

## Original Article

# Smooth muscle atrophy and colon pathology in SMN deficient mice

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**Abstract:** Spinal muscular atrophy (SMA) is an autosomal recessive genetic disorder characterized by loss of motor neurons in the ventral horn of the spinal cord. Clinical features such as progressively lethal respiratory weakness and associated muscle wasting have been extensively studied but less attention has been given to gastrointestinal (GI) dysfunction, which is common symptomatology in SMA patients with 43% constipation, 15% abdominal pain, and 14% meteorism. In the current study, the PrP92-SMN mouse model of SMA was utilized, to complement previous studies in which cells of the Enteric Nervous system (ENS) were susceptible to *Smn* (survival motor neuron) deficiency and could possibly be the basis of the observed GI symptoms. Necropsy of our mouse model showed impairment in feces excretion and smaller bladder mass, compared to Wild-Type (WT) animals. Along with the reduction in bladder mass, we also observed a decrease in the size of smooth muscles, due to reduction in Cross-Sectional Area (CSA). Interstitial cells of Cajal (ICC) provide important regulatory functions in the GI tract. To investigate if ICC are implicated in *Smn* deficient-induced colonic dysmotility, we assessed ICC distribution and abundance, by c-Kit, a well-established marker. SMA mice exhibited fewer c-Kit positive cells with altered localization, compared to WT. In conclusion, the observed histopathological abnormalities of our mouse model, can be secondary to SMN deficiency and could possibly underlie the GI symptoms observed in SMA patients. Future therapeutic approaches for SMA, must address not only CNS symptoms, but also non-motor-neuron-related symptoms. The PrP92-SMN mouse model could be a useful model for assessing therapeutic rescue of GI dysfunction in SMA.

**Keywords:** Spinal muscular atrophy, gastrointestinal dysfunction, smooth muscle atrophy, colon pathology, interstitial cells of Cajal, c-kit

## Introduction

Spinal muscular atrophy (SMA) is an autosomal recessive genetic disorder characterized by loss of the anterior horn motor neurons of the spinal cord [1]. This rare neuromuscular disease is caused by mutation in the *SMN1* (survival motor neuron) gene that encodes the full-length SMN protein [2, 3]. In humans a second form of the *SMN* gene exists, *SMN2* [4]. *SMN1* gene differs from *SMN2* gene by only 5 nucleotides (nt); more specifically 1 nt at exons 7 and 8 and intron 6 and 2 in intron 7. The nucleotide exchange in exon 7, results in the creation of a

new exonic splice silencer (ESS) and consequently 90% of the pre-mRNAs resulting from *SMN2* are alternatively spliced, lacking exon 7. A small percentage (10%) of *SMN* proteins arising from *SMN2* are fully functional [5]. The clinical manifestation of SMA includes pediatric progressive muscle wasting and paralysis, eventual respiratory failure [6] or even death based on the maximal motor function achieved [7, 8].

In SMA, the pathogenic mechanisms of frequently reported clinical features such as progressively lethal respiratory weakness [6] and other skeletal muscle wasting have been exten-

sively investigated. However, the gastrointestinal dysfunction enuresis and some other autonomic-nerve-associated symptoms like bradycardia hyperhidrosis and abnormal vasodilation that account for a proportion of symptoms in SMA patients [9-12] are rarely studied.

According to the 2007 Consensus Statement for Standard of Care in SMA, patients with spinal muscular atrophy suffer from gastroesophageal dysmotility problems; such as delayed gastric emptying, and potentially life-threatening gastroesophageal reflux (GER) [13]. It has been shown by clinical studies that 4% of patients with SMA type II have been diagnosed with GER and another 7% with GER related symptoms. Furthermore, 43% of patients complain of constipation, 15% had abdominal pain, and 14% had meteorism [14].

In the gastrointestinal (GI) tract, one of the major Enteric Nervous System (ENS) components is Auerbach's plexus (or myenteric plexus), which provides motor innervation to both longitudinal and circular muscular layers of the GI tract, with parasympathetic and sympathetic input [15, 16]. Besides direct innervation of motor neurons to smooth muscle cells, the innervation can also occur through interstitial cells of Cajal (ICC), a type of interstitial cell that interacts with and forms electrical connectivity with neurons and smooth muscle cells [17-20]. ICC provide important regulatory functions in the GI tract including: (a) pacemakers of electrical slow wave activity [21-24], (b) coordination of pacemaker activity and active propagation of slow waves [25-29], (c) neurotransmission by facilitating motor neural innervation from the myenteric ganglion [30], and (d) mechano-sensation to stretch of GI muscles [31-34].

There are different types of ICC: myenteric ICC (MY-ICC) are multipolar shape cells serve as a pacemaker which creates the bioelectrical slow wave potential that leads to contraction of the smooth muscle [35-38]. Intramuscular ICC (IM-ICC) are bipolar shaped cells involved in the stimulation of smooth muscle cells, and that neurotransmitters act through [39-42]. Submucosal interstitial cells of Cajal (SM-ICC) are multipolar shaped cells still thought to be pacemaker cells in the colon [35, 43]. It is reasonable to hypothesize that the ENS and ICC innervating the gastrointestinal smooth muscles may be vulnerable to SMN deficiency, as are

motor neurons in the spinal cord innervating skeletal muscles.

In the current study, in order to investigate whether ENS (Auerbach's plexus) and ICC innervating the GI smooth muscle are vulnerable to SMN deficiency, we utilized the *SMN2; Smn; PrP92-SMN* mutant mice (which we refer to as "SMA" mice). In this strain of mice, SMN production is localized to the CNS. Homozygous *SMN2; Smn; PrP92-SMN* mice are rescued from the severe SMA phenotype, have significantly increased lifespan (average of 210 days) and have normal lumbar motor neuron root counts and body weight [44]. They can potentially be utilized for studies evaluating SMN restoration only in the brain and spinal cord, thus modeling some of the current human therapeutic strategies. In these mice, we observed significant alterations in micro-pathohistology of the GI tract, including a decrease of smooth muscle cross sectional area in the SMN-deficient bladder and colon. More specially, c-kit positive ICC volume was decreased in the SMN-deficient colon. Both pathological alterations may cause reduced contractile force and problems with neuromuscular activation and transmission, leading to GI dysmotility, as is observed in SMA patients.

### Materials and methods

#### *Animal breeding and genotyping*

Animal studies were performed in accordance with the Institutional Animal Care and Use Committee and approved by the University of Florida. (30 weeks) Male and female mice were used in all studies. Age and sex-matched C57BL/6J were used as controls.

SMN1-deficient, *SMN2; Smn; PrP92-SMN* mutant mice were utilized as the SMA mouse model. When maintaining a colony, mice heterozygous for the *Smn1tm1Msd* targeted mutation, homozygous for the *SMN2* low copy line 89 transgene, and homozygous for the *PrP92-SMN* transgene were bred together. Unlike the delta7 mouse, which is homozygous for the *Smn1tm1Msd* targeted mutation (*Smn* null allele) and human *SMN2* transgene (*SMN2* low copy line 89) and exhibits symptoms, neuropathology, and early lethality similar to human type I proximal spinal muscular atrophy (SMA) patients, this strain carries the additional

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**Table 1.** Antibodies utilized for immunostaining

Antibodies	Host	Dilution	Company	Catalog number
Primary antibodies				
PGP 9.5	Rabbit	1:1000	Thermo Scientific	RB-9202
C-kit	Rat	1:500	Thermo Scientific	MA5-17836
Dystrophin	Rabbit	1:1000	Abcam	AB15277
Secondary antibodies				
Goat anti-Rabbit IgG, Alexa Fluor 594	Goat	1:1000	Thermo Scientific	A11037
Goat anti-Rat IgG, Alexa Fluor 488	Goat	1:1000	Thermo Scientific	A11006
Goat anti-Rabbit IgG, Alexa Fluor 488	Goat	1:1000	Thermo Scientific	A11034

*PrP92-SMN* transgene (Figure S1). With the mouse prion protein (*PrP92*) promoter directing full-length human SMN, SMN is expressed at high levels in neurons, but low levels in cardiac muscle, skeletal muscle, kidney, and lower GI tract, with no expression in upper GI tract (Figure S1), liver and spleen [44-46]. With this approach, homozygous *SMN2*; *Smn*; *Prp92-SMN* mice are CNS rescued, have normal lumbar motor neuron root counts and much increased lifespan [44], which should be a good model to investigate the impact of SMN deficiency on organs that have low or no SMN expression.

The genotyping of the mice was completed according to the protocol of The Jackson Laboratory (JAX) [47]. Genomic DNA was extracted from tail clips and amplified with PCR using the following primers: Common primer, 5'-CTC CGG GAT ATT GGG ATT G-3'; Mutant reverse primer, 5'-GGT AAC GCC AGG GTT TTC C-3'; Wild type reverse primer, 5'-TTT CTT CTG GCT GTG CCT TT-3.

### Colon tissue processing

Mice were sacrificed at 30-weeks of age, which is the average reported lifespan (210 days) of these animals [44] and weighed. Various tissues were dissected, weighed and processed. The entire colon was collected, its length was measured and dissected along the mesenteric border. The colon was divided into three pieces: one piece was fixed in ice cold acetone at 4°C for whole mount as previously described [48], another was embedded in optimal cutting temperature (OCT, Fisher HealthCare, US) and frozen in liquid nitrogen-cooled iso-pentane, the rest was snap frozen and stored at -80°C for western blotting.

### Histopathology

Transverse and longitudinal cryosections (10 µm) of distal regions of the colon were prepared using a cryostat (Leica, Germany) and were stained with haematoxylin and eosin (H&E). Images were captured on a light microscope (Leica DM4 B, Leica, Germany).

### Immunohistochemistry

The preparation and immunostaining for whole mount samples and cryosections were performed according to the protocol from JOVE [48]. All sections were analyzed by immunohistochemistry using the primary and secondary antibodies listed in Table 1. Smooth muscle and bladder CSA was determined on 10 µm cryosections stained with dystrophin. For PGP9.5 primary antibody staining, sections were subjected to antigen retrieval by boiling slides in 10 mM citrate buffer (pH 6.0) for 20 minutes.

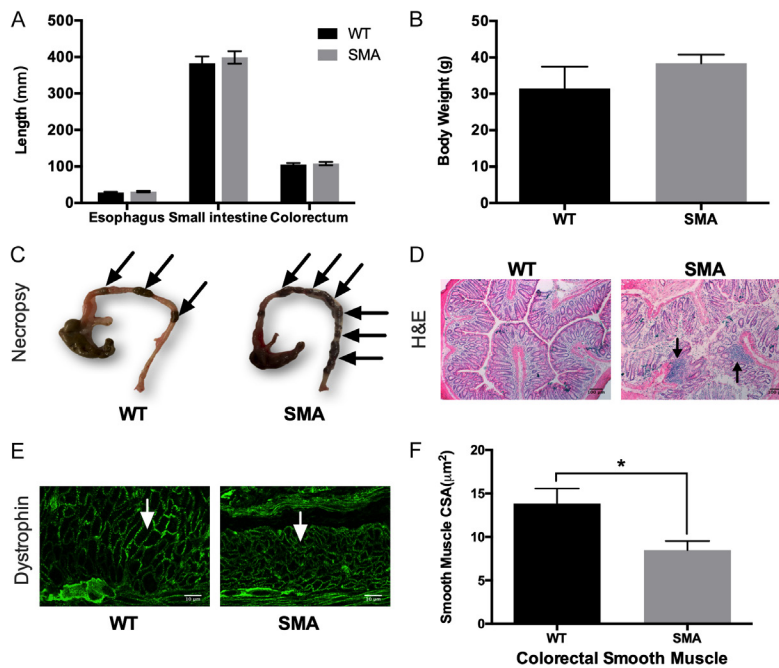
### Imaging and quantification

Images were captured on a confocal laser scanning microscope (TCS SP8, Leica, German). All the images were quantified with ImageJ (Fiji 1.0) software. For the smooth muscle, the cellular membrane outline was identified by dystrophin positive pixels (green), and the cross-sectional area (CSA) was determined and qualified by combined use of 'Trainable weka segmentation 3D', 'Binary' and 'Particles' analysis. Similarly, the PGP9.5 positive pixels and DAPI stained nuclei were used to identify Auerbach's plexuses (enteric nervous ganglia included). The ganglia cells and ganglionic cross section areas were counted and measured. The numbers of ICC, the c-kit positive (green) labeled

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**Table 2.** Antibodies utilized for Western blot

Antibodies	Host	Dilution	Company	Catalog number
SMN	mouse	1:1000	Cell Signaling Technology	12976S
Goat anti-Mouse IgG, Alexa Fluor 488	Goat	1:1000	Thermo Scientific	A-11001
C-kit	Goat	1:2000	Bio-Techne Corporation	AF1356
Rabbit anti-Goat IgG, Alexa Fluor 790	Rabbit	1:1000	Thermo Scientific	A27019
Anti-Actin Antibody, Alexa Fluor 488	Rabbit	1:1000	Merck millipore	ABT1485-AF488



**Figure 1.** Anatomical features of the SMN2; Smn; PrP92-SMN mouse (SMA mouse) strain. (A) No difference was observed in Esophagus, Small Intestine and Colorectum lengths or (B) Body weight between SMA and WT mice. (C) The colon was dissected from SMA and WT mice. The arrows points to well-pelleted feces. SMA mice displayed increased numbers of pelleted-feces, indicating constipation. (D) H&E staining of SMA and WT colon. Secondary lesions (arrow) were present in SMA mice. (E) Representative images of colon smooth muscles stained for dystrophin. (F) Quantification of colon smooth muscle CSA. SMA mice have approximately 35% smaller smooth muscle CSA, compared to WT ( $8.476 \pm 1.052 \mu\text{m}^2$  N=7 vs  $13.82 \pm 1.735 \mu\text{m}^2$  N=7,  $P=0.0218$ ), exhibiting a significant shift in distribution towards smaller CSA fibers (Kolmogorov-Smirnov  $D=0.2584$ ,  $P < 0.0001$ ).

interstitial cells were counted separately in each anatomical layers of colon tract.

### Immunoblot analysis

Tissues extracted for immunoblot analysis were snap frozen in liquid nitrogen and stored at  $-80^\circ\text{C}$  until further processing. The expression of proteins of interest was measured by western blotting as previously described [49]. Actin was used as loading control in all sam-

ples. The blot images were acquired on the LI-COR Imaging System. Quantification of band intensity was analyzed by Image Studio™ Software (LI-COR, USA). All the primary and secondary antibodies are listed in **Table 2**. Values are expressed as the ratio of protein of interest to loading control.

### Statistical analysis

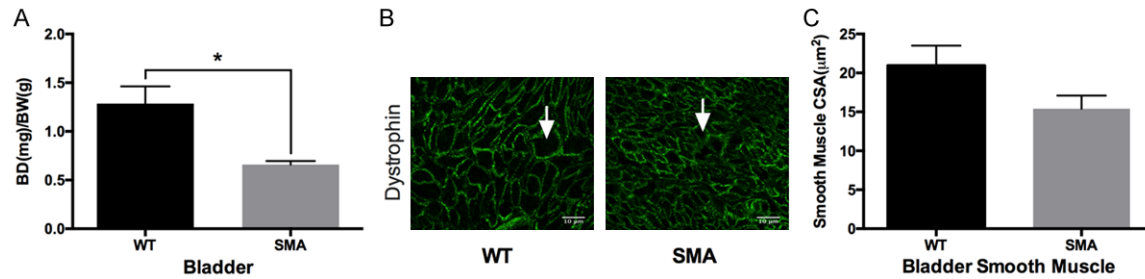
All data are presented as means  $\pm$  SEM. All analyses were performed with PRISM 6.0c (GraphPad Software, Inc, USA). A Student's t test was performed for comparison of 2 groups, and 2-way ANOVA was performed for comparison of  $\geq 3$  groups to determine significance, followed by Sidak's multiple comparisons post hoc test. Kolmogorov-Smirnov test was used to compare cumulative distributions of smooth muscle CSA. A  $P$  value of  $< 0.05$  was considered significant.

## Results

### Impaired feces excretion and reduced colorectal smooth muscle fiber size in SMA mice

It has been reported that patients with SMA type II have constipation and GI symptoms [14]. To determine the state of the GI system in our animal model, we measured the length of "major" parts of the gastrointestinal system (esophagus, small intestine and colorectum) (**Figure 1A**) and body weight (**Figure 1B**). No significant changes were observed in either part of

## Colon pathology in SMA mice



**Figure 2.** The bladder morphology of SMA mice. A. Normalized bladder mass to body mass. SMA mouse bladder is 50% smaller as compared to WT ( $0.6589 \pm 0.03764$  N=7 vs  $1.285 \pm 0.1773$  N=7,  $P=0.0015$ ). B. Representative images of bladder smooth muscles stained for dystrophin. C. Quantification of bladder smooth muscle CSA. No difference was observed in bladder smooth muscle CSA between SMA and WT ( $15.41 \pm 1.691 \mu\text{m}^2$  N=5 vs  $20.94 \pm 2.557 \mu\text{m}^2$  N=5,  $P=0.1086$ ), but with a significant shift in distribution towards smaller CSA smooth muscle cells (Kolmogorov-Smirnov  $D=0.1647$ ,  $P < 0.0001$ ).

the GI system in our SMA mice (esophagus:  $28.52 \pm 2.72$  mm, small intestine:  $382.6 \pm 26.7$  mm, colorectum:  $105.2 \pm 5.56$  mm; N=2) compared to their controls (esophagus:  $31.18 \pm 2.58$  mm, small intestine:  $399.1 \pm 24.13$  mm, colorectum:  $107.9 \pm 6.24$  mm; N=2). Also, no significance difference was observed in body weight in SMA mice ( $38.38 \pm 6.74$  g, N=8), compared to control ( $31.43 \pm 12.01$  g, N=4), neither is weight in specific muscles, because the skeletal muscles have similar SMN expression (Figure S1B and S1C). However, increased fecal loading was observed in SMA mice (Figure 1C). To determine the origin of the impaired fecal excretion, transverse sections of SMA mice colon were stained with Hematoxylin and Eosin (H&E) and examined for morphological alterations and possible inflammation. Evaluation of colon sections show the presence of secondary lesions in SMA mice and infiltration by inflammatory cells (Figure 1D). Another possible etiology, of the gastrointestinal problems observed in SMA patients is the involvement of smooth muscles of the GI system. We found that the CSA of smooth muscle in the colon was approximately 40% lower in SMA compared to control mice (Figure 1E, 1F). From the above we conclude, while the *PrP92-SMN* transgene rescues the SMA motor neuron and CNS phenotypes, dysfunctions of the GI system are still present.

### *SMA mice exhibit reduction in bladder and smooth muscle size*

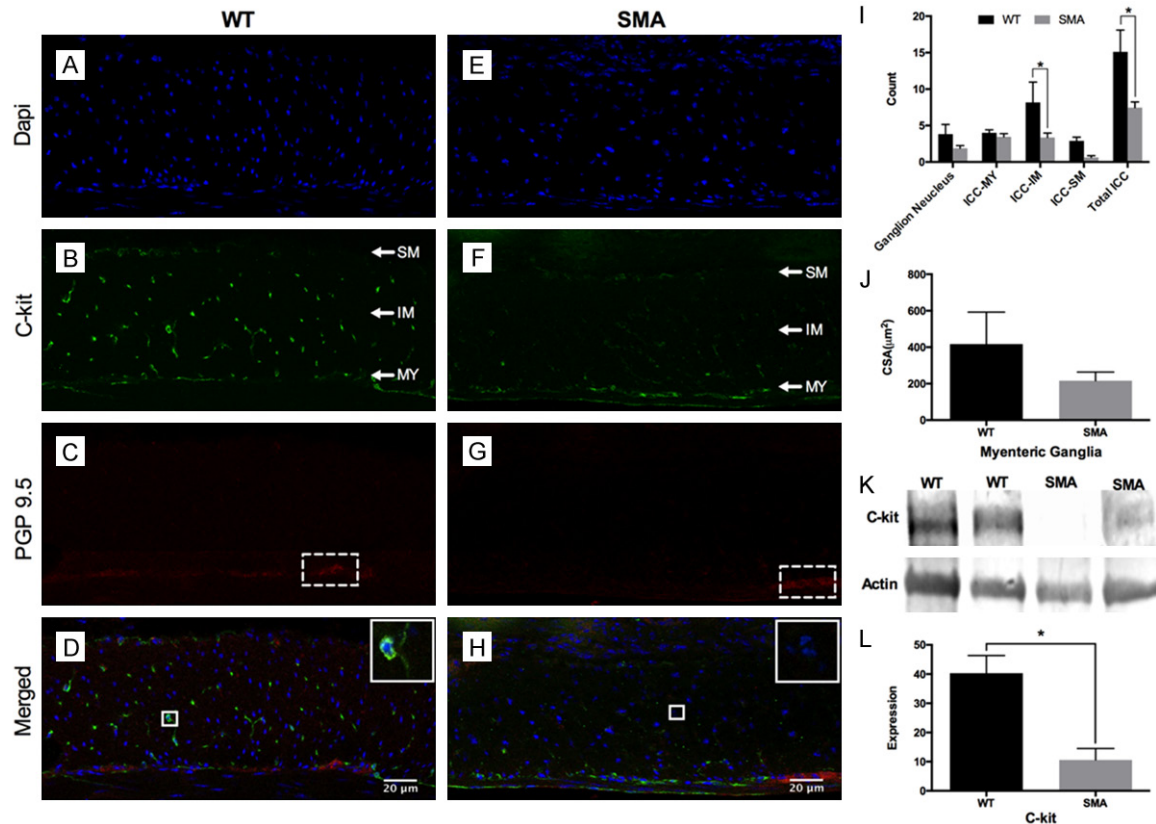
Another area that the role of SMA has been overlooked is enuresis and urinary incontinence. To the best of our knowledge there is

just one clinical study that has examined that issue and reported that a third of all children diagnosed with SMA have signs of urinary incontinence [12]. To investigate whether a similar phenomenon is present in our animal model, we measure the bladder weight (mg)/body weight (g) (BD/BW), as a possible indication of the phenomenon. We found that BD/BW of our SMA mice was nearly 50% lower compared to WT mice (Figure 2A). Furthermore, based on immunohistological analysis, SMA mice bladder smooth muscle fibers were by 20% smaller than WT mice (Figure 2B, 2C). The smaller sized smooth muscle fibers may cause poor contraction and can be the etiology for urinary incontinence.

### *SMA mice have a lower volume of c-kit positive ICC in colon*

The GI tract is innervated by extrinsic (sympathetic, parasympathetic and visceral afferent) axons and intrinsic neurons of the enteric nervous systems (ENS). Defects of these innervations or ICC facilitation may cause GI dysmotility [50-55]. In the current study, we examined enteric neurons and ICC in our animal model. Neuron density in the colon was identified and quantified by PGP9.5 staining. ICC abundance and distribution were determined by immunoblot and staining, respectively [48]. Examining the cryosections from serosa to mucosa, we found that no significant difference was observed in the mesh formed by PGP9.5-positive nerve cells, between SMA and WT mice. However, SMA mice exhibited 50% fewer c-Kit positive stained ICCs compared to WT mice (Figure 3A-J). The reduction of c-Kit pro-

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**Figure 3.** Histological investigation on distribution and abundance of ENS and ICC. (A-H) are representative images of enteric neurons/ganglions and ICC in colons from WT and SMA mice. Enteric neurons were stained with the neuronal marker PGP9.5 (Red), ICC with c-Kit (Green) and cell nuclei were stained with DAPI (Blue). (J) The WT mice have a non-significantly larger ganglionic CSA ( $417.2 \pm 175.3 \mu\text{m}^2$ , N=6 vs  $216.2 \pm 47.72 \mu\text{m}^2$ , N=10, P=0.1916) and ganglionic nuclei number ( $3.82 \pm 1.335$ , N=6 vs  $1.89 \pm 0.372$ , N=10, P=0.7390) than SMA mice; (I) The SMA mice had a decreased trend for c-kit positive ICC compared to WT mice in the MY and SM layers, and presented significantly decreased numbers in the IM layer ( $3.38 \pm 0.593$ , N=10 vs  $8.18 \pm 2.779$ , N=6, P=0.0206) and a striking 50% reduction in total ICC numbers ( $7.47 \pm 0.779$ , N=10 vs  $15.10 \pm 2.997$ , N=6, P < 0.0001). (K) The immunoblots of c-Kit protein and actin. (L) Semi-quantification of c-Kit protein relative to an actin loading control. The c-kit protein level is four-fold higher in WT colon tissues compared to the SMA mice ( $40.31 \pm 6.025$  N=5 vs  $10.61 \pm 3.892$  N=5, P=0.0032), consistent with the immunofluorescence imaging results.

tein levels in the SMA mice was confirmed by immunoblot (Figure 3K, 3L). Whole-mount immunofluorescence at three different tract layers (Myenteric (MY), Intramuscular (IM) and submucosa (SM)) (Figure 4), showed that there is a lower abundance (approximately 50%) of c-kit positive ICC in SMA mice compared to controls. These findings could be an indication that ICC may play a role in facilitating motor neural innervation from the myenteric plexus, and SMN deficiency might cause c-kit positive ICC maldevelopment and reduced abundance impacting neurotransmission.

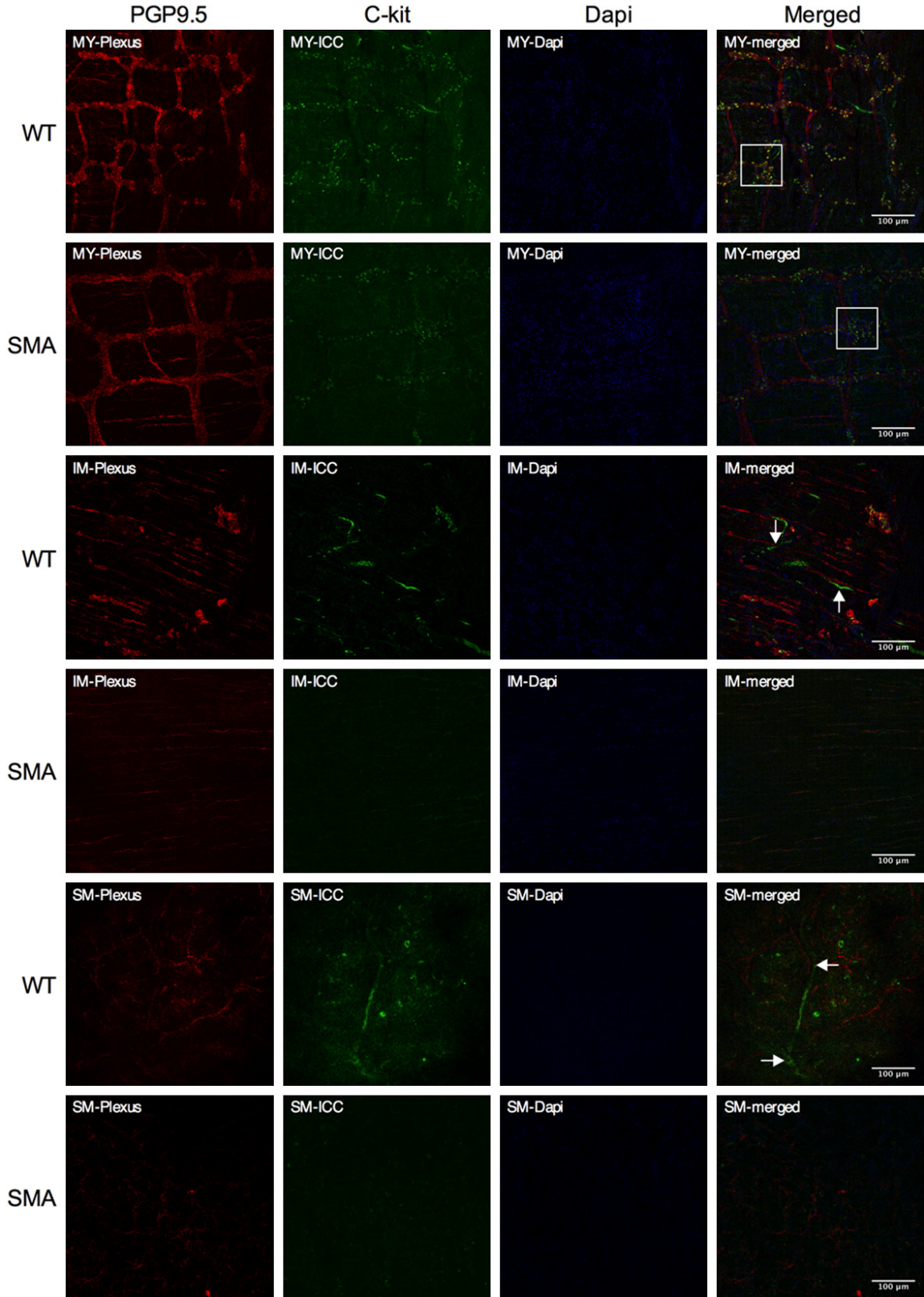
### Discussion

Spinal Muscular Atrophy patients present with GI-related symptomatology, which include gas-

troesophageal reflux, constipation and delayed gastric emptying. These phenotypes have received limited attention in SMA animal model studies. In the current study, 30-week old *Prnp92-SMN* mice (SMA mice) were used for investigation, allowing organs to be larger and better developed for histological analysis than in Delta 7 mice, which have an average survival of only 13 days [56]. Using the *Pr92-SMN* mice model of SMA, that exhibits a mild phenotype due to CNS and motor neuron rescue, we were able to examine the phenotypic and morphological alterations in the GI tract associated with the loss of SMN.

We found that the body mass of WT and SMA (*Pr92-SMN*) mice had no significant difference (Figure 1B), in contrast to two other stud-

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**Figure 4.** Whole mount immunofluorescence of colon. Whole mount of colon with PGP9.5 (Red) staining for neurons, c-Kit (Green) staining for ICC and with DAPI (Blue) staining for cell nuclei in three focal planes: (Myenteric (MY), Intramuscular (IM) and submucosa (SM)): Compared to the SMA mice, the WT mice present stronger green intensity

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of c-kit positive ICC (indicated by white solid square) surrounding the myenteric ganglion ( $12.47 \pm 0.01$  vs  $9.990 \pm 0.01$ ,  $P < 0.0001$ ). Both in the intramuscular (IM) and submucosa (SM) layers, stronger staining, larger c-kit positive fibers (white arrows) were found in WT mice, paralleling the PGP 9.5 positive structures, as compared to SMA mice.

ies that examined the GI phenotype of SMA mice [57, 58]. The reason for that discrepancy can be attributed to PrP92-promoter used to CNS specific expression for SMN. Motor neuron rescue in the spinal cord prevents the skeletal muscle atrophy caused by denervation. Also, some of the mice were found to have leaky expression from the PrP92-SMN transgene outside of the neuronal compartment, especially in skeletal muscles (Figure S1C). This may be another reason for skeletal muscle restoration and no weight loss. However, leaky expression was not present in GI tissues such as colon, stomach and small intestine tissues (Figure S1C).

In a previous study [58], SMN deficiency was found to lead to disrupted ENS signaling to the smooth muscle of colon with a 10-fold greater contractile response with high-frequency electrical field stimulation. However, no neurodegeneration was found to be associated with abnormal ENS signaling in that study. This is consistent with our observation of no significant differences in ganglionic nucleus number and CSA were in our study. But this observation is contrary to a study in Taiwanese SMA mice that exhibited an increase of enteric neuron numbers in the small intestine [57]. Limited clinic data from 8 infants with Werdnig-Hoffmann disease (SMA type I) showed low values of neural tissue in the myenteric plexus in the small intestine and colon [59].

ICC [60] connects motor neurons to local effector cells, such as smooth muscle cells. The current study examines the impact of SMN deficiency on smooth muscle and ICC in SMA. A reduction of c-Kit positive ICC was observed in SMA mice, suggesting that SMN deficiency could lead to loss of ICC that resembles loss of motor neurons in spinal cord. ICC was previously found to integrate excitatory and inhibitory neurotransmission with slow-wave activity to orchestrate peristaltic motor activity [61], therefore ICC loss could be a potential reason for the abnormal ENS signaling in SMA mice. In addition, decreased smooth muscle CSA was seen in colon smooth muscle in our SMA mice. This muscular alteration could be directly caused by SMN-deficiency, as is the case in

skeletal muscle, where it has been reported that SMN-deficiency impairs myotube formation by altering myogenic gene expression and focal adhesion dynamics that affect muscle cell migration and organization of the actin cytoskeleton [62]. The muscular alterations might also be the result of denervation resulting from the c-kit positive ICC loss.

In a previous study, macrophage infiltration was observed in the small intestine from the Taiwanese SMA mouse model [57]. Another animal study [63] reported that under the pro-inflammatory conditions of the muscularis-associated GI obstruction, the release of bioactive substances from activated resident macrophages may affect smooth muscle contractility. In addition, under these conditions, both the number and the function of neighboring ICC may also be affected [63, 64]. Thus, the macrophage infiltration in SMA colon tract could be a potential contributor to the ICC volume reduction and smooth muscle atrophy of the colon from SMA mice. Therefore, for the SMN deficiency-associated observed GI symptoms (smooth muscle atrophy, ICC loss, and macrophage infiltration), we cannot distinguish which are the primary pathological alterations due to SMN loss and which are secondary consequences. But in the future, cellular level studies could help determine if these defects are primary or secondary in SMN-depleted smooth muscle cells, ICC and macrophage cells.

In addition to GI symptoms, children with SMA types I and II have a high rate of urinary incontinence [12]. This has been identified as “stress incontinence” due to striated muscles weakness and atrophy in the pelvic floor muscles and the external urethral sphincter, but not the attributed to smooth muscles of the detrusor [12]. However, in our study, we observed a 50% lower bladder weight/body weight and 20% smaller bladder smooth muscle fibers in the SMA-deficient mice, suggesting that SMN deficiency may cause poor contraction of the smooth muscle may contribute to overflow incontinence.

In SMA, besides the well-known motor-neuron-related symptoms, symptoms like GI dysmotili-



ty, bradycardia, and hyperhidrosis make up a non-motor-neuron-related symptomatic group. The histopathological abnormalities we found in colon and bladder of SMA mice, ICC loss and smooth muscle atrophy secondary to SMN deficiency, could be responsible for the pathological changes in SMA patients. In the future, the therapies for SMA will need to not only target the CNS and motor neurons, but also address such non-motor-neuron-related symptoms. The mice we described herein will be a useful model for testing the ability of therapies to rescue GI dysfunction at different post-natal stages.

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### Disclosure of conflict of interest

None.

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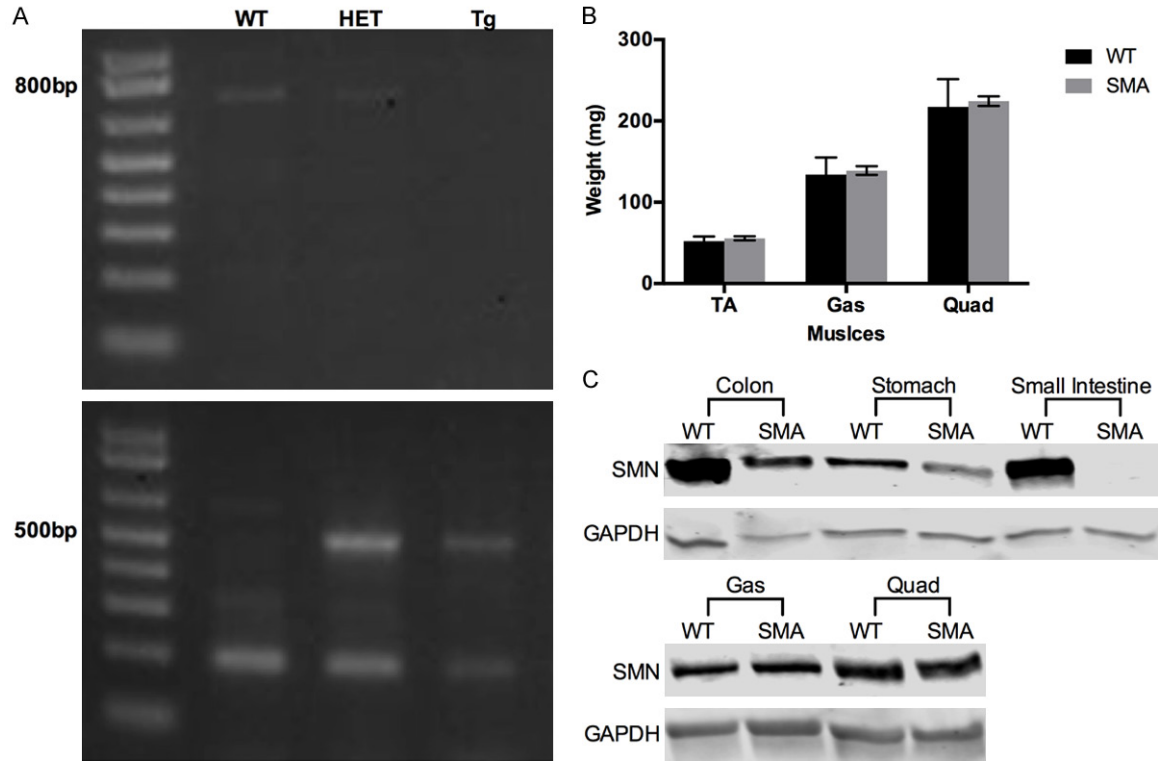
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**Figure S1.** A. In genotype, the functional *SMN* is 800 bp length DNA, the mutated *Smn* is 500 bp length. B. The weights in specific muscles of tibialis (TA) ( $52.10 \pm 5.738$  mg N=4 vs  $55.625 \pm 2.616$  mg N=8,  $P=0.997$ ) gastrocnemius (Gas) ( $134.15 \pm 20.935$  mg N=4 vs  $138.9725 \pm 5.459268$  mg 4=N,  $P=0.995$ ) and quadriceps femoris (Quad) ( $217.33 \pm 34.139$  mg N=4 vs  $224.38 \pm 5.933$  mg N=4,  $P=0.984$ ) had no difference between WT and SMA. C. SMA mice had lower or even deleted expression level of SMN protein in GI tissues, such as colon, stomach and small intestine, compared to WT. But SMA mice were found having leaky expression from the *PrP92-SMN* transgene, outside of the neuronal compartment, especially in gastrocnemius (Gas) quadriceps femoris (Quad) and etc.