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

**Muscle**
- Mouse: cKO – dystrophy
- Human: Smaller in SMA fetuses

**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

**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

<table>
<thead>
<tr>
<th>Muscle defect</th>
<th>Source</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased satellite cell number</td>
<td>Biopsies from older SMA patients C/C mice</td>
<td>Lee Sweeney, unpublished</td>
</tr>
<tr>
<td>Premature satellite cell differentiation</td>
<td>Smn(^{-/-}); SMN2(^{+/+}) mouse satellite cells</td>
<td>Hayhurst et al, 2012</td>
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<tr>
<td>Defects in cell migration, cytoskeleton organization and focal adhesions</td>
<td>Delta7 mouse myoblasts</td>
<td>Bricceno et al, 2014</td>
</tr>
<tr>
<td>Muscle maintenance defects</td>
<td>HSA-Cre; Smn(^{F7/F7}) mice Pharmacological model</td>
<td>Nicole et al, 2003 Chien-Ping Ko, unpublished</td>
</tr>
<tr>
<td>Muscle regeneration defects</td>
<td>CreER; Smn(^{F7/-}) mice C/C mice</td>
<td>Kariya et al, 2014 Lee Sweeney, unpublished</td>
</tr>
</tbody>
</table>

Courtesy of SMA Foundation
# Hypomorphic Allelic Series of Mouse Models of SMA

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Allele 1</th>
<th>Allele 2</th>
<th>Total Smn protein (% WT)</th>
<th>Phenotype</th>
<th>Median survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smn&lt;sup&gt;+&lt;/sup&gt;/+</td>
<td>50</td>
<td>50</td>
<td>100</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>Smn&lt;sup&gt;+&lt;/sup&gt;/-</td>
<td>50</td>
<td>0</td>
<td>50</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>Smn&lt;sup&gt;2B&lt;/sup&gt;/+</td>
<td>15</td>
<td>50</td>
<td>65</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>Smn&lt;sup&gt;2B&lt;/sup&gt;/2B</td>
<td>15</td>
<td>15</td>
<td>30</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>Smn&lt;sup&gt;2B&lt;/sup&gt;/- (BL6)</td>
<td>15</td>
<td>0</td>
<td>15</td>
<td>severe SMA</td>
<td>25 days</td>
</tr>
<tr>
<td>Smn&lt;sup&gt;2B&lt;/sup&gt;/- (FVB)</td>
<td>15</td>
<td>0</td>
<td>15</td>
<td>severe SMA</td>
<td>19 days</td>
</tr>
<tr>
<td>Smn&lt;sup&gt;-/-&lt;/sup&gt;;SMN2/SMN&lt;sup&gt;Δ7&lt;/sup&gt;</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>very severe SMA</td>
<td>14 days</td>
</tr>
<tr>
<td>Smn&lt;sup&gt;-/-&lt;/sup&gt;;SMN2/SMN2</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>very severe SMA</td>
<td>5 days</td>
</tr>
<tr>
<td>Smn&lt;sup&gt;-/-&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>pre-implantation lethal</td>
<td>0 days</td>
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</table>
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

Hayhurst et al, 2012

Arnold et al, 2004
MOLECULAR MODIFICATIONS IN SKELETAL MUSCLE FROM HUMAN SMA PATIENTS – IMPORTANCE OF SMN IN MAINTAINING MOLECULAR HOMEOSTASIS

**A**

<table>
<thead>
<tr>
<th>Protein</th>
<th>Control</th>
<th>SMA</th>
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</thead>
<tbody>
<tr>
<td>SMN</td>
<td>![Image](99x66 to 638x433)</td>
<td>![Image](99x66 to 638x433)</td>
</tr>
<tr>
<td>Beta V Tubulin</td>
<td>![Image](99x66 to 638x433)</td>
<td>![Image](99x66 to 638x433)</td>
</tr>
<tr>
<td>VDAC 2</td>
<td>![Image](99x66 to 638x433)</td>
<td>![Image](99x66 to 638x433)</td>
</tr>
<tr>
<td>Parvalbumin</td>
<td>![Image](99x66 to 638x433)</td>
<td>![Image](99x66 to 638x433)</td>
</tr>
</tbody>
</table>

**C**

- **VDAC 2**
  - Control 1: ![Image](99x66 to 638x433)
  - Control 2: ![Image](99x66 to 638x433)
  - Control 3: ![Image](99x66 to 638x433)
  - Control avg: ![Image](99x66 to 638x433)
  - SMA patient 1: ![Image](99x66 to 638x433)
  - SMA patient 2: ![Image](99x66 to 638x433)
  - SMA patient 3: ![Image](99x66 to 638x433)
  - SMA avg: ![Image](99x66 to 638x433)

**B**

- **SMN**
  - Control 1: ![Image](99x66 to 638x433)
  - Control 2: ![Image](99x66 to 638x433)
  - Control 3: ![Image](99x66 to 638x433)
  - Control avg: ![Image](99x66 to 638x433)
  - SMA patient 1: ![Image](99x66 to 638x433)
  - SMA patient 2: ![Image](99x66 to 638x433)
  - SMA patient 3: ![Image](99x66 to 638x433)
  - Control avg: ![Image](99x66 to 638x433)

**D**

- **Parvalbumin**
  - Control 1: ![Image](99x66 to 638x433)
  - Control 2: ![Image](99x66 to 638x433)
  - Control 3: ![Image](99x66 to 638x433)
  - Control avg: ![Image](99x66 to 638x433)
  - SMA patient 1: ![Image](99x66 to 638x433)
  - SMA patient 2: ![Image](99x66 to 638x433)
  - SMA patient 3: ![Image](99x66 to 638x433)
  - Control avg: ![Image](99x66 to 638x433)
  - SMA avg: ![Image](99x66 to 638x433)
SUMMARY

- Smn expression is temporally down-regulated in skeletal muscle
- Total myofiber number is comparable in Smn\textsuperscript{2B/-} mice
- Myonuclear number already reduced in Smn\textsuperscript{2B/-} mice suggesting a problem in myoblast fusion
- Fiber caliber and length smaller in myofibers from Smn\textsuperscript{2B/-} mice consistent with fusion defects
- Satellite cell number reduced in myofibers from Smn\textsuperscript{2B/-} mice
- Satellite cell activation is normal in myofibers from Smn\textsuperscript{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

\[ \text{Smn}^{2B/2B}; \text{SMN2}^{-/-} \times \text{Smn}^{+/+};\text{SMN2}^{+/+} \]  
(Eshraghi & al. 2016)  
(Monani & al. 2000)

\[ \begin{align*} 
\text{Smn}^{2B/+}; \text{SMN2}^{-/-} & \\
\text{Smn}^{2B/-}; \text{SMN2}^{-/-} & 
\end{align*} \]

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

Deguise et al. unpublished
REASON FOR SMALLER MEAN FIBER SIZE REDUCTION?

(Deguise & al. 2016)

↑ Atrophy

↓ Myogenesis or Hypotrophy

(Boyer & al. 2014)
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)
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)
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