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Molecular crosstalk between non-SMN-related and SMN-related spinal muscular atrophy

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Abstract:	Most cases of spinal muscular atrophy are caused by functional loss of the survival of motor neuron 1 (SMN1) gene, while less than 5% of cases are attributed to genes other than SMN. Mutations in LMNA, the lamin A/C encoding gene, cause an adult form of SMA, and in our recent work we highlight a role for lamin A/C in SMN-related SMA pathways. Here, we discuss this apparent molecular crosstalk between different types of SMA in context with previous work, showing that dysregulation of proteins produced by other SMA-causing genes, including UBE1, GARS and SETX, are also implicated in SMN-related SMA pathways. The perturbation of UBE1, GARS and lamin A/C help explain mechanisms of tissue-specific pathology in SMA, and we propose Wnt/ β -catenin signalling as a common molecular pathway upon which they each converge. Therapeutic strategies directed at these proteins, or their convergent pathways, may therefore offer a new approach to targeting tissue-specific pathology in SMN-related SMA.

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3 **Molecular crosstalk between non-SMN-related and SMN-related spinal**
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43 **Keywords:** spinal muscular atrophy, SMA, SMN, LMNA, lamin A/C, GARS, UBE1, UBA1,
44 SETX, β -catenin, Wnt/ β -catenin
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Abstract

Most cases of spinal muscular atrophy are caused by functional loss of the survival of motor neuron 1 (*SMN1*) gene, while less than 5% of cases are attributed to genes other than *SMN*. Mutations in *LMNA*, the lamin A/C encoding gene, cause an adult form of SMA, and in our recent work we highlight a role for lamin A/C in SMN-related SMA pathways. Here, we discuss this apparent molecular crosstalk between different types of SMA in context with previous work, showing that dysregulation of proteins produced by other SMA-causing genes, including *UBE1*, *GARS* and *SETX*, are also implicated in SMN-related SMA pathways. The perturbation of *UBE1*, *GARS* and lamin A/C help explain mechanisms of tissue-specific pathology in SMA, and we propose Wnt/ β -catenin signalling as a common molecular pathway upon which they each converge. Therapeutic strategies directed at these proteins, or their convergent pathways, may therefore offer a new approach to targeting tissue-specific pathology in SMN-related SMA.

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3 Spinal muscular atrophies (SMAs) are a heterogeneous group of neuromuscular disorders,
4 clinically characterised by lower motor neuron loss, and muscle weakness and atrophy, but
5 with varied age of symptom onset and severity of motor function impairments, and differential
6 involvement of other organs. SMAs are generally classified as either proximal- or distal-SMA
7 based on the limb region primarily affected by muscle weakness. Approximately 95% of SMA
8 cases are caused by homozygous functional loss of the survival of motor neuron 1 (*SMN1*)
9 gene,¹ resulting in insufficient levels of survival of motor neuron (SMN) protein. A minority
10 (i.e. <5%) of SMA cases involve genes other than *SMN1*, and to date, at least 30 different genes
11 have been attributed to cases of non-SMN related SMA (reviewed by Farrar and Kiernan²).
12 Intriguingly, experimental evidence has emerged from our recent work³ and the work of others
13 that proteins produced by several non-SMN-related SMA genes including *LMNA*,³ *UBE1*,^{4,5}
14 *GARS*,⁵ and *SETX*,⁶ are implicated in SMN-related SMA pathways.
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33 Mutations in *LMNA*, the lamin A/C encoding gene, cause a range of neuromuscular conditions
34 with prominent cardiac involvement, including an adult form of SMA,^{7,8} and our recent study
35 also proposes a role for lamin A/C in SMN-related SMA pathways.³ Frequent reports of cardiac
36 abnormalities in SMN-related SMA patients and mouse models of SMN-dependent SMA⁹
37 prompted us to study the underlying molecular pathways using quantitative proteomics
38 analysis. A key finding from this study was the identification of increased lamin A/C levels as
39 a robust molecular phenotype in the heart of SMN-related SMA mice.³ This increase would
40 inevitably increase rigidity of nuclei leading to disrupted contractile activity in cardiomyocytes,
41 and thereby provides a mechanism to explain previous reports of morphological and functional
42 cardiac defects in patients and SMA mice.⁹ Lamin A/C dysregulation was also apparent in
43 fibroblast cells from individuals with severe SMN-dependent SMA and in other tissues from
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3 SMN-dependent SMA mice, but with differing directions of expression change depending on
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5 the tissues examined.³
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10 A role for lamin A/C in SMN-dependent SMA is further strengthened by experiments in which
11 we demonstrated a mechanistic link between lamin A/C and ubiquitin-like modifier activating
12 enzyme 1 (UBA1) protein.³ Mutations in the *UBE1* gene, which encodes the ubiquitin-like
13 modifier activating enzyme 1 (UBA1) protein, cause a form of X-linked infantile SMA
14 (SMA_{X2}),¹⁰ and involvement of UBA1 in SMN-dependent pathways has also been well
15 characterised across several models of SMN-dependent SMA. UBA1 levels were reduced in
16 mouse⁴ and zebrafish models of SMN-dependent SMA,⁵ and in induced pluripotent stem cell-
17 derived motor neurons from individuals with severe SMN-related SMA.^{5,11} Pharmacological
18 or genetic suppression of UBA1 / *UBE1* phenocopied the SMA motor neuron phenotype in
19 zebrafish, thus demonstrating that UBA1 contributes directly to SMN-related SMA disease
20 pathways.⁴ Systemic restoration of UBA1 levels increased motor performance in zebrafish and
21 mouse models of SMA, as well as increased survival and improved systemic pathology in SMA
22 mice.⁵
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42 The link between lamin A/C and UBA1 is likely to involve β -catenin, since both lamin A/C¹²
43 and UBA1 are implicated in regulating β -catenin signalling,⁴ and β -catenin itself contributes to
44 SMN-dependent SMA pathways.⁴ Defective Wnt/ β -catenin signalling, for example, was shown
45 to contribute to the pathology of dilated cardiomyopathy caused by mutations in the *LMNA*
46 gene.¹² Decreased expression of several components of Wnt/ β -catenin pathway, including β -
47 catenin, was identified in the heart from a mouse model of *LMNA* cardiomyopathy, and
48 pharmacological activation of Wnt/ β -catenin signalling improved cardiac pathology in these
49 mice.¹² In a separate study, lamin A/C overexpression increased nuclear levels of β -catenin and
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3 activated the Wnt signalling pathway to promote osteoblast differentiation.¹³ Our recent study³
4 further expanded on the link between lamin A/C and β -catenin by demonstrating that they
5 interact in mouse heart extracts under normal physiological conditions. UBA1, on the other
6 hand, controls the stability of β -catenin through the canonical ubiquitin-proteasome pathway,
7 and deficiency in UBA1 protein levels leads to β -catenin accumulation and neuromuscular
8 pathology in SMN-dependent SMA.⁴ Pharmacological inhibition of β -catenin signalling, using
9 quercetin, ameliorated neuromuscular pathology in *Drosophila*, zebrafish and mouse models
10 of SMN-dependent SMA.⁴
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24 Mutations in *GARS*, the gene encoding glycine-tRNA synthetase (GARS), are responsible for
25 Charcot-Marie-Tooth disease Type 2D (CMT2D), typically characterised by sensory
26 impairment, but also cases of both childhood and adult forms of distal SMA, termed distal
27 spinal muscular atrophy type V.² Studies of GARS, which was found to be another downstream
28 target of UBA1 pathways, provided further insights into the fundamental molecular
29 mechanisms driving pathology in SMN-dependent SMA tissues.⁶ Increased expression of
30 GARS in spinal cords from a mouse model of severe SMN-dependent SMA was restricted to
31 sensory neurons and increasing the expression of UBA1 was sufficient to restore GARS levels
32 and correct sensory neuron defects in severe SMN-dependent SMA mice.⁶ UBA1/GARS
33 pathway dysregulation is therefore likely to be responsible, at least in part, for disrupted
34 sensory neuron fate and altered sensory-motor connectivity in these mice.⁶ The amino acid-
35 tRNA synthetases (ARS), of which GARS is one, form a multi-synthetase complex with three
36 scaffold proteins called aminoacyl-tRNA synthetase-interacting multifunctional proteins
37 (AIMP1, 2 and 3). A study of the intestinal epithelium and tumorigenesis found evidence of
38 enhanced Wnt/ β -catenin signalling in mice harbouring a hemizygous deletion of AIMP2,¹⁴
39 which therefore indirectly implicates ARS proteins in β -catenin signalling pathways too.
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3 Further work would be required to confirm and verify this across other cells and tissues, but it
4 does, nonetheless, raise the possibility that convergence upon Wnt/ β -catenin signalling
5 pathways could well be a common mechanistic link between UBA1, lamin A/C, and GARS in
6 SMN-dependent SMA (Figure 1).
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14 Mutations in the SETX gene, which encodes the (probable helicase) senataxin protein, can
15 cause several conditions including ataxia with oculomotor apraxia type 2, a type of
16 amyotrophic lateral sclerosis, and an autosomal dominant form of proximal spinal muscular
17 atrophy (ADSMA).¹⁵ Like SMN, senataxin is involved with maintaining RNA transcriptome
18 homeostasis¹⁵ and is another non-SMN related SMA candidate implicated in SMN-related
19 SMA pathways. Decreased senataxin protein levels were identified in fibroblasts and spinal
20 cords from individuals with SMN-dependent SMA, cultured spinal cord neurons from a mouse
21 model of SMN-dependent SMA and in SMN-deficient HeLa cells.¹⁶ Consistent with one of its
22 known functions, the decreased expression of senataxin *in-vitro* coincided with increased
23 accumulation of R-loops and double-strand breaks (DSBs).¹⁶ Restoration of SMN *in-vitro*
24 restored senataxin levels and decreased R-loop accumulation - and overexpression of senataxin
25 alone was sufficient to decrease R-loop accumulation - in SMN-related SMA patient fibroblasts
26 and cultured mouse spinal cord neurons.¹⁶ This evidence therefore suggests that senataxin is,
27 at least partially, responsible for genomic instability in SMN-related SMA *in-vitro*, most likely
28 via its helicase activity. Mutations in the gene encoding immunoglobulin mu DNA binding
29 protein 2 (IGHMBP2) – a protein with high sequence homology to the helicase domain of
30 senataxin – cause a type of severe SMA with respiratory distress,¹⁵ which further highlights
31 the importance of helicase activity in SMA disease pathways.
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3 There is no cure for any type of SMA but significant progress has been made recently in the
4 development of therapies aimed at raising full-length SMN protein levels for SMN-related
5 SMA.¹⁷ Nusinersen (Spinraza™), an antisense oligonucleotide drug, is now widely available
6 for children and young adults with SMA, and most recently, Zolgensma™, an adeno-
7 associated virus-based gene replacement therapy, was given approval by the FDA for the
8 treatment of SMA children in the United States under 2 years of age. Despite these incredible
9 advances, both strategies are extremely expensive, neither show complete efficiency,¹⁷ and
10 the long-term outcomes remain unknown. In addition, where neuronal tissue is the only target
11 of the treatment (i.e. in the case of Spinraza™), peripheral pathologies may still be a concern.
12 Consequently, there is a need for a new generation of SMA therapies that could, in
13 combination with SMN-targeted therapy, offer maximal benefit to SMN-related SMA
14 patients. Recent preclinical work has positioned five non-SMN-related SMA proteins as key
15 players in SMN-related SMA disease pathways, some of which help explain the mechanisms
16 of tissue-specific pathology in SMN-related SMA. Therapeutic strategies directed at these
17 proteins, or pathways upon which they converge, may therefore offer a new approach to
18 targeting tissue-specific pathology in SMN-related SMA.
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48 constraints.
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Author contributions: DS and HRF wrote the manuscript.

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38 **Figure 1. Proteins produced by several non-SMN-related SMA genes are implicated in**
39 **SMN-related SMA pathways.** Alterations to lamin A/C, UBA1, GARS, and SETX have been
40 reported as downstream consequences of reduced SMN. Further work has shown that UBA1
41 is able to modulate lamin A/C and GARS expression independently of SMN. UBA1 converges
42 on β -catenin signalling pathways in SMN-related SMA, and evidence from studies unrelated
43 to SMA also implicate GARS and lamin A/C in the modulation of Wnt/ β -catenin signalling.
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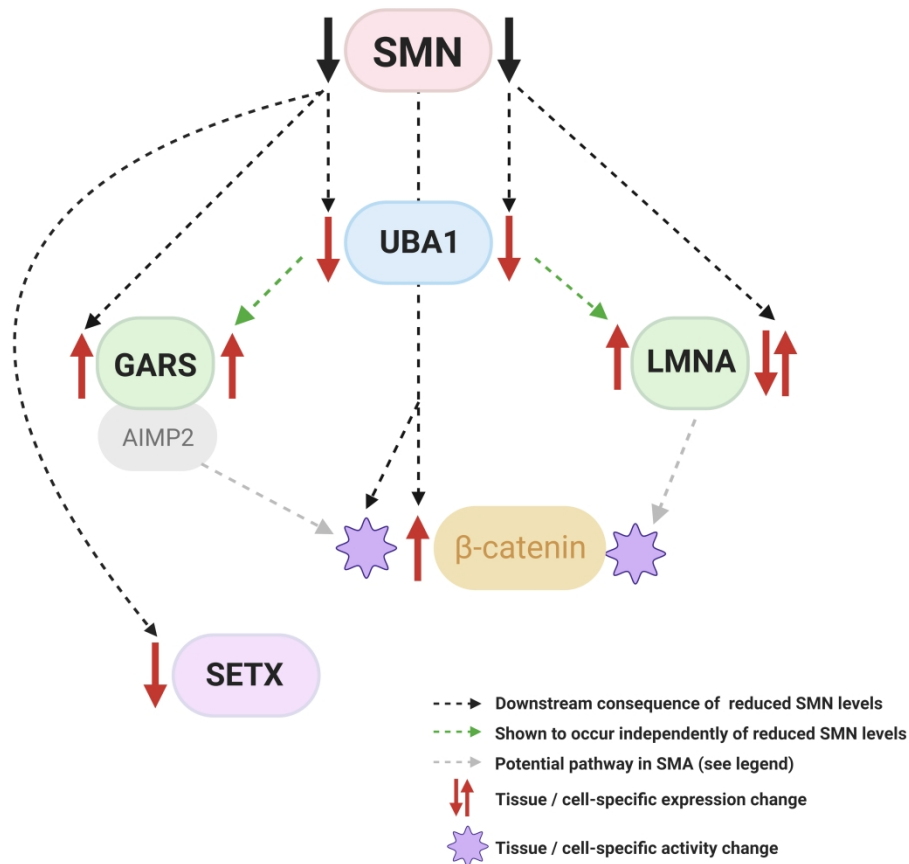


Figure 1. Proteins produced by several non-SMN-related SMA genes are implicated in SMN-related SMA pathways. Alterations to lamin A/C, UBA1, GARS, and SETX have been reported as downstream consequences of reduced SMN. Further work has shown that UBA1 is able to modulate lamin A/C and GARS expression independently of SMN. UBA1 converges on β -catenin signalling pathways in SMN-related SMA, and evidence from studies unrelated to SMA also implicate GARS and lamin A/C in the modulation of Wnt/ β -catenin signalling. Image created with BioRender.com.

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