Pathogenic commonalities between spinal muscular atrophy and amyotrophic lateral sclerosis

Citation for published version:

Digital Object Identifier (DOI):
10.1016/j.ejmg.2017.12.001

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
European journal of medical genetics

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
Pathogenic commonalities between spinal muscular atrophy and amyotrophic lateral sclerosis: converging roads to therapeutic development

Melissa Bowerman¹,², Lyndsay M Murray³, Frederique Scamps⁴, Bernard L Schneider⁵, Rashmi Kothary⁶, Cedric Raoul*⁷

¹ Department of Physiology, Anatomy and Genetics, University of Oxford, UK

² Current address: Institute for Science and Technology in Medicine, Keele University, Staffordshire, UK and; Wolfson Centre for Inherited Neuromuscular Disease, RJAH Orthopaedic Hospital, Oswestry, UK

³ Euan McDonald Centre for Motor Neuron Disease Research and; Centre for Integrative Physiology, University of Edinburgh, Edinburgh, UK

⁴ The Institute for Neurosciences of Montpellier, Inserm UMR1051, Saint Eloi Hospital, Montpellier, France

⁵ Brain Mind Institute, Ecole Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

⁶ Regenerative Medicine Program, Ottawa Hospital Research Institute, Ottawa, Canada and; Departments of Medicine and Cellular and Molecular Medicine, University of Ottawa, Ottawa, Canada

* corresponding author: cedric.raoul@inserm.fr
ABSTRACT

Spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS) are the two most common motoneuron disorders, which share typical pathological hallmarks while remaining genetically distinct. Indeed, SMA is caused by deletions or mutations in the survival motor neuron 1 (SMN1) gene whilst ALS, albeit being mostly sporadic, can also be caused by mutations within genes, including superoxide dismutase 1 (SOD1), Fused in Sarcoma (FUS), TAR DNA-binding protein 43 (TDP-43) and chromosome 9 open reading frame 72 (C9ORF72). However, it has come to light that these two diseases may be more interlinked than previously thought. Indeed, it has recently been found that FUS directly interacts with an Smn-containing complex, mutant SOD1 perturbs Smn localization, Smn depletion aggravates disease progression of ALS mice, overexpression of SMN in ALS mice significantly improves their phenotype and lifespan, and duplications of SMN1 have been linked to sporadic ALS. Beyond genetic interactions, accumulating evidence further suggests that both diseases share common pathological identities such as intrinsic muscle defects, neuroinflammation, immune organ dysfunction, metabolic perturbations, defects in neuron excitability and selective motoneuron vulnerability. Identifying common molecular effectors that mediate shared pathologies in SMA and ALS would allow for the development of therapeutic strategies and targeted gene therapies that could potentially alleviate symptoms and be equally beneficial in both disorders. In the present review, we will examine our current knowledge of pathogenic commonalities between SMA and ALS, and discuss how furthering this understanding can lead to the establishment of novel therapeutic approaches with wide-reaching impact on multiple motoneuron diseases.
INTRODUCTION

Motoneuron diseases (MNDs) encompass a group of devastating neurodegenerative disorders characterized by the progressive and selective degeneration of motoneurons in the spinal cord and/or the brain. Amongst MNDs, spinal muscular atrophy (SMA) and amyotrophic lateral sclerosis (ALS) are the most common in children and adults, respectively. SMA is a monogenic disease whereby over 95% of cases are due to deletions or mutations within the survival motor neuron 1 (SMN1) gene (Lefebvre et al., 1995). ALS can be sporadic (~80%) or familial (~20%) (Andersen and Al-Chalabi, 2011), and in the latter case can be caused by numerous genetic mutations with the most common being in chromosome 9 open reading frame 72 (C9ORF72) (DeJesus-Hernandez et al., 2011; Renton et al., 2011), superoxide dismutase 1 (SOD1) (Rosen et al., 1993), Fused in Sarcoma (FUS) (Kwiatkowski et al., 2009; Vance et al., 2009) and TAR DNA-binding protein 43 (TDP-43) (Gitcho et al., 2008; Kabashi et al., 2008; Sreedharan et al., 2008). SMA and ALS are thus genetically distinct (Andersen and Al-Chalabi, 2011; Lefebvre et al., 1995) and have therefore traditionally been considered as separate disorders. However, accumulating evidence suggests that functional interactions between the protein products of causative genes of both of these MNDs may influence each other's pathological traits. Indeed, FUS directly interacts with Smn, whereby ALS-linked FUS mutations stabilize the FUS-SMN interaction and lead to cytosolic redistribution of SMN, reduction of intracellular small nuclear bodies (called Gemini of coiled bodies, Gems), altered levels of small nuclear RNA and axonal defects (Groen et al., 2013; Sun et al., 2015; Yamazaki et al., 2012). Furthermore, mutant SOD1 perturbs Smn localization (Gertz et al., 2012; Kariya et al., 2012), Smn depletion aggravates disease progression of SOD1 mutant mice (Turner et al., 2009), overexpression of SMN in SOD1 and TDP-43 mice significantly improves their phenotype and lifespan (Perera et al., 2016; Turner et al., 2014), TDP-43 overexpression enhances correct splicing of the SMN gene (Bose et al., 2008) and duplications of SMN1 have been linked to sporadic ALS (Blauw et al., 2012). Recently, mutations in profilin 1, a monomeric actin binding protein that inhibits the assembly of filamentous actin, have been identified in a subset of ALS patients (Wu et al., 2012). Interestingly, profilin 1 interacts with SMN and is found within the cytoplasm and Gems of motoneurons (Giesemann et al., 1999). While studies have mainly focused on the role of profilin 2 in SMA (Bowerman et al., 2007; Nölle et al., 2011), profilin 1 highlights actin dynamics as a common denominator between ALS and SMA (Hensel and Claus, 2017).

It has thus become obvious that SMA and ALS share more similarities than initially thought and this implication has a direct and significant impact on how we pursue the development of therapeutical strategies that could be...
applicable to SMA and ALS as well as other MNDs. Indeed, uncovering of shared pathophysiological events has great potential for large-scale clinical applications. While SMA and ALS are classified as canonical MNDs, several investigations in pre-clinical models and patients have demonstrated the contribution of tissues and cells from both the central nervous system (CNS) and the periphery to disease severity and progression (Hamilton and Gillingwater, 2013; Loeffler et al., 2016). In the present review, we will thus take a whole-body approach to discuss the pathological commonalities between SMA and ALS, focusing on skeletal muscle, neuroinflammation, immune organ dysfunction, metabolic perturbations, defects in neuronal excitability and selective motoneuron vulnerability. In addition, we will expand on how targeted gene therapy approaches could be exploited to treat shared symptoms in both diseases.

SELECTIVE MOTONEURON VULNERABILITY

One of the key commonalities that unite SMA and ALS is the selective vulnerability of motoneurons. Importantly, however, not all populations of motoneurons are equally vulnerable as some are lost very early in disease while others remain relatively intact even at late stages. A good example is the motoneurons that innervate the extraocular muscles, which are typically spared in SMA and ALS patients and animal models (Comley et al., 2016; Gizzi et al., 1992; Kubota et al., 2000; Spataro et al., 2014; Tjust et al., 2012; Valdez et al., 2012). This preserved eye movement is frequently exploited as a means of communication by SMA and ALS patients (Kubota et al., 2000; Spataro et al., 2014). The observation that selective pools of motoneurons are resistant in MNDs is intriguing. Understanding the basis for this selective resistance and vulnerability could provide insight into the molecular effectors that dictate vulnerability, and uncover novel therapeutic approaches. Identifying common patterns or mechanisms of selective vulnerability between SMA and ALS motoneurons would allow for the development of treatment strategies that may have a wide-reaching potential in several MNDs.

A number of studies have profiled the patterns of selective vulnerability in motoneurons of both patients and animal models of SMA and ALS. The pattern of motoneuron loss in SMA is described as extremely stereotyped (Deymeer et al., 2008), whereby motoneurons innervating proximal lower and upper limb muscles are lost before those innervating distal limb muscles, highlighted by the highly consistent pattern of motor unit loss within the muscles of the arm and thigh (Deymeer et al., 2008). There is also a selective vulnerability of motoneurons innervating the core muscles of the abdomen and back while cranial motoneurons, as discussed above, are spared (Kubota et al., 2000). The differential vulnerability of motoneurons has been much more extensively documented in mouse models of SMA. Indeed, motor units fall somewhere on a spectrum of vulnerability and
display a predictable pattern of motoneuron loss in distinct muscles (Ling et al., 2012; Murray et al., 2013; Thomson et al., 2012). For example, synaptic defects occur earlier and are more pronounced in medial motoneurons innervating axial muscles compared to lateral motoneurons innervating distal limb muscles (Mentis et al., 2011). Interestingly, selective vulnerability has even been observed within the same muscle, whereby the caudal band of the *levator auris longus* muscle is consistently more vulnerable than its rostral band in severe SMA mice (Murray et al., 2008).

Conversely, the pattern of motoneuron loss in ALS is somewhat less predictable and is perhaps due to the variety of genetic and sporadic causes of this disease (Andersen and Al-Chalabi, 2011). Furthermore, ALS can manifest as both a bulbar or spinal onset and the location of onset in the spinal forms can equally be highly variable (Shellikeri et al., 2017). Despite this, mouse models of ALS based on a single genetic mutation have revealed a consistent and predictable pattern of motoneuron loss (Valdez et al., 2012).

Predictable patterns of motoneuron loss as well as selective vulnerability and resistance of particular pools of motoneurons therefore seem to be common features between SMA and ALS. Whether there is commonality in the patterns of selective vulnerability between SMA and ALS is somewhat more difficult to address. Indeed, whilst it is easy to find examples of muscles which commonly demonstrate high levels of denervation (e.g. *tibialis anterior*) it is equally simple to identify disparities, such as with the *extensor digitorum longus*, which is seemingly very vulnerable to denervation in ALS, but comparatively resistant in SMA (Boyd et al., 2017; Thomson et al., 2012; Valdez et al., 2012). Interestingly, a recent study has profiled the vulnerability of tongue, extraocular and deep lumbrical muscles in mouse models of SMA and ALS, and identified a remarkable similarity in the relative vulnerability associated with each muscle (Comley et al., 2016), an approach that could be further extended to include additional muscles and models. Ultimately, however, the different patterns of selective vulnerability between SMA and ALS are perhaps not surprising. Multiple factors impact the vulnerability status of an individual motor unit, one of which is age (Murray et al., 2011) as demonstrated by distinct patterns of selective vulnerability in SMA mouse models at differing ages (Murray et al., 2008, 2013).

It is therefore perhaps more relevant to look at the properties of motoneurons to determine whether there are common factors that render them more or less vulnerable to disease. Work on mouse models of SMA and ALS have eliminated morphological factors such as body position, motor unit size and fiber type of innervated muscle (Thomson et al., 2012; Valdez et al., 2012). However, it has been suggested that sprouting competence may
play a role in directing relative vulnerability as the analysis of three distinct ALS mouse models revealed that motor units with a relatively increased capacity to sprout were comparatively less vulnerable to degeneration (Frey et al., 2000). Motoneurons thus fall into different developmental categories distinguished in adulthood by their sprouting competence (Pun et al., 2002). Differential sprouting competence also correlates with selective neuronal vulnerability in mouse models of SMA (Murray et al., 2008, 2013). Interestingly, two independent studies aimed at identifying transcriptional differences between differentially vulnerable motoneurons in SMA and ALS reported that the expression of IGF-2, a factor that promotes motoneuron sprouting, is a predictive indicator of resistance to disease-induced degeneration (Hedlund et al., 2010; Murray et al., 2015). Furthermore, overexpression of IGF-2 prevents motoneuron loss in mouse models of SMA and ALS, and extends lifespan of ALS mice (Allodi et al., 2016).

The IGF-2 work indicates that shared molecular mechanisms may underlie selective vulnerability in SMA and ALS. In an attempt to identify vulnerability modifiers, recent studies have investigated transcriptional differences between motoneuron populations, whereby differentially susceptible pools of motoneurons were defined in patients or mouse models of SMA or ALS, and the equivalent populations were isolated from neurologically healthy humans, rats or mice (Boyd et al., 2017; Brockington et al., 2013; Hedlund et al., 2010; Kaplan et al., 2014; Murray et al., 2015). Transcriptional analysis of these motoneuron populations revealed a large number of differentially expressed transcripts with previously validated functions in neurodegenerative pathways. We have recently re-analyzed this collection of transcriptional screens to identify commonalities between the data sets (Kline et al., 2017), thus producing a refined list of transcripts commonly altered in differentially vulnerable motoneurons in SMA and ALS. One candidate modifier was alpha-synuclein, which in addition to its well characterized role in Parkinson’s disease, also has previously described neuroprotective properties (da Costa et al., 2000; Hashimoto et al., 2002; Manning-Bog et al., 2003). Indeed, overexpression of alpha-synuclein in a mouse model of SMA led to a significant extension in lifespan and a marked preservation of neuromuscular junctions (NMJs) (Kline et al., 2017). Thus, understanding the molecular mechanisms that regulate the differential vulnerability of healthy and diseased motoneurons can lead to the identification of neuroprotective pathways, give insight into the mechanistic similarities between SMA and ALS (Figure 1), and ultimately provide the potential to develop therapeutic strategies beneficial in both diseases.
DEFECTS IN NEURONAL EXCITABILITY

An additional shared pathological feature between SMA and ALS is an abnormal excitability of motoneurons due to extrinsic and intrinsic factors. The loss of synapses on spinal motoneurons is one of the key pathological features seen in SMA mice. The primary synapses affected in SMA are formed by glutamatergic VGlut1+ proprioceptive afferent axons on the soma and dendrites of motoneurons, inducing a decrease in monosynaptic excitatory potentials (Mentis et al., 2011; Neve et al., 2016). In addition, an increased frequency of motoneuron postsynaptic events is also observed, which could be attributed to the VGlutT2+ afferent terminals (Gogliotti et al., 2012). Interestingly, motoneuron-specific overexpression of Smn in SMA mice abolished the increased frequency of postsynaptic potentials and prevented the loss of VGlut1+ synapses, which supports a motoneuron-dependent effect on the loss of these synaptic contacts (Gogliotti et al., 2012).

SMA motoneurons are also intrinsically hyperexcitable (Gogliotti et al., 2012; Mentis et al., 2011), which has been attributed to a lower voltage threshold for action potential triggering and variable increase in input resistance. SMA motoneurons also display an increased persistent inward current (PIC) amplitude that contributes to firing pattern (Heckman et al., 2009). Specific restoration of Smn in SMA mice allowed for the correction of SMA motoneuron hyperexcitability and decreased the post-synaptic excitatory potentials (Gogliotti et al., 2012). This study strongly supports the proposal that defects in intrinsic motoneuron excitability also contribute to synaptic abnormalities. Induced pluripotent stem cells (iPSCs) from SMA patients also display hyperexcitability due to increased membrane input resistance, hyperpolarized threshold, larger action potential amplitude and increased firing frequency (Liu et al., 2015). The increase in Na⁺ current amplitude associated with decreased time for reactivation in SMA iPSCs is expected to participate in the increased propagation of excitability towards the NMJ. Thus, intrinsic defects in SMA neuronal excitability may have a pathological impact both at the soma and at the nerve terminal.

In ALS, excitotoxicity appears to be a major mechanism leading to motoneuron death and contributing significantly to disease pathogenesis (King et al., 2016). Contrary to SMA, the influence of other cell types on altering motoneuron electrical activity in ALS is well established and has lead to the hypothesis of a non-cell-autonomous mechanism as a key contributor to disease progression and presentation. Notably, in ALS patients and animal models, glutamate clearance by astrocytes is defective due to decreased expression of the excitatory amino acid transporter 2 (EAAT2), leading to glutamate-induced excitotoxicity (Van Den Bosch et al., 2006). Moreover, the low expression of the GluR(2) α-Amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
(AMPA) receptor subunit by vulnerable motoneuron populations may render them unduly susceptible to calcium-mediated toxic events following glutamate receptor activation (Williams et al., 1997). Expression of atypical calcium-permeable AMPA receptors by human motoneurons provides a possible mechanism whereby disturbances of glutamate neurotransmission in ALS may selectively injure this cell group.

Similar to SMA motoneurons, neuronal cells in ALS also demonstrate intrinsic excitability perturbations. Indeed, there is epidemiological evidence showing that increased axonal excitability related to PIC is strongly associated with shorter survival rates in ALS patients (Kanai et al., 2012). Furthermore, the only therapeutic approved for use in ALS, riluzole, is thought to block PIC (Schuster et al., 2012). The increase in PIC is also observed in murine ALS motoneuron primary cultures (Kuo et al., 2005) and iPSCs-derived motoneurons from ALS patients demonstrate hyperexcitability characteristics (Wainger et al., 2014). Several experimental and computational studies point to relationships between motoneuron dendritic morphology and membrane biophysical properties that either reduce or increase membrane resistance and thus excitability (Amendola and Durand, 2008; Elbasiouny et al., 2010; Martin et al., 2013). However, in vivo recordings of murine adult ALS motoneurons reveals no changes or hyperexcitability (Delestrée et al., 2014). Thus, while intrinsic hyperexcitability seems to be a hallmark of SMA motoneurons, some controversy still exists for ALS that could be due to differential motoneuron susceptibility, the time course of disease progression and dendritic morphological changes. Having a better understanding of the similarities between the intrinsic electrical activities of SMA and ALS motoneurons (Figure 1) could help in the design of pharmacological treatment approaches that could potentially restore pathologies in both diseases.

**INTRINSIC MUSCLE DEFECTS**

As a consequence of motoneuron loss, both SMA and ALS muscles display significant NMJ defects in both the pre- and post-synaptic compartments, defined by denervation, neurofilament accumulation, reduced endplate size, immature endplate morphology and aberrant neurotransmission (Mélissa Bowerman et al., 2012; Fischer et al., 2004; Kariya et al., 2008; Kong et al., 2009; Murray et al., 2008; Rizzuto et al., 2015; Sharma et al., 2016). Although muscle pathology in both SMA and ALS has traditionally been considered as a consequence of motoneuron degeneration, accumulating evidence strongly suggests that intrinsic muscle defects exist and contribute to disease progression and presentation.
One of the first indications of the role of muscle in SMA was the report that conditional knockout of Smn in skeletal muscle results in a severe muscle dystrophy phenotype (Cifuentes-Diaz et al., 2001). Conversely, Smn restoration specifically in muscle through the use of the myogenic regulator factor (MRF) MyoD promoter improved survival and motor behavior to a similar extent than that obtained with Smn restitution under the motoneuron-specific promoter ChAT (Martinez et al., 2012). Interestingly, complete myofiber size rescue was only observed when using the MyoD promoter, despite no NMJ improvement (Martinez et al., 2012), suggesting a distinct role for SMN in muscle. Histopathological muscle abnormalities have also been highlighted in SMA mice, some of which occur pre-symptomatically, revealing reduced myofiber size, increased number of centrally located nuclei, muscle weakness, increased cell death and fewer myofibers in hind limb muscles (Mélissa Bowerman et al., 2012; Boyer et al., 2013, 2014; Cifuentes-Diaz et al., 2001; Dachs et al., 2011; Hammond et al., 2010; Hsieh-Li et al., 2000; Le et al., 2005; Monani et al., 2000; Nicole et al., 2003). Furthermore, muscle satellite cells, the primary source of progenitors for postnatal muscle growth and regeneration (Wang and Rudnicki, 2011), differentiate abnormally and display a reduced efficiency in myotube formation in severe SMA mice (Hayhurst et al., 2012).

The myogenic developmental program is typically characterized by satellite cells undergoing a sequential repression of Pax7 and activation of MRFs, including myogenic factor 5 (Myf5), myoblast determination protein (MyoD), myogenin, and muscle-specific regulatory factor 4 (Le Grand and Rudnicki, 2007; Seale et al., 2000). Central nucleation is a common feature of newly generated muscle fibers and the observation of increased centrally nucleated fibers in SMA muscle suggests that Smn deficiency may stimulate myogenesis and/or slow down maturation of newly generated fibers. Indeed, immortalized myoblasts with reduced Smn expression levels display abnormal proliferation, aberrant myoblast fusion and malformed myotubes (Shafey et al., 2005), most likely a consequence of abnormal MyoD, myogenin and Pax7 expression (Bricceno et al., 2014). Combined, these in vitro experiments further support a role for Smn in myoblast proliferation and differentiation, independently from the neuronal input. Analysis of SMA patient muscle biopsy cultures shows an aberrant Myf-5 expression (Guettier-Sigrist et al., 2002). We have also demonstrated a pronounced dysregulated expression of Pax7 and MRFs in muscle of SMA mice, even in non-denervated muscles (Boyer et al., 2014). Muscle precursor cells also display defects in SMA, whereby satellite cells with reduced Smn protein levels differentiate abnormally (Hayhurst et al., 2012). Moreover, elevated levels of Smn in satellite cells markedly increase the number of regenerating myofibers following muscle-specific depletion of Smn in mice (Nicole et al., 2003).
strongly indicating a tight association between Smn and the function of satellite cells. Myogenic program abnormalities have also been documented at the level of muscle microRNAs (miRNA), with the reported dysregulation of miR-206, -486, -9 and -132, in Smn-depleted myoblast cells and SMA mouse muscle (Bricceno et al., 2014; Catapano et al., 2016). There is therefore clear evidence that loss of Smn results in intrinsic muscle defects, although it remains to be further defined what are the molecular mechanisms involved and how alterations of the myogenic program affect myofiber maturation, satellite cell formation and muscle homeostasis.

Not surprisingly, emerging evidence also supports an active role for skeletal muscle in ALS pathogenesis. Indeed, the muscle-restricted expression of mutant SOD1 results in skeletal muscle atrophy, reduced muscle strength, activation of anti-oxidant response, mitochondrial dysfunction, motor function deficits, increased cell death, contractile apparatus, NMJ and motoneuron degeneration (Dobrowolny et al., 2008; Wong and Martin, 2010). Similar to SMA mice, aberrant genetic, biochemical and physiological changes in ALS muscle are observed prior to motoneuron loss. Indeed, investigations in pre-symptomatic ALS mice demonstrate an altered electrophysiological activity in diaphragm (Rocha et al., 2013), aberrant expression of proteins involved in cellular metabolism and cytoskeletal processes (Capitanio et al., 2012), differential expression of genes implicated in Wnt, phosphoinositide 3-kinase, and epithelial-mesenchymal transition signaling cascades (de Oliveira et al., 2014) and increased muscle weakness (Derave et al., 2003). The impact of ALS-causing mutations on the myogenic regulatory program has also been addressed. Analysis of skeletal muscle from SOD1 mutant mice reveals aberrant expression of the Pax7, Myf5, MyoD and Myogenin transcripts at various stages of disease (Manzano et al., 2011). Furthermore, satellite cells isolated from these mice have a reduced proliferation rate (Manzano et al., 2013), an observation that was also noted in satellite cells from ALS patients (Pradat et al., 2011; Scaramozza et al., 2014). Interestingly, adenoviral-mediated overexpression of myogenin in muscle of SOD1 ALS mice improved motoneuron survival and NMJ innervation while using the same strategy to increase MyoD had a negative influence on the same parameters (Park et al., 2013). Finally, analysis of miRNA profiles of muscle from ALS rodents and patients has identified several candidates such as miR-206, -1, -133a, -133b, -145, -21, -24, -424 and -214 as being significantly differentially expressed compared to healthy control tissue (de Andrade et al., 2016; Sumitha et al., 2014; Toivonen et al., 2014).

Skeletal muscle is a highly plastic tissue, adapting its structure and metabolism in response to diverse physiological and pathological conditions. Accumulating evidence suggests shared intrinsic muscle pathologies between SMA and ALS (Figure 2), such as early muscle weakness and fatigability, dysregulated myogenic
program and altered satellite cell function, all of which contribute to muscle dyshomeostasis. The disrupted interactions between satellite cells, myonuclei, myofibers and NMJs may therefore exacerbate disease pathology in these MNDs. Regardless of whether the muscle defects are intrinsic or not, therapeutic targeting of muscle should be a critical consideration when devising treatment plans for these devastating diseases. Indeed, muscle-targeted therapeutic strategies (e.g. myostatin/follistatin pathway, skeletal muscle troponin activator, IGF-1, PGC-1α, Tweak/Fn14) (Bosch-Marcé et al., 2011; Bowerman et al., 2015; Holzbaur et al., 2006; Morrison et al., 2009; Rose et al., 2009; Shefner et al., 2012; Thau et al., 2012) have already resulted in significant improvements in survival and/or pathological features of SMA and ALS pre-clinical models, some of which are currently in clinical trials.

**NEUROINFLAMMATION AND IMMUNE ORGAN DYSFUNCTION**

More than 30 years ago, the first signs of astrocytic disturbance were observed in the nervous system of SMA and ALS patients (Brock and Mcllwain, 1984). Since, a wealth of studies has demonstrated that both astrocyte and microglial cells shift to an activated phenotype, defining a neuroinflammatory context that accompanies the neurodegenerative process. Non-cell-autonomous components that mediate neuro- and peripheral inflammation are thus taking a key role in the selective and progressive loss of motoneurons.

In experimental models of ALS, different strategies have been employed to explore cell-autonomous and non-cell-autonomous pathogenic mechanisms in the context of neuroinflammation (Kanning et al., 2010). Chimeric mice comprising a mixture of wild-type and mutant cells revealed that both non-neuronal and neuronal cells act in concert to provoke disease (Clement et al., 2003). While a toxic action of mutant SOD1 within motoneurons is crucial for onset and early phase of the disease (Boillée et al., 2006; Wang et al., 2009), mutant SOD1 in astrocytes can affect, depending on the mutation, onset and early phase of the disease (Wang et al., 2011), or only disease progression (Yamanaka et al., 2008). In either case, astrocytic SOD1 mutant modulates the extent of the inflammatory response by controlling microglia activation (Wang et al., 2011; Yamanaka et al., 2008). In *vitro* studies with co-cultures of rat, mouse or human stem cell-derived motoneurons on SOD1 mutant expressing astrocytes further demonstrated that astrocytes release soluble factors that are selectively toxic for motoneurons (Aebischer et al., 2011; Di Giorgio et al., 2007, 2008; Marchetto et al., 2008). Several mechanisms that initiate or sustain the neuroinflammatory environment have been proposed, which puzzlingly encompass both deleterious and protective facets (Bowerman et al., 2013).
While not yet as extensive as in ALS, increasing evidence also underlines the importance of non-cell-autonomous factors in SMA pathogenesis with some striking commonalities between both diseases. Indeed, activated astrocytes, monocytes, macrophages and increased levels of pro-inflammatory cytokines are found in the spinal cord of SMA patients (Kuru et al., 2009; Rindt et al., 2015). Concordantly, an activated astrocyte phenotype is observed in the spinal cord of SMA mice (McGivern et al., 2013), which is similar to ALS, and takes place before motoneuron soma loss in SMA animals (Dachs et al., 2011; McGivern et al., 2013). Microglia activation is per contra detected later than astrogliosis in SMA mice (Tarabal et al., 2014), but their contribution to SMA pathogenesis remains largely elusive. Conversely, convincing lines of evidence support the contribution of astrocytes to SMA disease progression. Indeed, overexpression of SMN specifically in astrocytes of SMA mice led to a two- to three-fold increase in lifespan, decreased muscle atrophy, improved motor functions and increased NMJ innervation (Rindt et al., 2015). Interestingly, the loss of glutamatergic excitatory synapses, mainly from proprioceptive afferents, was significantly reduced (Rindt et al., 2015), which could be due to previously discussed contribution of proprioceptive pre-synaptic glutamate transmission on motoneuron excitability and functional integrity (Fletcher et al., 2017).

In addition to similar neuroinflammation histopathology, common molecular components between SMA and ALS neuroinflammatory networks can be identified. SMA iPSC-derived astrocytes show elevated basal calcium (Ca$^{2+}$) levels and deficits in internal Ca$^{2+}$ signalling that are associated with ERK activation and decreased production of a potent motoneuron survival factor, the glial-derived neurotrophic factor (GDNF) (McGivern et al., 2013). Aberrant Ca$^{2+}$ homeostasis is also detected in astrocytes expressing mutated SOD1 (Almad et al., 2016) and can influence neuronal excitability and synaptic transmission. Indeed, SMA astrocytes display defects in supporting synaptic formation and excitatory transmission in vitro, which was associated with decreased levels of the Ephrin ligand, Ephrin B2, known to control synapse formation and plasticity (Zhou et al., 2016). Aberrant expression of EphrinB2 was also found in reactive astrocytes in the spinal cord of SOD1 ALS mice (Urban, et al., 2015). Soluble factors released by SOD1 mutant astrocytes are known to increase motoneuron excitability though persistent sodium inward currents, increase firing rates and frequency of Ca$^{2+}$ transients prior to death of motoneurons (Fritz et al., 2013) (Figure 3).

Activation of ERK pathway in astrocytes might also contribute to the inflammatory environment by inducing the production of pro-inflammatory cytokines such as IL-1β, IL6 and TNFα, previously reported to be increased in the spinal cord of SMA mice (Rindt et al., 2015). Indeed, AAV-mediated overexpression of SMN in astrocytes
resulted in decreased levels of IL-6 and TNFα within the spinal cord of SMA mice (Rindt et al., 2015). Elevated levels of the inflammasome component IL-1β and phosphorylated ERK is also observed in spinal cord astrocytes of ALS mice and patients (Chung et al., 2005; Johann et al., 2015). The secretion of IL-6 by ALS astrocytes can be elicited by the pathogenic pro-inflammatory effector TWEAK (Bowerman et al., 2015), or by the cerebrospinal fluid (CSF) obtained from ALS patients, which in addition to promote TNFα production, leads to decreased astrocytic expression of GDNF by astrocytes (Mishra et al., 2016). The decreased ability of SOD1 mutant astrocytes to support motoneuron survival can be ameliorated by GDNF (Das and Svendsen, 2015). Thus, ERK-dependent neuroinflammation appears to be a shared pathological pathway in SMA and ALS spinal cords (Figure 3).

Additional shared non-cell-autonomous mechanisms have also been recently reported in a microglial cell line. In BV2 cells, SMN was shown to regulate the TRAF6-NF-κB pathway whereby SMN depletion leads to a sustained IL-1β-induced activation of the NF-κB subunit as well as increased production of TNFα and nitric oxide (NO) (Kim and Choi, 2017). In ALS mice, NF-κB activation occurs in microglial cells as disease progresses. SOD1 mutant-expressing microglial cells exert a cytotoxic effect toward motoneurons through an NF-κB-dependent mechanism, which is associated with TNFα and NO production in vitro. NO, through activation of the Fas (CD95) death pathway, as well as TNFα were both shown to trigger death of motoneurons (Raoul et al., 2002; Ugolini et al., 2003). In addition, microglial-specific reduction of NF-κB activation significantly reduced astrogliosis and microgliosis as well as delayed progression, but not onset, of the disease (Frakes et al., 2014). Interestingly, TDP-43 interacts with the NF-κB subunit p65 in neurons and glial cells, though predominantly in microglia, in spinal cord of ALS patients. Overexpression of mutant or wild-type TDP-43 enhanced the cytotoxicity of LPS-challenged primary microglial cells and was accompanied by increased production of NO (Swarup et al., 2011). Finally, TRAF6-NF-κB involvement in activated ALS microglia is supported by the observation that its activation is potentiated by the presence of extracellular SOD1 mutant (Kinsella et al., 2016) (Figure 3). The link between neuroinflammatory events in ALS and SMA is further highlighted by the decreased activation of astrocytes and microglia cells in mutant TDP-43 ALS mice overexpressing S11n (Perera et al., 2016).

More systemic factors, including defects in lymphoid organs, can be identified as being common between the two pathologies. Splenic pathology, abnormal architecture and differential size and weight of the spleen has been observed in SMA mice and patients (Deguise et al., 2017; Thomson et al., 2017). These spleen defects are
associated with pathologic pulp architecture and abnormal distribution of macrophages, B and T lymphocytes. An increased number of CD4^+ and CD8^+ T cells, B lymphocytes and altered ratio of F4/80-CD11b macrophage subpopulations was also documented in symptomatic SMA mice (Khairallah et al., 2017). Size reduction of the spleen and its abnormal architecture were also reported in ALS mice (Banerjee et al., 2008; Finkelstein et al., 2011; Seksenyan et al., 2010), accompanied by an increased proportion of T cells and activated natural killer (NK) T cells (a distinct subset of T lymphocytes), a reduced proliferative capacity of T cells, a diminished staining of the B-cell marker CD19, an increased percentage of apoptotic and necrotic T and B cells, a decreased number of CD4^+ T cells and an increased proportion of CD8^+ T cells. Despite some discrepancies between studies, the overall picture depicts a more systemic damage caused by genetic factors associated with ALS and SMA that should be attentively considered.

In addition to the spleen, abnormal architecture of the thymus is also observed in an SMA mouse model whereby thymic dysplasia is characterized by defective intrathymic T cell development, increased apoptosis as well as increased production of inflammatory cytokines such as IL-6, IL-1β and TNFα (Deguise et al., 2017). In ALS mice, thymic involution with loss of tissue structure is accompanied by a reduction of all thymocyte populations during the course of the disease while a decreased thymic output is strikingly observed in ALS patients (Seksenyan et al., 2010).

Primary defects in neuroimmunity and in the peripheral immune system or other peripheral defects should be therefore further studied in ALS and SMA to better ascertain shared pathological mechanisms. While in ALS, the findings accumulated over the years have started to reveal some interesting functions that could be therapeutically targeted, in SMA, this field of investigation has just begun and could lead to significant observations.

WHOLE-BODY METABOLIC DYSHOMEOSTASIS

SMA and ALS are both characterized by whole-body metabolic perturbations that if better understood, could lead to the development of therapeutic strategies to restore metabolic homeostasis. The influence of metabolism on SMA and ALS pathogenesis is highlighted by the fact that both dietary and exercise interventions, which are direct modulators of the metabolic state (López-Otín et al., 2016), have been demonstrated to impact disease progression.
In severe SMA mice that die pre-weaning, a maternal diet comprising 9% fat (PicoLab20) significantly increased lifespan and improved neuromuscular phenotype compared to a diet of 5.2% fat (Harlan-Teklad 22/5) (Butchbach et al., 2010). Furthermore, dietary modulation (PicoLab20) combined with an SMN-targeted pharmacological intervention (D156844) had a beneficial synergistic effect on survival in SMA mice (Butchbach et al., 2014). Similar observations were obtained in pre-clinical studies combining the histone deacetylase inhibitor trichostatin A (TSA) with a nutritional supplementation cocktail consisting of Vitamin B, infant formula, rodent diet softened with syrup, flavored jelly, whey protein, nutritional shakes and bacon softies (Narver et al., 2008). In patients, while direct effects of specific diet regimens on disease onset and progression have not been performed, it is clear that nutritional management of SMA patients is critical for overall health. There is an urgent need for research endeavours on how nutrient utilization and maintenance of metabolic homeostasis influences disease progression disease (Davis et al., 2014; Mehta et al., 2016).

In ALS, the available pre-clinical and clinical information on the role of diet is more extensive. Recently, a regression analysis of 302 ALS patients revealed an association between a diet high in fruits, vegetables, antioxidants and carotenes and functional measurements (Nieves et al., 2016). A smaller prospective randomized double-blind study in 16 ALS patients suggests that a diet enriched with milk whey protein results in weight gain and ameliorated biochemical serum markers (Silva et al., 2010). In TDP-43A315T mutant mice, adding a high-fat jelly to their diet significantly increased their lifespan while restoring the bioenergetic balance (Coughlan et al., 2016). In SOD1G93A mice, a high-fat diet (21% fat, 0.15% cholesterol) increased weight, survival and spinal cord motoneuron numbers while a calorie-restricted diet (60% of average ad libitum food intake) had the opposite effect compared to animals fed on a regular rodent chow (Zhao et al., 2015). Variable extents of improvement on lifespan and/or neuromuscular phenotype have also been reported in SOD1G93A and SOD1G86R mice on specific dietary regimens such as the Deanna protocol (Ari et al., 2014), a ketogenic diet (Zhao et al., 2006), extra virgin olive oil (Oliván et al., 2014), vitamin D3 (Gianforcaro and Hamadeh, 2012), a high calorie diet (Dupuis et al., 2004) and vitamin E (Gurney et al., 1996). Finally, similar to SMA studies, a combinatorial approach of dietary (21% fat and 0.15% cholesterol Calorie Energy supplemented Diet) and drug (M30) interventions has a synergistic benefit on survival and motor function (Golko-Perez et al., 2016).

As mentioned above, activating metabolic pathways via exercise also impacts SMA and ALS disease pathogenesis. In SMA mice, daily wheel running from post-natal day 10 prolongs survival, prevents motoneuron loss and improves both motor function and cardiac defects (Biondi et al., 2012; Grondard et al., 2005). A follow-
up study further showed that Smn-depleted animals subjected to a 10-month running or swimming program had improved neuromuscular pathology, energetic metabolism, motor function and muscle fatigue compared to sedentary animals (Chali et al., 2016). In ALS patients, various exercise regimens demonstrate similar benefits on muscle strength (Bohannon, 1983), spasticity (Drory et al., 2001), ALS functional rating scale (Bello-Haas et al., 2007; Drory et al., 2001; Lunetta et al., 2016), and quality of life defined by Short Form-36 (Bello-Haas et al., 2007). In SOD1<sup>G93A</sup> mice, a 10-week treadmill program significantly increased lifespan compared to sedentary animal (Kirkinezos et al., 2003). Similarly, swimming-based training improves motor function, delays motoneuron death and also increases survival (Deforges et al., 2009). It is important to note that the positive effects of exercise on survival and neuromuscular phenotype appear to be dependent on training intensity and type of activity (Deforges et al., 2009; Mahoney et al., 2004). There are presently several clinical trials for SMA and ALS patients to determine the therapeutic benefits of exercise and their results will most likely provide added enlightenment to the discussed pre-clinical and small-scale clinical studies.

While it remains unclear whether intrinsic metabolic defects in specific cells and tissues such as motoneurons, skeletal muscle, glial cells and lymphoid organs or a systemic metabolic dysregulation are the source of dyshomeostasis in SMA and ALS, patients nevertheless present syndromes that have well-documented severe functional consequences. Indeed, instances of hyperinsulinemia (Bowerman et al., 2014; Davis et al., 2015), insulin resistance (Davis et al., 2015; Reyes et al., 1984), hyperlipidemia (Dahl and Peters, 1975; Dedic et al., 2012), hyperglycemia (Melissa Bowerman et al., 2012; Shimizu et al., 2011), hyperleptinemia (Kölbel et al., 2017), aberrant fatty acid metabolism (Pradat et al., 2010; Zolkipli et al., 2012), hypoglycemia (Bruce et al., 1995), hyperglucagonemia (Melissa Bowerman et al., 2012; Hubbard et al., 1992), glucose intolerance (Davis et al., 2015; Pradat et al., 2010) and development of diabetes (Borkowska et al., 2015; Hamasaki et al., 2015) have all been reported in SMA and ALS patients and animal models. As we move along therapeutic progress for both diseases, it will be interesting to see if the gene-targeted therapies correct the metabolic abnormalities described herein or if they will have to be complemented with interventions aimed at restoring metabolic homeostasis.

TARGETED GENE THERAPIES

Because of the lack of effective pharmacological treatments, the possibility of gene therapy has attracted particular attention in the context of fatal MNDs. In particular, the possibility to restore SMN in SMA patients by delivering a transgene encoding a fully functional SMN protein has appeared as a rational approach for this monogenic disease. Identifying a vector-based system to deliver the transgene specifically in motoneurons has
long remained a major hurdle to successful gene therapy. Indeed, targeting motoneurons along the entire spinal cord as well as other neurons involved in spinal and supraspinal motor circuits, represents a major challenge. In addition, although motoneurons display a selective vulnerability in both SMA and ALS, it is evident, as discussed above, that other CNS and non-CNS cell types also have important pathological contributions and optimally should be targeted by gene therapy approaches (Hamilton and Gillingwater, 2013; Imlach et al., 2012; Lalancette-Hebert et al., 2016; Lobsiger and Cleveland, 2007; Simone et al., 2016).

The development of vectors derived from the adeno-associated virus (AAV) was a major step towards effective gene therapy against MNDs. AAVs are small-sized viral particles that have the capability to efficiently transduce post-mitotic cells within the rodent and primate CNS. To achieve widespread transduction of the CNS, the vector can be delivered either directly into the CSF or systemically via the bloodstream, an approach that is also applicable to non-human primates (Bevan et al., 2011). In particular, AAV9 vectors have a remarkable ability to cross the blood-brain barrier (BBB), a feature which has been further evolved by modifying the AAV9 capsid to generate the PHP.B variant (Deverman et al., 2016; Duque et al., 2009; Foust et al., 2010). Importantly, when delivered to the bloodstream or to the CSF, AAV vectors also transduce peripheral organs such as the liver, which may have implications for the treatment of SMA (Bevan et al., 2011; Dirren et al., 2014). Intravenous injection of a self-complementary AAV9-SMN vector has shown therapeutic efficacy in mouse models of SMA (Dominguez et al., 2011; Foust et al., 2010; Valori et al., 2010). These proof-of-principle experiments have prompted a phase I clinical trial in 1-8 months old severe SMA Type I patients with a high-dose administration of the vector (clinicaltrials.gov: NCT02122952). Although the long-term outcome of this treatment is still unknown, clear therapeutic efficacy has already been reported. Some of the treated children are now able to sit unassisted and all of them have reached month 13.6 without any adverse event, an age at which only 25% would have been predicted to survive the disease. If the dramatic effect of the treatment is confirmed over longer term, this trial will be a milestone achievement, opening the path for further gene therapy approaches for MNDs.

It is however unclear if a similar gene therapy can be applied to ALS patients. While AAV-SMN gene therapy can provide some therapeutic benefits in a mouse model of TDP-43-mediated ALS (Perera et al., 2016), gene therapy for ALS faces additional challenges. As the treatment will be administered to patients near the time of disease onset, vector systems need to be adapted for delivery to the adult CNS. It is unlikely that intravenous injections can be considered as the dose of AAV9 particles needed to target the adult CNS via this route of delivery will be too high, unless more efficient vectors can be developed. Instead, AAV vectors can be injected
directly into the CSF of adult mice and non-human primates to target either motoneurons or astrocytes (Dirren et al., 2014; Meyer et al., 2015; Samaranch et al., 2012). Alternatively, injection of AAV vectors into skeletal muscle can target the innervating motoneurons. This approach can be used to treat individual muscles that are critically affected by the disease, such as the diaphragm, but cannot be envisaged for large portions of the skeletal musculature, which consists of more than 300 bilateral muscles in the human body (Towne et al., 2010, 2011).

The genes directly associated with ALS such as SOD1, TARDBP, FUS and C9ORF72, are the most evident targets for gene therapy. AAV-based therapeutic vectors for RNA interference against SOD1 mutants are under development for the treatment of this familial form of ALS (van Zundert and Brown, 2017). However, when it comes to treating sporadic ALS, which represents 90% of the cases, other effectors should be considered. It is therefore critical to identify gene targets that may support the survival and function of diseased motoneurons. As discussed above, a rational approach to identify key factors is to explore cell- and non-cell-autonomous pathways similarly affected across MNDs such as SMA and ALS. It is therefore likely that treatments will need to be designed to manipulate these molecular targets in a cell type-specific manner. By combining expression systems that are preferential for a given cell type with AAV capsids with adequate tropism, it is possible to engineer vectors that selectively induce transgene expression either in certain types of neurons or glial cells, or in the skeletal muscles (Figure 4) (Colin et al., 2009; Dirren et al., 2014; Kügler, 2016; Wang et al., 2008). These vector systems could be used in both ALS and SMA to rescue the activity of glial cells that support motoneurons or at the level of skeletal muscle to protect NMJs. Overall, gene therapy increasingly appears as a promising approach to tackle degenerative MNDs. As we move forward, it will be critical to identify the key molecular targets that control motoneuron dysfunction and death, and devise precise and effective vector systems to rescue the cell types that are therapeutically relevant.

CONCLUSION

In the present review, we have discussed the cellular and mechanistic pathological similarities between SMA and ALS (Figure 5), two common and devastating MNDs. From the primary motoneuron target to peripheral tissues and systems, understanding the commonalities between both diseases will be of utmost benefit for the development of wide-reaching therapeutic strategies. It is also important to consider that the similarities between the two MNDs do not lie in specific molecular effectors but in general dysfunctional pathways, which are easier to modulate with one single treatment approach. In addition, identifying key dysregulated pathways may elucidate regulatory networks between cells and tissues, potentially further uncovering primary and secondary
causes of disease etiology. Using non-genetic injury-induced models of neurodegeneration, muscle atrophy and neuroinflammation will also provide insight into general vs disease-specific mechanisms. Nevertheless, an integrated combinatorial approach encompassing targeted gene therapy as well as pharmacological, dietary and exercise interventions at various stages during disease progression, will most likely become the optimal strategy to alleviate the CNS and non-CNS defects that arise during the lifetime of SMA and ALS patients.
REFERENCES


ACKNOWLEDGEMENTS
This work was supported by a grant from the E-Rare-2 program (FaSMALS 31ER30_160673), institut national de la santé et de la recherche médicale (Inserm). M.B. was an SMA Trust Career Development Fellow while at the University of Oxford. C.R. and B.S. are supported by a joint research grant from the Swiss National Science Foundation and ANR (grant 310030L_156460). We are grateful to Angelo Lepore for sharing information about the contribution of EphrinB2 in ALS.
FIGURE LEGENDS

Figure 1. Similarities between SMA and ALS motoneurons.

Figure 2. Similarities between SMA and ALS skeletal muscle.

Figure 3. Schematic illustrating the common cellular and molecular events that can influence motoneuron integrity. Astrocytic- or microglia-derived signals include IL-6, IL-1β, TNFα and NO. NO can perpetuate inflammatory status and, similar to TNFα, directly act on motoneurons to trigger death signaling. Reduced levels of the astrocyte-derived GDNF, a potent neurotrophic factor, can influence motoneuron survival while reduced levels of astrocytic EphrinB2 can influence motoneuron synaptic plasticity. Intracellular and extracellular mechanisms converge in both SMA and ALS to NF-κB signaling, which plays an important function in governing microglia reactivity.

Figure 4. AAV characteristics for optimal SMA and ALS gene therapy.

Figure 5. Tissues and cells that share common functional, physiological and molecular pathologies in SMA and ALS.
Selective vulnerability of tongue, extraocular and deep lumbrical motoneurons

Correlation between vulnerability and sprouting competence

IGF-2 as a marker of differentially vulnerable motoneurons

α-synuclein as a marker of differentially vulnerable motoneurons

Abnormal electrophysiological properties
Pre-symptomatic functional, histological and molecular intrinsic defects

Aberrant expression of the myogenic regulatory program

Satellite cell dysregulation

Differential expression of miRNAs
ERK activation

[Ca^{2+}]_i homeostasis

IL-6

IL-1β

TNFα

GDNF

Ephrin B2

neurotrophic support

synaptic plasticity

death signalling

motoneuron

astrocyte

microglia

mutant SOD1

TRAF6

NF-κB

SMN

(IKK)

TNFα

NO

Figure 1
Bowerman M et al.
SMA and ALS pathogenic commonalities

- Spinal cord
- Motoneuron intrinsic excitability
- Neuroinflammation
- Resistance of extraocular muscles
- Sprouting competence
- Denervation
- Endplate morphology
- Thymic output
- Architecture
- Thymic output
- Size and architecture
- Distribution of lymphocytes

- Neuromuscular junction
- Muscle fiber
- Myogenic regulatory program
- Metabolism

Figure 2
Bowerman M et al.