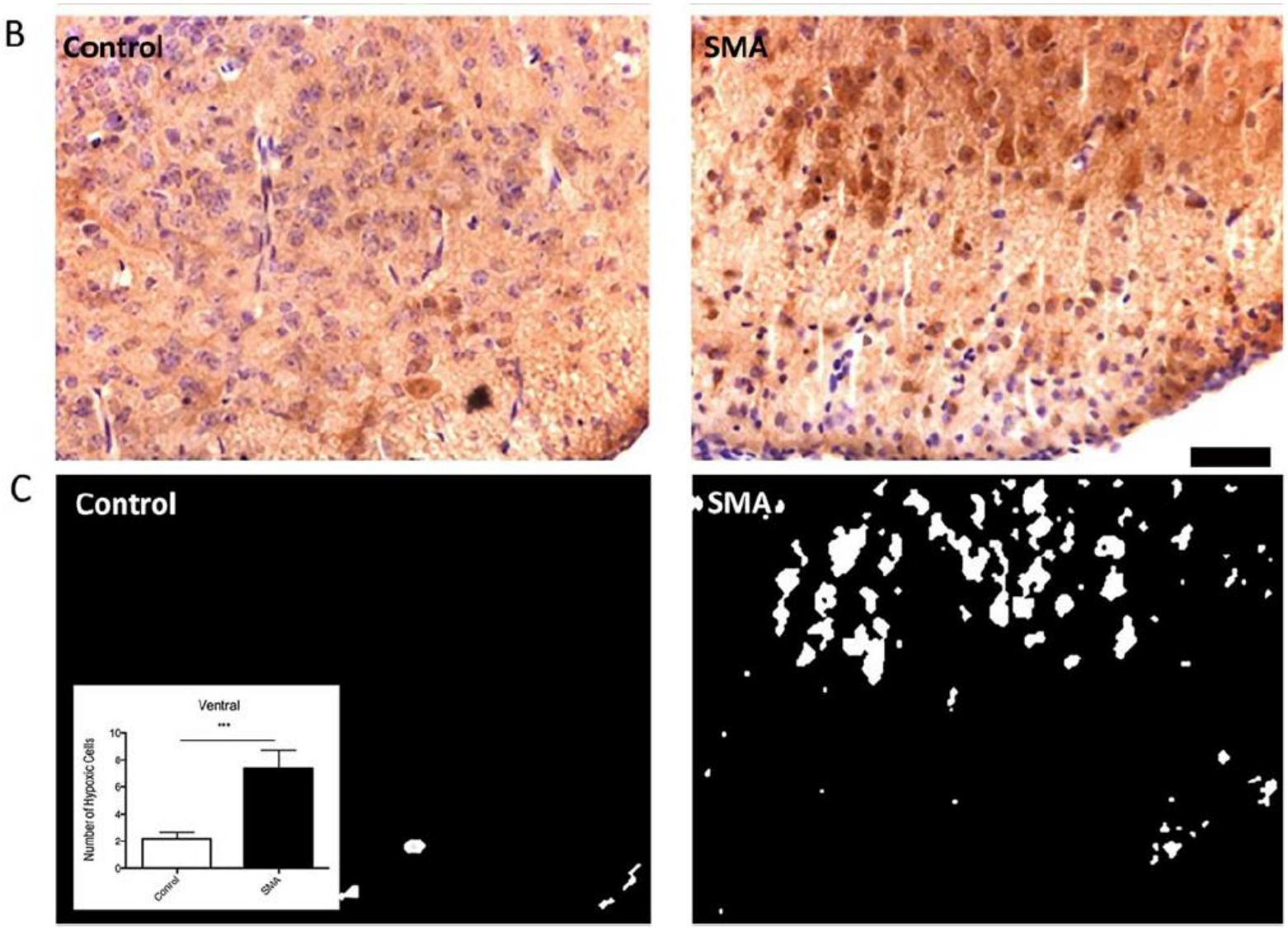
Vascular defects lead to hypoxia in spinal cord



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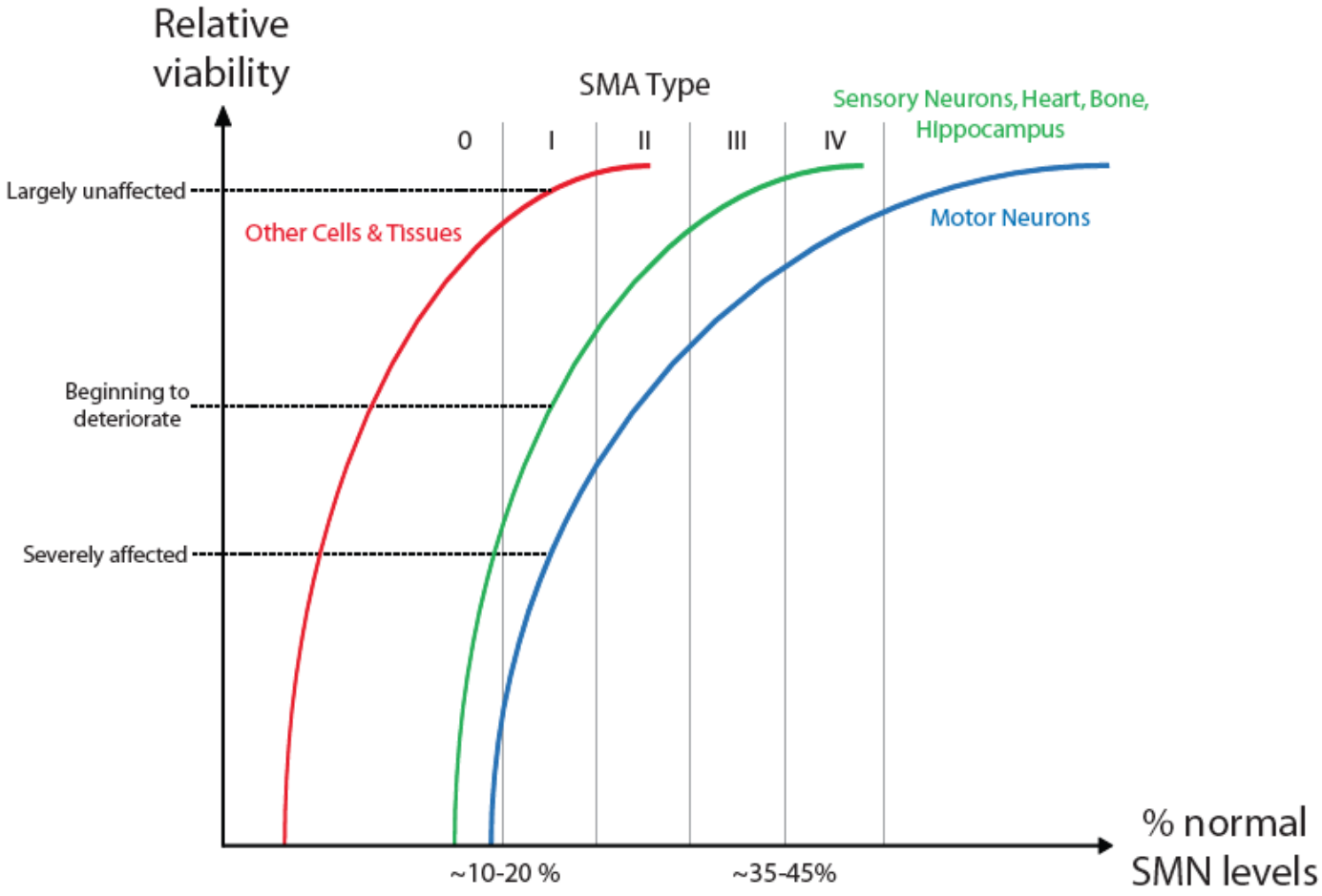
Model of systemic susceptibility to low SMN levels



Sleigh et al 2011. Disease Models & Mechanisms

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Acknowledgements

UKSMA RESEARCH CONSORTIUM

Muscular Dystrophy UK
Fighting muscle-wasting conditions

The SMA Trust
Funding Research into Spinal Muscular Atrophy
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