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Michael Groden  
*Touro College*

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# Amyotrophic Lateral Sclerosis (ALS) and Spinal Muscular Atrophy (SMA): A Genetic Linkage?

Michael Groden

Michael will graduate in June 2015 with an Honors Biology B.S. degree and a minor in Psychology.

## Abstract

Neurodegenerative motor neuron disorders (MNDs) have devastating effects. Spinal Muscular Atrophy (SMA), for example, is a debilitating and sometimes lethal disease in children. SMA is monogenic, autosomal recessively inherited disorder caused by a loss-of-function mutation of surviving motor neuron 1 (SMN1). SMN2 is an identical copy of this gene and produces abbreviated transcripts without exon 7 though some full transcripts are produced that ameliorate the disease. Previous clinical trials for this disease have not produced consistent results. However, in a recent cross-sectional study, biomarkers for SMA (BforSMA), protein candidates and metabolite markers were identified (Finkel et al., 2012). These markers can be used for clinical assessment, identification of molecular pathways, and may guide response to treatment. Clinical trials of amyotrophic lateral sclerosis (ALS), another motor neuron disorder, have been uniformly disappointing without the benefit of a full understanding of ALS's mechanisms. Numerous theories attempt to explain ALS's selectivity for motor neuron degeneration, but none are conclusive. One hypothesis, gem depletion, emerges from the studies of superoxide dismutase 1 (SOD1) transgenic mice that have been discovered to contain low levels of SMN, thereby potentially linking SMA and ALS. Furthermore, SMN1 and SMN2 are seen as risk factors for ALS (Andersen & Al-Chalabi, 2011). Biomarker identification may also help in identifying ALS's pathogenesis and pathophysiology as it has begun to do for SMA. ALS and SMA may be more similar than previously thought. Both MNDs may interact in a variety of genetic and mechanistic pathways unknown at present. If so, this may serve to link seemingly disparate crippling diseases, and thereby promote efforts by government agencies and pharmaceutical companies to pursue research and development for these "orphan" diseases.

## Introduction

Progressive muscular atrophy (PMA) was first described in 1848 by F.A. Aran, who reported 11 cases of weakness and paresis of the upper limbs (Bonduelle, 1989). However, it was not until 1869 that French neurologist Jean-Martin Charcot classified amyotrophic lateral sclerosis (ALS) as a separate neurodegenerative motor neuron disorder (MND) from PMA. It was not until 1874 that ALS was given the name of amyotrophic lateral sclerosis in Dr. Charcot's "Oeuvres Completes." In his earliest studies, he noted that, "lesions within the lateral column in the spinal cord resulted in chronic progressive paralysis and contractures (no atrophy of muscles), while lesions of the anterior horn of the spinal cord resulted in paralysis without contractures (with atrophy of muscles)" (Goetz, 2000). ALS shares many clinical symptoms to other MNDs such as: spinal muscular atrophy, primary lateral sclerosis and bulbar palsy. The key to ALS's understanding was due to Dr. Charcot's unique method known as "anatomy-clinical method." Using this technique he was able to determine the correlation between clinical signs detected during life and anatomical lesions seen at death. Furthermore, post-mortem studies revealed both the anterior horn cell lesion typical of acute amyotrophy, and also the distinctive bilateral and symmetric sclerosis of the lateral spinal cord columns. Hence, he named the syndrome ALS, since it incorporated the two aspects of gray matter involvement (amyotrophy) and white matter damage (lateral sclerosis). Dr. William Gowers, though, argued with Charcot's terminology since it suggested that lateral sclerosis was primary and amyotrophy was secondary, and instead, postulated they are one event (Goetz, 2000). This is still debated over a century later.

ALS is an incurable disease that has an incidence of approximately

2 in every 100,000 people. The mean age of onset for ALS is 55-60 and affects men more than women. No diagnostic test exists for ALS. Physicians only diagnose ALS when both upper and lower motor neurons are affected and after ruling out all other causes. At first, one may notice weakness in an arm or leg, described as "limb onset" of ALS, or difficulty with speech production, known as "bulbar onset" ALS, which quickly spread to other parts of the body. Eventually, all limbs and movement cease, leading to complete paralysis. Since ALS shares several common symptoms with spinal muscular atrophy and other neurological conditions, several tests are required to diagnose. Presently, the only tests, aside from limited genetic testing, vary from electromyography (EMG) and nerve conduction study (NCS) to magnetic resonance imaging (MRI). After all these tests are performed, physicians still may not know until later stages of the disease.

Presently, the cause of ALS is unknown. Some research points out that ALS results from inaccurate protein formation. Other research demonstrates there is an excess of glutamate in the synapses causing neurite toxicity. Still other research shows changes to RNA processing. A fourth theory suggests environmental factors since U.S. military personnel in the Gulf War had higher incidence of developing ALS (Haley 2003).

Genetic mutations in ALS are numerous, for instance, superoxide dismutase 1 (SOD1), chromosome 9 open reading frame 72 (C9orf72), TAR DNA-binding protein 43 (TDP-43), senataxin (SETX) and fused in sarcoma (FUS) are a few of the growing list of genetic mutations associated with ALS. In contrast, spinal muscular atrophy (SMA) has only one genetic mutation in survival

motor neuron 1/2 (SMN1/2). SMA shares some common features with ALS, such as lower motor neuron degeneration, gem depletion and possibly biomarkers.

ALS and SMA share a biochemical pathway in gem depletion related to each disease's respective genetic mutation (TDP-43 and SMN) (Yamazaki, 2012; Groen et al., 2013; Turner, 2014; Rafałowska et al., 2014). Also, both selectively target motor neurons although SMN1, SMN2, TDP-43, SETX and FUS are ubiquitously expressed (Cauchi, 2014; Achsel et al., 2013; Tsuiji et al., 2013). A project called: Biomarker identification for SMA (BforSMA) has yielded significant results for future clinical studies (Finkel et al., 2012). ALS biomarker identification would also accelerate future clinical trials. SMA has had much more success in diagnosis and prognosis than ALS due to its monogenic nature, while many of ALS's genetic mutations are still being discovered (Keller et al., 2014). Nonetheless, there appears to be a strong relationship between these two disparate MNDs. By researching the overlap of these two MNDs a common therapeutic approach may be possible. As of yet, it remains unknown why ALS and SMA, which have genes that are ubiquitously expressed, selectively destroy motor neurons.

### Methods

Peer-reviewed articles from PubMed and UptoDate (a division of Wolters Kluwer Health) using keywords "Amyotrophic Lateral Sclerosis" "ALS" "Genetic basis of ALS" "Biomarkers for ALS" "Spinal Muscular Atrophy" "SMA" "SMA and ALS" were utilized as background for this paper. Dr. Alex Pearlman and Dr. Harry Ostrer also provided guidance in developing this thesis.

### Theories on Targeted Motor Neuron Degeneration in ALS

ALS is a heterogeneous disease with multiple pathogenic mechanisms and variable sites of disease onset and progression. Researchers currently are searching for the starting point of the disease and the reason why motor neurons are specifically targeted. Present research focuses on aberrant protein formation in axons, excess glutamate activity in neuromuscular synapses and gem depletion in cells as being responsible for ALS pathology.

The first hypothesis on ALS pathogenesis is faulty protein formation in axons. According to Dr. Zhang, at the University of Madison-Wisconsin, misfolded protein in neurons causes a cascade of events (Chen et al., 2014). Eventually, the protein is shuttled to the distal part of the axon, but becomes tangled in transport and axonal degeneration occurs. This may also explain Alzheimer's and Parkinson's disease and their pathophysiology. Through use of induced pluripotent stem cells (iPSCs), Dr. Zhang formed new nerve cells in vitro and tested his hypothesis (Chen et al., 2014). Indeed, when patient iPSCs were used, neurofilament

(NF) aggregation together with neurite swelling in spinal motor neurons (MN) resulted. "Such MN-selective NF changes were mimicked by expression of a single copy of mutant SOD1 (D90A) in human embryonic stem cells (hESCs) and prevented by genetic correction of the SOD1 mutation in patient iPSCs" (Chen et al., 2014). "A4V is the most common SOD1 mutation in the US and D90A is most common in Europe" (Giannini et al., 2010; Saeed et al., 2009). In ALS MNs bead like structures form along neurites. These bead like structures have heavy immunostaining for plasma phosphorylated neurofilament-H. Plasma phosphorylated neurofilament-H levels closely reflect disease progression in SOD1 (G93A) mice and are regarded as an ALS biomarker (Calvo et al., 2012). Therefore, the bead like structures that were heavily phosphorylated with plasma phosphorylated neurofilament-H, indicated pathogenicity. As opposed to control MNs and non-MNs that have an even staining pattern. This finding highlights the possibility of targeting NF regulation for therapeutic intervention.

A second hypothesis on the pathogenesis of ALS is excess glutamate accumulation, which causes neuro-degeneration. "Glutamate is generally acknowledged to be the most important transmitter for normal brain function" (Purves et al., 2001). Glutamate is an excitatory neurotransmitter. However, high extracellular glutamate can have toxic effects on neurons. It is synthesized in neurons from precursors. In a Human Molecular Genetics paper by Dr. Guo he explains that glial glutamate transporter, excitatory amino acid transporter (EAAT2 also known as GLT1), is responsible for removing glutamate from the synaptic cleft (Guo et al., 2003). In ALS patients, the glutamate transporter EAAT2 has been found inactive. Defective glutamate transport and loss of EAAT2 protein have also been observed in affected brain regions of patients with Alzheimer's disease (Masliah et al., 1996). Overactivation of glutamatergic neurons can result in a neurodegenerative process known as excitotoxicity i.e. cell death. (Guo et al., 2003). "However, it is still unknown whether it is a primary cause in the cascade leading to neuron degeneration or a secondary event to cell death" (Guo et al., 2003). After experimentation with transgenic mice, containing increased expression of EAAT2 and SOD1 variation (G93A) degeneration of neurons was slowed, but did not cease. The results "suggest that the loss of EAAT2 may contribute to, but does not cause, motor neuron degeneration in ALS" (Guo et al., 2003). Transgenic mice with the common mutations of SOD1 exhibit neurodegeneration comparable to ALS. The SOD1 mutation results in a toxic gain of function rather than a loss of enzymatic function (Wong et al., 1995). Furthermore, in a Nature paper by Dr. Rothstein he demonstrates  $\beta$ -lactam antibiotics, for example, ceftriaxone, a semi-synthetic, third generation cephalosporin antibiotic, has been used to treat bacterial infections (Rothstein et al., 2005). These antibiotics also show no substantial toxic CNS effects. Additionally, they have found it to increase gene expression

of GLT1. Leading to neuroprotection by increasing glutamate transporter expression. The mechanism of this overexpression appears to be activation of the genetic promoter for GLT1, although the pathway for promoter activation is, as yet, unknown. (Rothstein et al., 2005). This too provides another possible intervention of ALS, which is currently in phase 3 clinical trials (Berry et al., 2013). Note that the only drug currently available for ALS is Riluzole. It provides no relief of symptoms, but slows the progress of the syndrome by decreasing glutamate accumulation.

### ALS and SMA Overlap

A third hypothesis for ALS disease onset is gem depletion. Gems or gemini of Cajal bodies are protein products of SMN, TDP-43, FUS and SETX located in nuclear foci and in the cell cytoplasm responsible for the assembly of small nuclear ribonucleoproteins (snRNPs) (Tsuiji et al., 2013; Achsel et al., 2013; Cauchi, 2014). In spinal motor neurons with TDP-43 mutations there are depleted gems, snRNPs and small nuclear ribonucleotides (snRNAs) (Tsuiji et al., 2013). TDP-43 and FUS localize within nuclear gems together with the SMN complex and are involved in the maintenance of the spliceosome by controlling levels of snRNA (Tsuiji et al., 2013). It was further shown that accumulation of spliceosomes cause aberrant splicing of mRNAs resulting in motor neuron death in ALS and SMA. To clarify what gems are, how they relate to SMN, TDP-43 and FUS and the link between spliceosomes and motor function, Dr. Ruben Cauchi explains succinctly the RNA processing pathway. The pathway starts with the SMN-Gemins complex consisting of a nine-membered union of diverse proteins. More specifically, these are SMN, seven Gemin proteins (Gemin2-Gemin8) (Carissimi et al., 2006) and Unrip (Carissimi et al., 2005). The SMN-Gemins complex establishes the snRNP assemblyosome. "snRNPs are composed of one or two short noncoding RNA molecules (snRNAs) bound to a set of seven Smith (SM or Sm-like (Lsm) proteins, and a unique set of snRNP-specific proteins" (Matera, Terns, & Terns, 2007).

Working together with numerous non-snRNP splicing factors, U1, U2, U4/U6 and U5 snRNPs form the more abundant spliceosome that is responsible for splicing introns from pre-mRNA; as opposed to, U11, U12, U4atac/U6atac and U5 snRNPs that constitute the less available spliceosome (Patel & Steitz, 2003). The Lsm-class U6 and U6atac snRNPs, though, are synthesized in the nucleus while the core structure of the remaining Sm-class snRNPs is assembled in the cytoplasm. The published work on these interactions is fairly recent, and it is still not fully understood why *in vivo* requires the action of the SMN-Gemins complex (Otter et al., 2007) and involves the uploading of a heptameric Sm D1/D2/E/F/G/D3/B ring-shaped core domain onto the "Sm site," a conserved uridine-rich sequence motif intrinsic to snRNAs (Cauchi, 2014).

The snRNAs expelled from the nucleus following transcription are tagged by SMN-Gemins complex-independent Gemin5 protein. Once Gemin5 is charged with snRNAs it binds with SMN-Gemin complex, proximate to Gemin2, to form the Sm core assembly. Gemin2 is essential for the majority of Sm proteins recognition to form the ring-shaped domain. It also blocks foreign RNA binding supposedly until bona fide RNA substrates, snRNAs, are identified. (Zhang et al., 2011) (Figure 1). SMN's function in snRNP assembly, according to many, is the most decisive of all the SMN-Gemins complex members (Cauchi, 2014) and is probably linked to axonal mRNP trafficking (Fallini, Bassell, & Rossoll, 2012; Briese, Esmaeili, & Sattelle, 2005). Proper Sm core assembly is necessary not only for stability and function of snRNPs, but also for snRNP biogenesis, including cap hypermethylation, 3' terminal trimming and eventual import into the nucleus. Once in the nucleus, snRNPs mature in Cajal Bodies (CBs) prior to pre-mRNA splicing. After multiple splicing procedures, snRNPs return to the CBs where they are regenerated or recycled (Staněk et al., 2008).

Mutations in the copy numbers of SMN1 and the identical gene of SMN2 are the principal etiologic basis of SMA cases. The number of copy numbers in SMN2 is inversely correlated with disease severity. One of the main theories explaining SMA is improper mRNA processing. According to the above, the fewer normal SMN proteins cause a lack of SMN-Gemins complex essential for pre-mRNA splicing (Borg & Cauchi, 2013; Briese et al., 2009). Phenotypic SMA effects have been modeled in fly, zebrafish and mouse models with insufficient levels of SMN protein (Burghes & Beattie, 2009; Briese et al., 2009). Gemins mutations, however, have not been associated with SMA. Investigators hypothesize SMN is unique in human genetics and a SMN2-like pseudogene in any of the Gemins would be incompatible with life. Dr. Cauchi conducted an experiment with organisms containing reduced levels of Gemins selectively in motor neurons. Organisms with this defect develop similar motor deficits found in the attenuation of SMN. Therefore, inadequate levels of any member in the SMN-Gemins complex can cause motor deficits. Decreased capacity of the SMN-Gemins complex can account for neuromuscular selectivity, based on sequencing of RNA from microdissected motor neurons of presymptomatic SMA mice; there are specific transcriptome abnormalities that link SMN deficiency to motor neuron pathology in SMA (Zhang et al., 2013).

The function of Gems is thought to colocalize with CBs in later developmental stages. Gems are viewed as storage depots for supplementary SMN-Gemins complexes since upregulation of SMN or Gemins induces gem formation or increases gem numbers (Cauchi 2011, Turner et al., 2014). Additionally, when there is loss of SMN or Gemins there is substantial decrease in gems and CBs.

ALS and SMA phenotypes affect lower motor neurons in the anterior horn of the spinal tract. There is a common tract and biochemical pathway that both MNDs share. ALS can also affect the upper motor neurons. Two of the more common genetic mutations found in ALS are TDP-43 and FUS. (Millecamps et al., 2010; Tsai et al., 2011). Surprisingly, these two genes engage in RNA processing. Under pathogenic conditions, the proteins of each are found concentrated in the cytoplasm and not in the nucleus in both neuronal and glial cells. This suggests there is a loss of proper nuclear function or toxic gain of function pertinent to ALS pathogenesis (Andersen & Al-Chalabi, 2011; Ferraiuolo et al., 2011; Lagier-Tourenne, Polyimenidou, & Cleveland, 2010).

SMN has been found in decreased levels in transgenic SOD1 murine models (Turner, 2009) and SMN protein levels were reduced in ALS patients (Turner, 2014). This agrees with previous evidence relating decreased copy numbers of SMN2 to increased severity of ALS. (Veldink, 2005). Furthermore, loss of the SMN2 protein caused gem depletion in motor neurons, and knockout mice of TDP-43 showed altered numbers of gems (Shan et al., 2010). In ALS patient derived cells with TDP-43 or FUS mutations, the gem numbers are significantly reduced too (Yamazaki et al., 2012). Also, biochemical experiments have shown that TDP-43 and FUS interact with the SMN-Gemins complex (Yamazaki et al., 2012, Tsuiji et al., 2013).

### Biomarker Identification

Since both ALS and SMA are swift and crippling diseases, early diagnosis is essential for prognosis and future treatment options. Although, genetic testing is a current diagnostic tool for SMA, it does not test for all mutations in SMA, and is a premium that most cannot afford. Additionally, it does not provide a test for treatment response. Previous clinical trials for both ALS and SMA have produced no complete treatments for humans. There is a need to develop biomarkers that can help deliver quicker results from research into practice. At the same time, biomarkers facilitate the discovery of novel targets and pathways in pathogenesis.

One of the most successful approaches for diagnosis is biomarker identification, incorporating: proteomics, metabolomics and transcriptomics. Biomarker identification is not new to translational medicine. Biomarkers have been used tremendously in the cancer field and they are beginning to show promise in SMA too. After a recent cross-sectional study in Biomarkers for SMA (BforSMA), the top 5 biomarker candidates: CILP2, TNXB, COMP, ADAMTSL4 and CLEC3B may be used for diagnosis and testing response to treatment after further testing (Finkel (2012)). A longitudinal study will be necessary to qualify the results of BforSMA. The BforSMA isolated the candidate plasma proteins, metabolites based on type of SMA presented since type I will produce possible differences than type II or III. Transcripts, though, did not

provide significant candidates in the BforSMA. The potential from the BforSMA is tremendous for future clinical trials and reduction in patients and costs associated with studies. ALS would also benefit from biomarker identification.

All of the BforSMA are only from peripheral blood mononuclear cells. As a result, there was an absence of change in gene expression or splicing suggesting that the degree of reduction of the SMN protein in this tissue is not enough to cause dramatic changes. This would be consistent with the fact that blood and other tissues do not exhibit the change of cellular or organ function except in Type I SMA. Since SMN protein is known to have reduction concurrent with genetic mutations in ALS, some of the biomarkers not tested for in BforSMA may serve as an interesting study for a common biomarker between these two seemingly disparate diseases.

Following the BforSMA study, a publication by Dr. Robert Bowser outlines biomarkers that have been discovered recently in transgenic models that may best represent ALS patients, along with prognostic determinants for ALS. Some of the biomarkers highlighted are SOD1 in the cerebrospinal fluid (CSF), increased levels of NF in blood/CSF, increased expression of CD4+ T cells (Bakkar, Boehringer, & Bowser, 2014). Some of the positive prognostic markers for ALS are low pNF-H, high sCD14 and low S100B in blood/CSF (Bakkar, Boehringer, & Bowser, 2014). A disadvantage of these biomarkers is that they are only tested from transgenic models unlike the BforSMA, which tested human SMA patients. As with the BforSMA, longitudinal studies are necessary to qualify these findings.

There is significant evidence of genetic and mechanistic interaction between SMA and ALS. Unfortunately the low rate incidence of ALS – approximately 2 in every 100,000 people – has limited the expenditure of resources into the understanding of the pathogenesis and treatment of this disease. Thus any linkage between ALS and the more common disorder of SMA may prove extremely beneficial. With further research on the shared pathways and possible common biomarkers of ALS and SMA, therapies of MNDs may emerge that may combat the devastating consequences of both of these neurodegenerative disorders.

### Future Directions

To further clarify the potential linkage, between SMA and ALS, both the limb- and bulbar- onset types of ALS must be brought under a more uniform classification scheme that recognizes the homogeneity of these diseases which may then be applied to SMA. The elucidation of the genetic make-up of ALS may also lead to a common gene for multiple sites of origin. Moreover, some genetic mutations in ALS may also be shown to augment symptoms common in SMA. The fact that both of these MNDs affect

lower motor neuron function and share a common biochemical pathway in pathogenesis may lead to other insights into the interaction between these MNDs. Biomarker identification will likely prove extremely useful in future clinical trials as a potential possible diagnostic tool.

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