MUSCULOSKELETAL DEFECTS IN SMA

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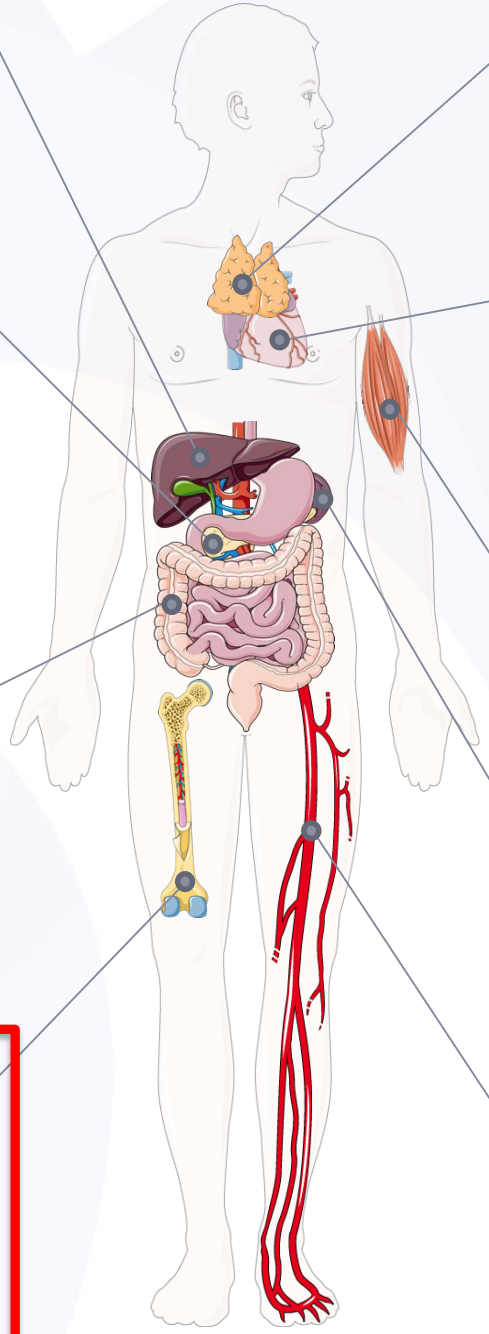
SMA AS A MULTI-ORGAN DISORDER?



Liver

Mouse
- cKO – embryonic lethal
- Iron homeostasis defect
- Impaired development
- ↑ megakaryocytes

Human
- Case reports of fatty liver

Pancreas

Mouse
- Altered proportion of α and β cells
- Glucose resistance

Human
- Altered proportion of α and β cells
- Report of hyperinsulinemia, insulin resistance, impaired glucose tolerance

Gastrointestinal

Mouse
- Constipation, delayed gastric emptying and slow liquid transit
- Altered GI neuromuscular transmission
- Reduced intestinal length

Human
- Constipation, delayed gastric emptying, gastroesophageal reflux

Bone

Mouse
- ↓ total bone area, bone mineral content and bone mineral density
- ↑ bone turnover

Human
- Low bone mineral density
- Prone to fracture
- Low 25-OH vitamin D levels

Thymus

Mouse
- Cortex thinning
- ↑ apoptotic bodies
- Impaired T-cell development

Human
- Atrophy

Heart

Mouse
- Bradycardia
- ↓ cardiac function
- ↓ vascularization and innervation

Human
- Case reports of ASD, VSD, and other cardiac defects

Muscle

Mouse
- Impaired myogenesis
- Intrinsic weakness
- cKO – dystrophy

Human
- Smaller in SMA fetuses

Spleen

Mouse
- Atrophy
- Abnormal histological structure
- Loss of B-cell follicles
- Fibrosis

Human
- Abnormal in some patients

Vasculature

Mouse
- Decrease muscle and SC capillary density
- Ear and tail necrosis

Human
- Decrease muscle capillary density
- Digital necrosis

ROLE OF SMN IN MUSCLE: OVERVIEW

Muscle defect	Source	Reference
Decreased satellite cell number	Biopsies from older SMA patients C/C mice	*Lee Sweeney, unpublished*
Premature satellite cell differentiation	$Smn^{-/-}$; $SMN2^{+/+}$ mouse satellite cells	*Hayhurst et al, 2012*
Abnormal expression of myogenic markers	Biopsies from SMA patients Delta7 mouse myoblasts Delta7 mice $Smn^{-/-}$; $SMN2^{+/+}$ and $2B/^{-}$ mouse myoblasts and mice	*Ripolone et al, 2015* *Bricceno et al, 2014* *Kong et al, 2009* *Boyer et al, 2014*
Myotube fusion defects	Type I SMA patient myoblasts $Smn^{-/-}$; $SMN2^{+/+}$ mouse myoblasts Delta7 mouse myoblasts $Smn^{-/-}$; $SMN2^{+/+}$ and $2B/^{-}$ mouse myoblasts C2C12 SMN-deficient myoblasts	*Arnold et al, 2004* *Hayhurst et al, 2012* *Bricceno et al, 2014* *Boyer et al, 2014* *Shafey et al, 2005*
Defects in cell migration, cytoskeleton organization and focal adhesions	Delta7 mouse myoblasts	*Bricceno et al, 2014*
Muscle maintenance defects	HSA-Cre; $Smn^{F7/F7}$ mice Pharmacological model	*Nicole et al, 2003* *Chien-Ping Ko, unpublished*
Muscle regeneration defects	CreER; $Smn^{F7/-}$ mice C/C mice	*Kariya et al, 2014* *Lee Sweeney, unpublished*

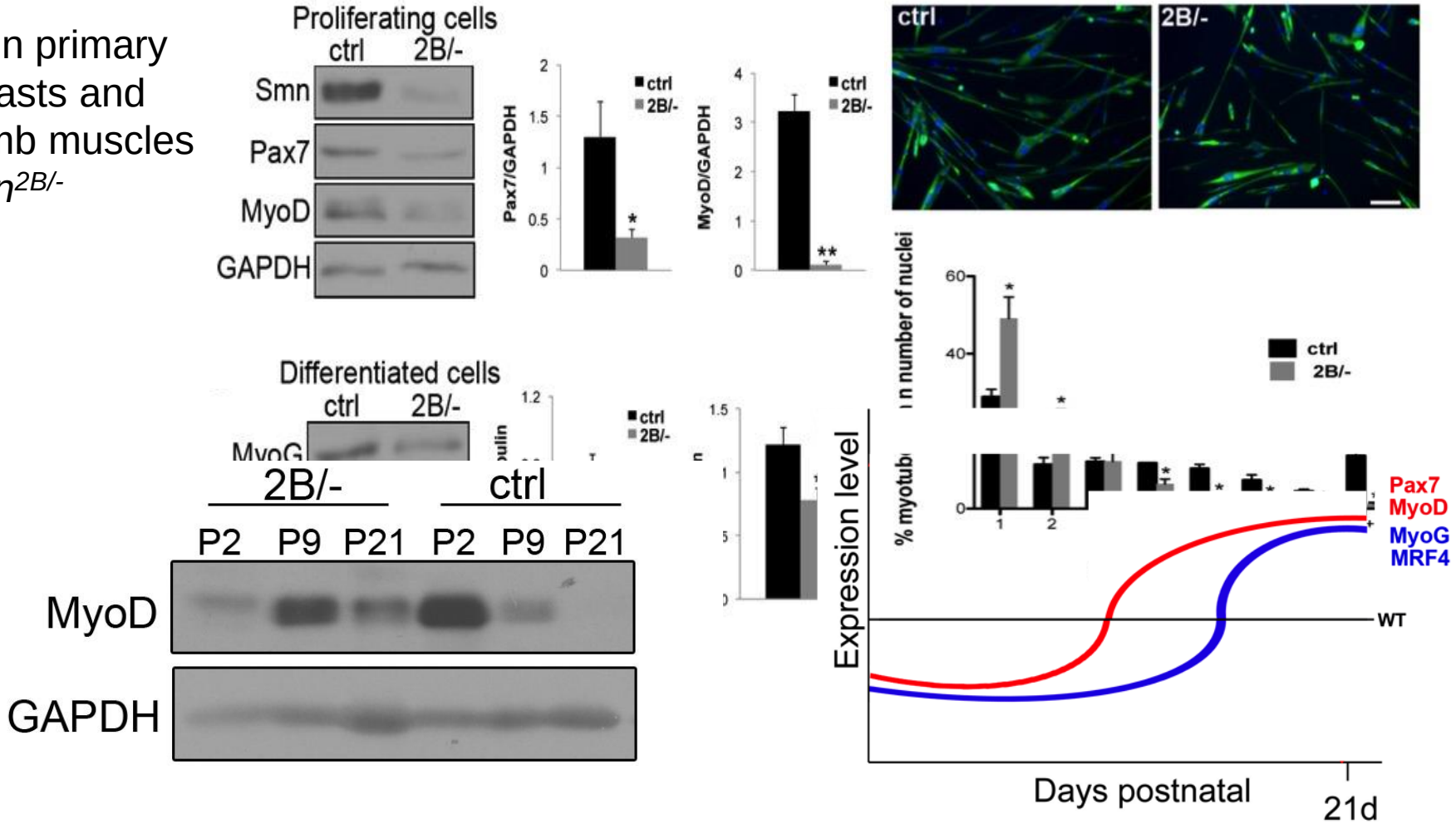
Courtesy of SMA Foundation

HYPOMORPHIC ALLELIC SERIES OF MOUSE MODELS OF SMA

Genotype	Allele 1	Allele 2	Total Smn protein (% WT)	Phenotype	Median survival
Smn$^{+/+}$	50	50	100	normal	normal
Smn$^{+/-}$	50	0	50	normal	normal
Smn$^{2B/+}$	15	50	65	normal	normal
Smn$^{2B/2B}$	15	15	30	normal	normal
Smn$^{2B/-}$ (BL6)	15	0	15	severe SMA	25 days
Smn$^{2B/-}$ (FVB)	15	0	15	severe SMA	19 days
Smn$^{-/-}$;SMN2/SMN2;SMNΔ7			10	very severe SMA	14 days
Smn$^{-/-}$;SMN2/SMN2			5	very severe SMA	5 days
Smn$^{-/-}$			0	pre-implantation lethal	0 days

MYOGENIC DEFECTS IN SMN DEPLETED MYOBLASTS AND IN MOUSE MODELS OF SMA

Seen in primary myoblasts and hindlimb muscles of *Smn*$^{2B/-}$

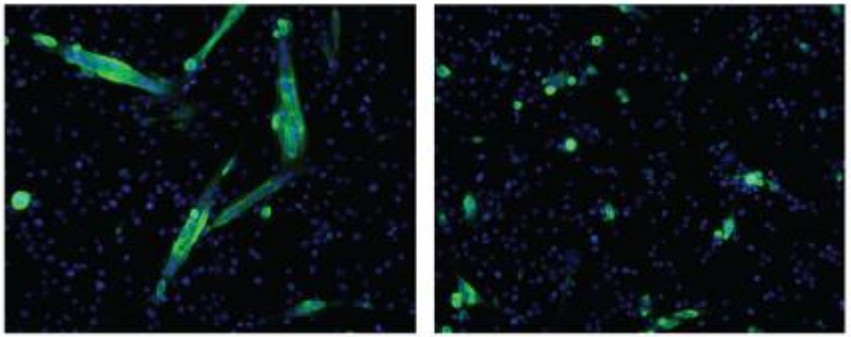


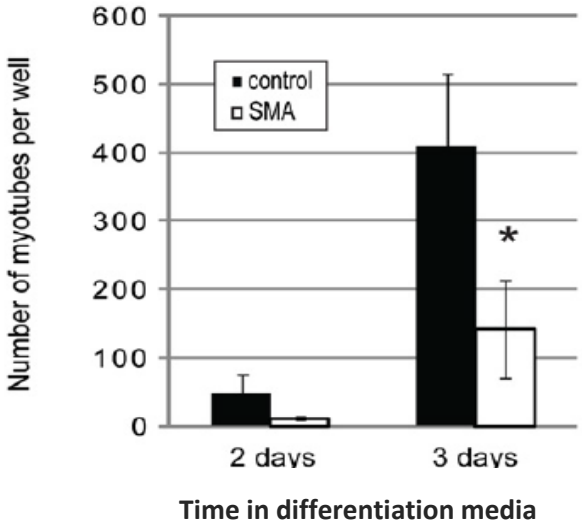
Boyer et al. (2014) HMG

SMA MYOBLASTS FORM FEWER MYOTUBES

Control

Severe SMA mouse model

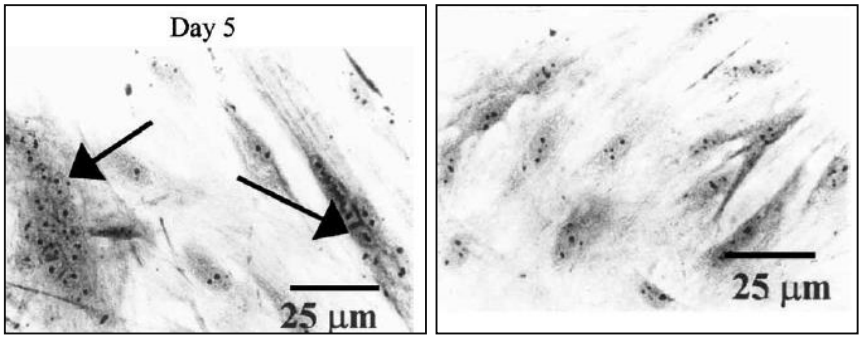


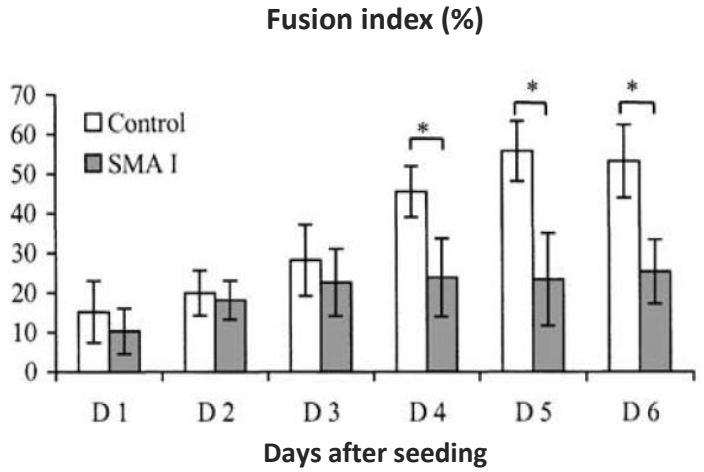


Hayhurst et al, 2012

Control

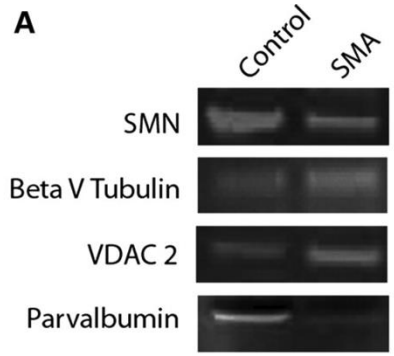
SMA Type I patient

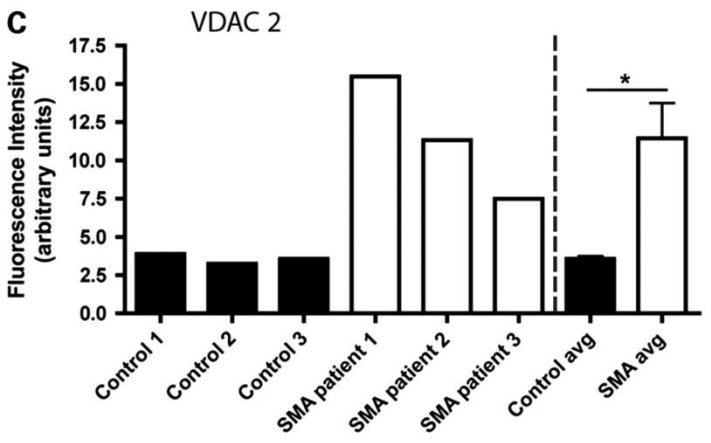


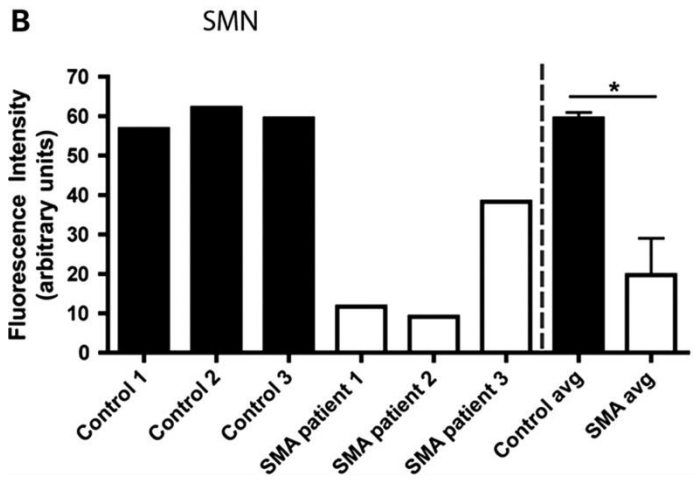


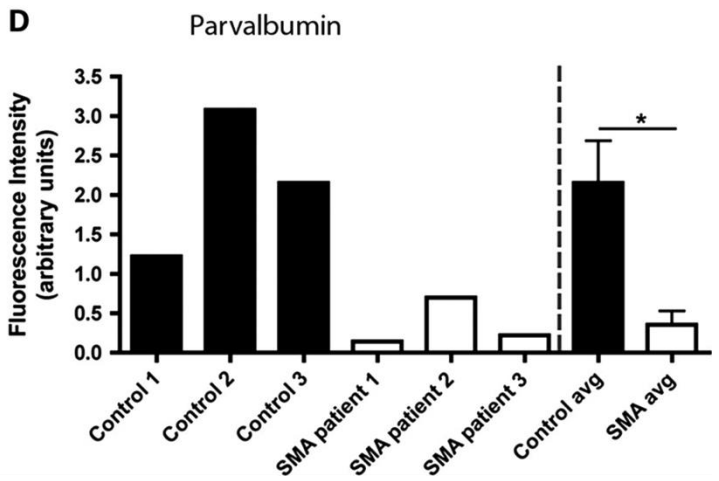
Arnold et al, 2004

MOLECULAR MODIFICATIONS IN SKELETAL MUSCLE FROM HUMAN SMA PATIENTS – IMPORTANCE OF SMN IN MAINTAINING MOLECULAR HOMEOSTASIS









Mutsaers et al. Hum mol Gen (2011) – Gillingwater lab

SUMMARY

- Smn expression is temporally down-regulated in skeletal muscle
- Total myofiber number is comparable in *Smn*$^{2B/-}$ mice
- Myonuclear number already reduced in *Smn*$^{2B/-}$ mice suggesting a problem in myoblast fusion
- Fiber caliber and length smaller in myofibers from *Smn*$^{2B/-}$ mice consistent with fusion defects
- Satellite cell number reduced in myofibers from *Smn*$^{2B/-}$ mice
- Satellite cell activation is normal in myofibers from *Smn*$^{2B/-}$ mice
- Overall, Smn depletion results in intrinsic muscle defects (delay in myogenic program, myoblast fusion, molecular homeostasis) together with muscle atrophy

DEVELOPMENT OF A NOVEL MILD MOUSE MODEL OF SMA – EXHIBITS FEATURES OF MYOPATHY IN THE ABSENCE OF MOTOR NEURON LOSS

$Smn^{2B/2B}; SMN2^{-/-}$ X $Smn^{+/-};SMN2^{+/+}$

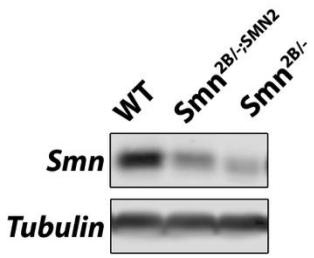
(Eshraghi & *al.* 2016) (Monani & *al.* 2000)

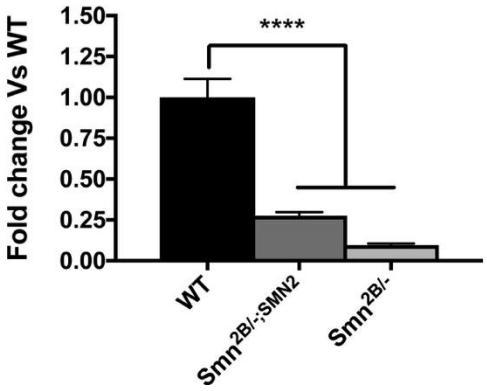
$Smn^{2B/+}; SMN2^{+/-}$

$Smn^{2B/-}; SMN2^{+/-}$

2B allele: 15% Smn
SMN2 transgene: 5-10% SMN

B



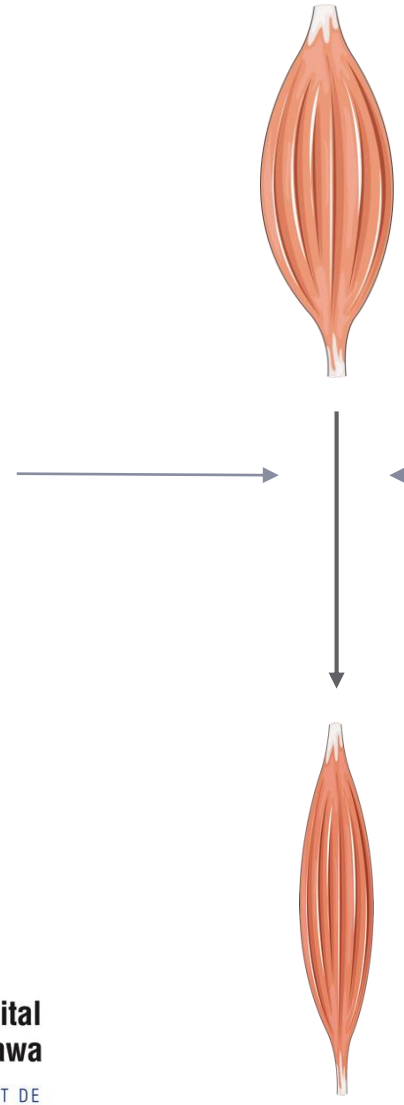


Deguise et al. unpublished

REASON FOR SMALLER MEAN FIBER SIZE REDUCTION?

(Deguise & *al.* 2016)

⇧Atrophy



(Boyer & *al.* 2014)

⇩Myogenesis

or

Hypotrophy

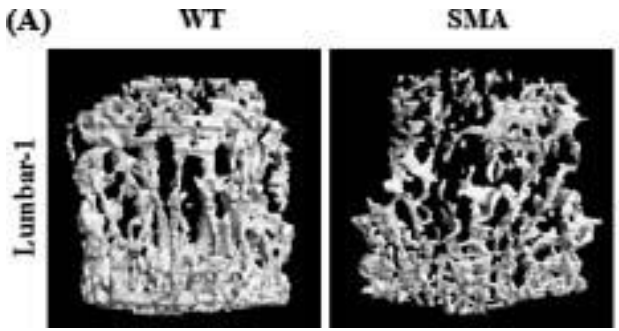
STRONG EVIDENCE FOR SMN ROLE IN MUSCLE

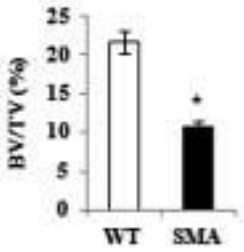
- SMA myoblasts have abnormal expression of myogenic markers and form fewer myotubes (mice and humans)
- Altered protein expression (mice and humans)
- Myopathy in the absence of a neuropathy in a new mild mouse model of SMA. Similar to the observation in the C/C mouse model.
- SMN-upregulating therapeutics restoring SMN levels in the muscle will likely be more advantageous to SMA patients compared to CNS only treatment

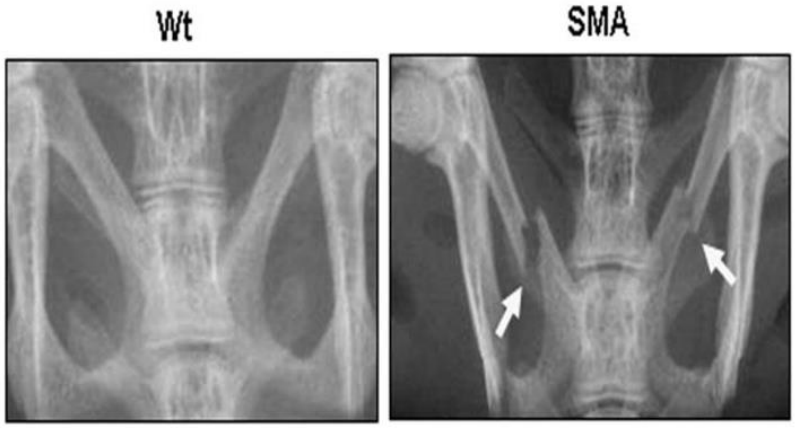
BONE DEFECTS ARE OBSERVED IN SMA MOUSE MODELS

- Decreased bone volume has been observed in SMA mouse models with various degrees of severity: *Smn$^{-/-}$SMN2* (*Shanmugarajan et al., 2009*), pharmacological model (*SMA Foundation data*), C/C (*Osborne, 2012*)

- Enhanced osteoclasts formation, bone resorption and fractures were observed in SMA mice (*Shanmugarajan et al., 2007, 2009*)







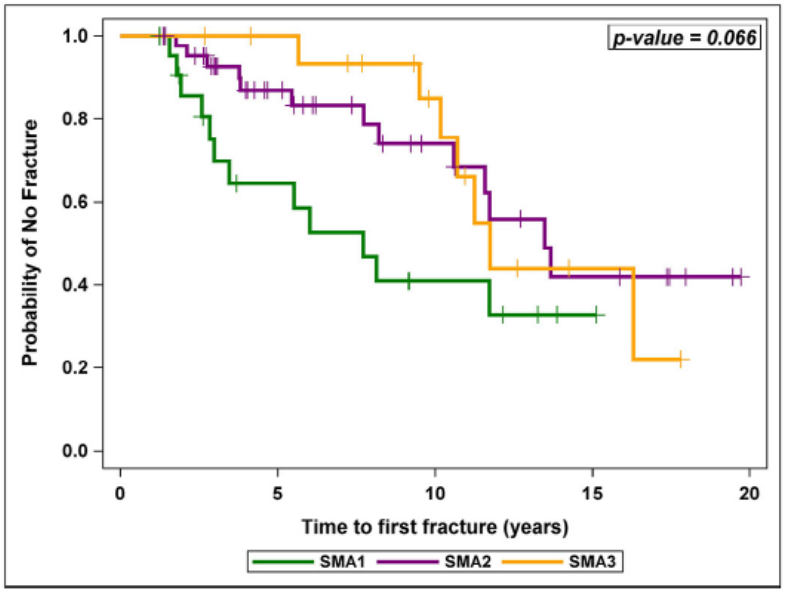
Smn$^{-/-}$SMN2

Smn$^{-/-}$SMN2

BONE COULD BE AFFECTED IN SMA PATIENTS

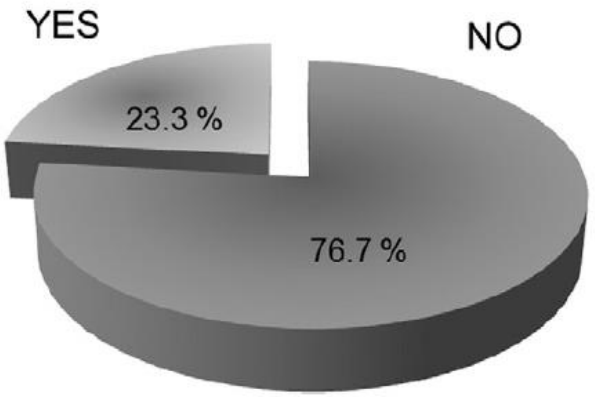
- Children with SMA Types 2 and 3 exhibit reduced bone density, increased bone resorption markers, and asymptomatic vertebral fractures (*Vai et al, 2015*)

- Children with SMA have a high prevalence of low bone mineral density and fractures (32/85 – 38%); 13% of patients fulfilled criteria of osteoporosis (*Wasserman et al, 2017*)



Wasserman et al., 2017

% of patients with vertebral fractures



Vai et al., 2015

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