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Impact of traumatic brain injury on amyotrophic lateral sclerosis: from bedside to bench

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Franz CK, Joshi D, Daley EL, Grant RA, Dalamagkas K, Leung A, Finan JD, Kiskinis E. Impact of traumatic brain injury on amyotrophic lateral sclerosis: from bedside to bench. *J Neurophysiol* 122: 1174–1185, 2019. First published May 22, 2019; doi:10.1152/jn.00572.2018.—Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by the loss of upper and lower motor neurons, which manifests clinically as progressive weakness. Although several epidemiological studies have found an association between traumatic brain injury (TBI) and ALS, there is not a consensus on whether TBI is an ALS risk factor. It may be that it can cause ALS in a subset of susceptible patients, based on a history of repetitive mild TBI and genetic predisposition. This cannot be determined based on clinical observational studies alone. Better preclinical models are necessary to evaluate the effects of TBI on ALS onset and progression. To date, only a small number of preclinical studies have been performed, mainly in the superoxide dismutase 1 transgenic rodents, which, taken together, have mixed results and notable methodological limitations. The more recent incorporation of additional animal models such as *Drosophila* flies, as well as patient-induced pluripotent stem cell-derived neurons, should facilitate a better understanding of a potential functional interaction between TBI and ALS.

amyotrophic lateral sclerosis (ALS); concussion; induced pluripotent stem cell (iPSC); superoxide dismutase 1 (SOD1); TAR DNA-binding protein 43 (TDP-43); traumatic brain injury (TBI)

INTRODUCTION

Patients with amyotrophic lateral sclerosis (ALS) may have a variety of initial presenting symptoms, but the vast majority will suffer a progressive deterioration that includes skeletal muscle weakness and atrophy, difficulties in swallowing and movement, and eventual death, usually resulting from neuromuscular respiratory failure (Kiernan et al. 2011). The progression of disease is attributable to the loss upper and lower motor neurons (MNs). Current treatments only have a small effect on progression, and, once diagnosed, patients typically live for another 2–5 yr. Therapeutic development has been stymied by

the fact that a unifying mechanism of disease has eluded ALS researchers and clinicians. Approximately 10–15% of patients suffer from heritable, familial forms with functionally diverse genetic etiologies including RNA metabolism, protein degradation, trafficking, and cytoskeletal homeostasis (Corcia et al. 2017; Ling et al. 2013). The majority of ALS cases are characterized as sporadic in nature, with recent evidence suggesting that a proportion of these can be explained by de novo mutations in known ALS-causing genes (Renton et al. 2014). Additionally, ALS may be oligogenic in nature (resulting from variants in >1 gene), and mutations in ALS-causing genes may exhibit pleiotropic effects, thereby confounding diagnosis. This, coupled with the limited penetrance of some known mutations, has led to a gradual departure from the classical familial/sporadic divisions, in favor of a multihit model that may require more than one genetic or environmental insult to

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elicit ALS disease symptoms (Hardiman et al. 2017; Ling et al. 2013; Renton et al. 2014).

The epidemiological factors that might increase susceptibility to ALS are poorly understood. Mild traumatic brain injury (TBI) has been proposed to be a risk factor (McKee et al. 2010); however, this association remains disputed (Armon and Nelson 2012). The purpose of this review is to summarize what is known about the relationship between TBI and ALS from a clinicopathological perspective, as well as to explore the lessons we can learn from preclinical models that may eventually clarify whether a functional interaction truly exists. In recent years, there has been great attention paid to mild TBI, which is the most frequent type of TBI and still often referred to as a concussion (Dixon 2017). The precise definition of mild TBI remains a subject of debate among neuroscientists and clinicians, so to simplify the nomenclature we will refer to most forms of head trauma as “TBI” and whenever possible make note of the different severities from mild to severe. In specific cases with neuropathology-based diagnosis of chronic traumatic encephalopathy (CTE), a progressive neurodegenerative disorder linked to repetitive head impacts, this term may be used instead of TBI.

EPIDEMIOLOGY AND CLINICAL CORRELATIONS

Anecdotal accounts relating TBI to the development of ALS are prevalent in clinical practice, with some of the earliest case series reported more than a century ago (Woods 1911). Since then, the association between head trauma and ALS has been a focus of many small to medium-size epidemiological studies. Early reports of a probable link between war veterans and ALS led to the assessment of ALS disease incidence within 690,000 young veterans of the 1991 Gulf War (Haley 2003). This study was well controlled and ultimately concluded that a war-related environmental trigger increased the incidence of ALS by as much as threefold relative to the expected frequency of cases. While the specific environmental trigger (i.e., injury, exposure to chemicals, etc.) was not identified, a follow-up study showed that the spike in ALS cases within veterans was restricted to the decade following the Gulf War (Horner et al. 2008). Another study in military veterans examined ones that had suffered a TBI of unspecified severity ($n = 241$ cases versus $n = 597$ controls) and found a greater than a twofold higher incidence of ALS in cases, further reinforcing a potential correlation between injury and ALS (Schmidt et al. 2010). Intriguingly, this risk was strongest in carriers of the apolipoprotein E type 4 allele (i.e., APOE-4) (Schmidt et al. 2010), which by itself has been shown to not be a risk factor for sporadic ALS or to affect disease onset and progression (Mui et al. 1995; Siddique et al. 1998). Several single nucleotide polymorphisms, including APOE-4, have been associated with clinical outcomes after TBI (Weaver et al. 2012). Recently, whole exome sequencing has been used to identify genetic mutations that appear to confer increased risk for developing ALS (Cirulli et al. 2015), and even genetic mutations known to be causal for ALS seem to have incomplete penetrance for unclear reasons (Al-Chalabi and Lewis 2011). This raises the possibility that an epidemiological factor, such as mild TBI, might play a role in onset of disease for at least some patients with ALS.

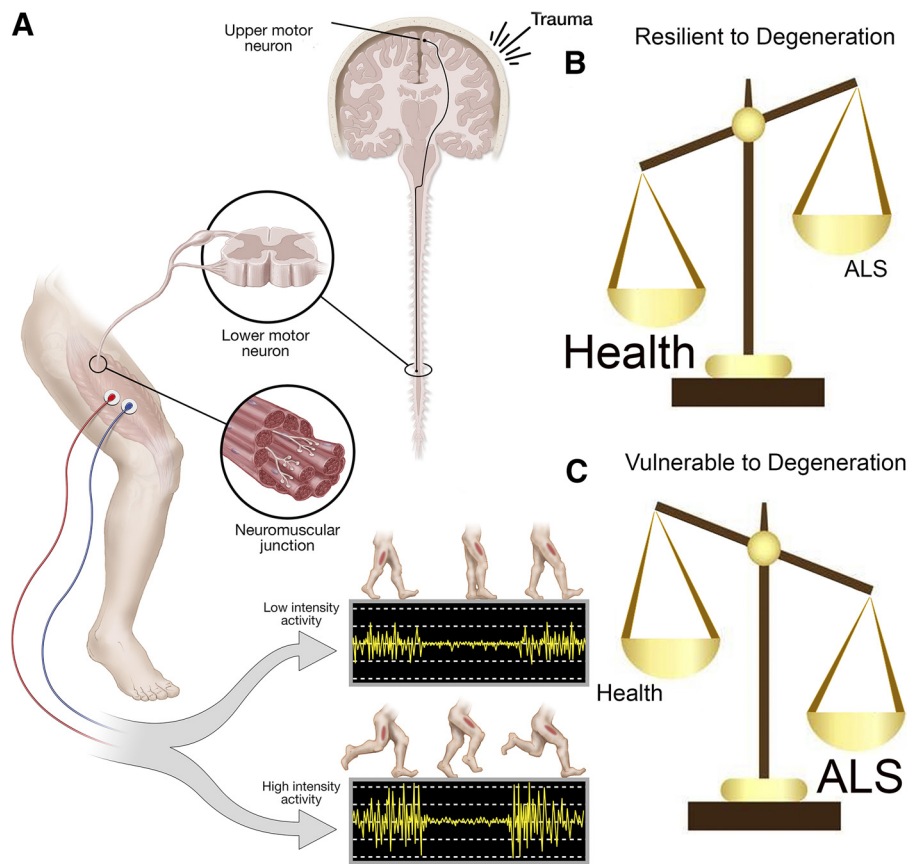
A 2007 study comparing 109 documented cases of head injury in New England soccer players against 55 age-, sex-, and

socioeconomic status-matched controls found no correlation between a single injury and rates of ALS, but a threefold increase in ALS risk was observed for subjects with a history of repeated injuries (Chen et al. 2007). More recently, the European ALS consortium reviewed the cases of 575 ALS patients and 1,150 healthy controls and again noted that a history of two + TBI events was associated with an almost threefold increased risk of ALS, and perhaps even greater risk when the injury occurred between the ages 35 and 54 yr (Pupillo et al. 2017). While TBI experienced in mid or late adulthood may have a greater impact on risk, there are other potential risk factors in need of consideration. For example, a previous study reported that ALS patients were approximately twice as likely as controls to have always been slim or to have been varsity athletes (Scarmeas et al. 2002). However, the link between high levels of physical activity and ALS remains controversial (Harwood et al. 2016; Lacorte et al. 2016). It is possible that high levels of physical activity are only harmful in association with TBI in vulnerable individuals who carry other risk factors for ALS such as genetic predisposition (Fig. 1).

At the same time, there have been a number of studies that did not find a clear association between TBI and ALS. A large retrospective study from the United Kingdom compared the rates of ALS in a cohort of patients who had a history of trauma ($n = 106,593$) against a large reference cohort ($n = 511,831$) and concluded that a remote history of TBI was not a significant risk factor for developing ALS (Turner et al. 2010). However, the study noted a significant association between acute or subacute TBI and ALS, if it occurred within one year of the ALS diagnosis. The interpretation of this result is not straightforward, because it may reflect reverse causation, in that traumatic injuries that occur within the year of formal diagnosis of ALS may simply reflect the early motor impairments of undiagnosed ALS, rather than trigger its onset. Another notable study from a single ALS clinic looked at 100 patients, of whom 24 had a documented history of head injury and 47 underwent autopsy (9 with head injury). The study concluded that a history of TBI was not a significant contributor to ALS progression (Fournier et al. 2015). This group went on to examine the expression patterns of pathological tau and TAR (transactive response) DNA-binding protein 43 (TDP-43) in the 47 autopsied brains but did not identify any substantial differences in expression of these neuropathological hallmarks of disease (Mackenzie et al. 2007; Maekawa et al. 2009) based on TBI history. Interestingly, extensive TDP-43 and tau pathology has been shown in the brains and spinal cords of athletes who had documented history of both repetitive mild TBI and MN disease (McKee et al. 2009, 2013).

Neither the study by Turner et al. (2010) nor that by Fournier et al. (2015) was designed to exclude repetitive mild TBI, such as occurs most frequently in collision sports like football or soccer (Pfister et al. 2016; Prien et al. 2018), as an ALS risk factor. This might account for some of the discrepant results to date. For example, a positive correlation between the duration of professional football play and the more extensive expression of pathological tau and TDP-43 has also been reported (McKee et al. 2013). In a systematic retrospective chart review of 1,835 ALS and primary lateral sclerosis (PLS) patients in Germany and Switzerland, 18 patients (14 ALS and 4 PLS) with remote history of frontal contusions or other frontal intracranial lesions

Fig. 1. The corticomotor pathway may be vulnerable to degeneration after traumatic brain injury (TBI), especially in people with high activity levels. **A**: amyotrophic lateral sclerosis (ALS) is a disease characterized by degeneration of both upper and lower motor neurons (MNs). Upper MNs reside in the motor cortex of the brain and have direct connections to lower MNs in the brainstem (not shown) and spinal cord. Lower MNs receive synaptic inputs from upper MNs as well as project their axons to muscle. Given the well-described focal clinical onset of ALS that often affects a single body region before spreading to anatomically contiguous regions with either type of MN within the neuroaxis, this clinical pattern is compatible with the concept that focal stress/damage after TBI may trigger neurodegenerative diseases like ALS. While the majority of people who suffer a TBI will not develop ALS, both the risk of TBI and ALS have been linked to participation in high intensity physical activities such as a collision sport (soccer, football) or military combat. These clinical associations support a multihit model in which occupational exposure to TBI and high-intensity activities, combined with patient-specific factors such as genetic predisposition, may determine whether MN health can be maintained (**B**) or whether ALS befalls an individual (**C**).



confirmed by brain MRI were analyzed (Rosenbohm et al. 2014). Remarkably, it was noted that focal onset of their motor symptoms occurred contralateral to the cortical lesion site in the majority of cases (15/18; 83.3%), suggesting spread might be mediated through connectivity of the corticomotor tracts. If true, this would fit with one proposed model of disease propagation, via corticospinal/bulbar connectivity from upper to lower MNs, perhaps related to a focal area of trauma (Fig. 1). This mechanism is associated with cortical hyperexcitability (Seeger et al. 2017) and perhaps propagation via perturbed synaptic connectivity or transmission of misfolded proteins such as TDP-43 through a prionlike mechanism.

The conflicting clinical results to date could be attributed to a number of factors, including the possible underdiagnosis and underreporting of milder TBI, as patients are likely to not choose to seek medical attention for transient symptoms. This is further compounded by the inconsistent history-taking practices between different physicians when it comes to the incidence of TBI, during evaluation in ALS specialty clinics. There is also the very wide range of normal for ALS disease onset and progression, which adds substantial variability between individual patients. As with many studies relying on clinical observation and epidemiology data, it is therefore difficult to definitively prove causation. In general, the studies performed to date were often small or moderate in sample size and were at risk for both recall bias and high rates of type II statistical error (i.e., “false negative”). Therefore, an increased emphasis on preclinical models should facilitate a better understanding of the potential interaction between TBI and ALS.

HISTOPATHOLOGY IN TBI AND ALS

The clinical histopathology of ALS and TBI are largely distinct. CTE is associated with repeated mild TBI and characterized by an extensive tauopathy, particularly in the outer layers of the cortex. Inclusions of amyloid beta are also observed in a large subset of patients, postmortem (Johnson et al. 2012; McKee et al. 2009). Conversely, tauopathy is rarely observed in ALS, and is largely associated with the related disorders frontotemporal dementia (FTD) (Ling et al. 2013) or ALS with severe cognitive impairment (Strong et al. 2006; Yang et al. 2003). The majority of ALS cases are characterized by typically cytosolic inclusions containing TDP-43, p62, and FUS, with the exception of specific ALS subtypes such as familial ALS with superoxide dismutase 1 (*SOD1*) mutations, which appear to have unique features (Shibata et al. 1996). Ubiquitin-positive inclusions appear to be a universal feature of all sporadic and familial ALS (Deng et al. 2010; Nakano et al. 2004). However, ubiquitin reactivity does not appear to be a feature in the few published cases of CTE with ALS overlap (McKee et al. 2010).

TDP-43 inclusions have also been observed in CTE with ALS (McKee et al. 2010, 2013), while a single TBI is sufficient to increase TDP-43 immunoreactivity, postmortem (Johnson et al. 2011). Aside from rare ALS cases caused by mutations in the *TDP-43* gene itself (Sreedharan et al. 2008), the mechanisms responsible for the cytosolic accumulation of TDP-43 in sporadic ALS or CTE remain poorly understood. The repeat expansion in *C9orf72*, which is the largest genetic contributor to ALS as well as FTD (DeJesus-Hernandez et al. 2011; Renton et al. 2011), has been associated with disruptions in

nucleocytoplasmic trafficking, which may contribute to cytosolic accumulation of TDP-43 (Freibaum et al. 2015; Jovičić et al. 2015; Zhang et al. 2015). Whether nucleocytoplasmic disruption also plays a role in TDP-43 accumulation after TBI remain to be seen. Overall, while the histopathology features of TBI and ALS are distinct, the common neuropathological feature of cytoplasmic TDP-43 aggregation suggests a potential mechanistic link that needs to be explored further. At the same time, although autopsy cases from subjects with clinical diagnoses of ALS and CTE show coexisting pathologic findings consistent both with ALS and CTE, these studies have no ability to examine cause and effect or the role of head injuries in an unselected ALS population.

TBI IN ALS ANIMAL MODELS

Few studies to date have attempted to address the influence of TBI on disease progression in ALS animal models. Transgenic mice and rats that overexpress mutant, human SOD1 protein demonstrate phenotypes that reproduce progressive MN disease, with features reminiscent of ALS in human patients, including selective vulnerability of MNs, impaired motor function, and death from neuromuscular respiratory failure (Turner and Talbot 2008). Consequently, mutant *SOD1* transgenic rodents remain the most well studied ALS model and are a standard bearer for animal modeling of neurodegenerative disease in general (Van Damme et al. 2017). Two studies have examined the effect of TBI in *SOD1* (G93A) transgenic rat models. In the first study, adult, presymptomatic rats were subjected to a focal controlled, cortical impact of “moderate” to “severe” magnitude centered over the motor cortex (Thomsen et al. 2015). Rats with the mutant SOD1 protein subjected to this form of TBI did not exhibit earlier disease onset, altered locomotor function, or shortened lifespan, compared with ones that underwent a sham procedure. It should be noted that, despite histological confirmation of extensive cortical tissue damage, there were no significant motor deficits detected after TBI, which is in stark contrast to motor deficits known to occur with motor cortex damage in human TBI patients. This may be a reflection of the well-known anatomical and functional differences between rodent and human pyramidal systems (Lemon 2008).

A follow-up study modified the injury paradigm such that each controlled cortical impact was reduced to mild severity but was performed on five separate occasions (weekly) starting in a young adult group of presymptomatic *SOD1* (G93A) rats, in an attempt to model repetitive mild TBI (Thomsen et al. 2016). The authors reported that rats subjected to repetitive mild TBI in the mutant *SOD1* genotype background demonstrated earlier ALS symptom onset, as defined by a decline in body weight and compared with sham control mutant rats. Unfortunately, there are a number of key limitations to this study as it pertains to understanding the relationship between TBI and ALS. The group sizes were relatively small ($n = 7$ TBI versus $n = 9$ sham), there were no motor functional measures reported, and the animals were not followed to disease end stage. The early disease onset upon mild TBI was not supported by any motor function assay or direct histopathological assessment. While tracking body weight has been shown to be a good measure of disease onset in mutant *SOD1* rats under standard conditions (Matsumoto et al. 2006), it is

unclear that this assumption is valid in the context of a superimposed repetitive injury paradigm. TBI may induce dysphagia or depression, which can directly contribute to the early weight disturbance. Importantly, the authors also reported that the same repetitive mild TBI paradigm led to motor deficits in wild-type rats (Thomsen et al. 2016).

There has been one study of TBI in presymptomatic *SOD1* (G93A) mice, that used a closed-head TBI paradigm (Evans et al. 2015). The authors noted an initial decline in body mass, much of which seemed to occur in the first 3 days after closed TBI, but the weight then proceeded to rise for several weeks before starting to progressively decrease in a similar fashion to controls. After TBI, the mutant *SOD1* mice, but not the wild-type mice, showed significantly decreased grip strength compared with sham control at 14 and 30 days. The authors also performed electromyography to evaluate for abnormal spontaneous activity such as fibrillation potentials or positive sharp waves, which was present even in wild-type mice acutely after closed TBI. Generally, this type of activity is thought to be generated by muscle fibers that are not innervated, either due to motoneurogenic or myopathic processes (Daube and Rubin 2009). The presence of either MN or muscle degeneration as a result of TBI is hard to account for in wild-type mice. Therefore, the significantly greater spontaneous activity pattern noted in mutant *SOD1* mice with TBI, at 1 and 7 days postinjury is hard to interpret. The mutant *SOD1* mice also exhibited an upregulation of inflammatory and oxidative stress biomarkers after TBI; however, there was no change in disease onset, as measured by motor behavioral evaluation, or overall survival.

Recent groups have employed the *Drosophila melanogaster* fruit fly to develop in vivo models of TBI. The flies are fairly advantageous for animal studies as they have a short lifespan, a wealth of genetic tools available, and relatively straightforward outcome measures related to neurodegeneration. In this paradigm, adult flies are placed in a vial, affixed to a standard compression spring. The spring is then bent to a predetermined angle and released against a semirigid surface to produce impact injury (Katzenberger et al. 2015). This method has previously been shown to reduce lifespan with repeated insult and can produce significant lesions in *Drosophila* brains (Katzenberger et al. 2013). Strikingly, repeated injury also leads to increased levels of phospho-tau and markers of immune activation in brain tissue, suggesting that it may recapitulate some of the hallmarks of CTE (Barekat et al. 2016; Katzenberger et al. 2013).

Unique among the studies is a recent publication examining the direct interaction between TBI and multiple models of ALS in *Drosophila* (Anderson et al. 2018). Using a sublethal injury scheme, the authors found that flies overexpressing ALS gene mutations in *FUS* or *C9orf72* exhibited an increase in mortality after injury, with concomitant increases in ubiquitinated protein species. Injury also unmasked persistent locomotor defects that were not observable in control animals. Interestingly, the authors also observed dramatic increases in TDPH-positive stress granules upon injury, which is the *Drosophila* homolog of TDP-43. While this phenomenon has been observed after axotomy in mouse peripheral nerves (Moisse et al. 2009), it is not yet a known hallmark of TBI. Given that stress granules have been observed in some models of ALS (Mackenzie et al.

2017; Taylor et al. 2016), this may suggest a common pathogenic mechanism.

Further studies will be necessary to definitively determine whether TBI can modulate ALS onset or progression. Inherently, animal model data must be interpreted with caution, as it is challenging to distinguish an actual alteration of ALS progression from any additive motor deficits caused by MN loss, which are known to occur early in presymptomatic ALS rodents (Franz et al. 2009; Frey et al. 2000; Pun et al. 2006). This is particularly important for ALS/TBI paradigms, as mild TBI may (Thomsen et al. 2016) or may not (Thomsen et al. 2015) cause detectable behavioral deficits in wild-type animals. Establishing a model of spinal MN degeneration in wild-type animals after a TBI paradigm is unlikely to be of high yield by itself, as the clinical experience has been that even upon induction of CTE-like disease only a small subset would go on to develop concurrent MN disease (McKee et al. 2013). Still, there remains ample opportunity to determine a cause-and-effect relationship between TBI and ALS through the use animal models. Larger study groups, detailed histopathology and electrophysiology, and variations in the timing of injury relative to ALS phenotypic onset should be considered. Additionally, over the last decade or so there have been dozens of new mouse lines described with ALS gene causing mutations beyond *SOD1*, including some that lack major motor deficits or even MN degeneration (De Giorgio et al. 2019). Detailed neuromuscular studies that combine a TBI paradigm with one of these recent mouse models that may have genetic vulnerability, rather than a predetermined fate, for lower MN degeneration could be highly insightful.

MODELING TBI IN VITRO

Although TBI pathology shares several mechanisms with other neurological disorders, it begins in a unique way: with rapid deformation of the tissue. Brain tissue is soft and incompressible, i.e., it is easy to change its shape but very difficult to change its volume (Holbourn 1943). Tension, compression, and shear are always coupled in an incompressible material, a phenomenon known as the Poisson effect. For example, if a piece of tissue is compressed on a vertical plane, it stretches on a horizontal plane and shears along a diagonal plane. If it is stretched vertically, it compresses horizontally and again shears along a diagonal plane. In fact, these two situations are not on average different from the perspective of a randomly oriented neuron inside the tissue. Neurons are long, slender structures. Slender structures can generally accommodate any type of loading except tension without failure because they can curl up without damage. Therefore, while compression, tension, and shear occur simultaneously on different planes through any point, neurons oriented along the plane of maximum tension are the most likely to fail. These are the basic principles underlying most in vitro models of TBI.

There are several well-established in vitro models of TBI (Morrison et al. 2011), including multiple 2D culture systems that apply tension and 3D culture system that apply compression (Bar-Kochba et al. 2016) or shear (LaPlaca et al. 2005). Organotypic slice cultures (Morrison et al. 2006) and 2D or 3D cultures (Ahmed et al. 2000; Cullen et al. 2007) of dissociated primary or immortalized cells have been employed in these models. Typically, 2D cultures are maintained on a silicone

membrane that is stretched to create a mechanical insult. Early in vitro TBI models induced stretch by applying air pressure to all (Ellis et al. 1995) or part of the silicone membrane (Smith et al. 1999). More recently, indentation with a rigid piston driven by an electromagnetic voice coil has been used to induce stretch (Morrison et al. 2003). Indentation systems achieve shorter pulse durations than pneumatic systems (Ellis et al. 1995; Morrison et al. 2006). These shorter pulses are more biofidelic (Hardy et al. 2007), which is significant because the trauma response is rate sensitive (Ahmadzadeh et al. 2014; Elkin and Morrison 2007). Also, the indentation approach has been scaled up to a 96-well format (Sherman et al. 2016) but technical challenges make it difficult to scale the pneumatic approach up to a multiwell format (Magou et al. 2011).

HUMAN IPSC MODELS FOR STUDYING THE RELATIONSHIP BETWEEN TBI AND ALS

In vitro modeling has proven to be a powerful preclinical tool in ALS. Experiments with patient-specific induced pluripotent stem cell (iPSC)-derived neurons have uncovered disease mechanisms (Barmada et al. 2014; Bilican et al. 2012; Chen et al. 2014; Devlin et al. 2015; Donnelly et al. 2013; Egawa et al. 2012; Kiskinis et al. 2014, 2018; Mitne-Neto et al. 2011; Sareen et al. 2013; Serio et al. 2013; Wainger et al. 2014; Yang et al. 2013), and enable relatively rapid clinical innovation through repurposing of clinically approved drugs (McNeish et al. 2015). Human iPSCs are easily generated after primary cells (typically skin cells or mononuclear, blood cells), which are harvested from a human patient, are converted by molecular reprogramming and then differentiated into relevant neural subtypes (Hunsberger et al. 2015). The utility of CRISPR/Cas9 gene editing for the generation of isogenic control iPSC lines (i.e. experiments comparing iPSC-derived neurons with genomes that differ only by a single genetic variant) can conclusively prove that a particular genetic variant causes a particular functional deficit. The combination of in vitro trauma experiments with patient-specific and isogenic iPSC-derived neurons have the potential to address the question of gene-trauma interactions in ALS pathology (Fig. 2). They could also be applied to a multihit, gene-trauma interaction model, i.e., the hypothesis that a given mutation is harmless in the absence of neurotrauma but leads to ALS in the wake of neurotrauma. This opportunity is particularly exciting in light of ongoing efforts to bank stem cells from up to 1,000 ALS patients (Progress & Updates: Answer ALS Research 2018).

At the same time cell culture models have limitations. They provide simple approximations of the likely complex in vivo disease processes, because they lack the cellular diversity and structural organization of an intact nervous system. This is particularly relevant in the case of TBI, which is known to involve interconnected dysfunction in all three components of the neurovascular unit, which consists of neurons, glia and associated vasculature (Xing et al. 2012). CNS glia, in particular, appear to play an essential role in neuronal degeneration and regeneration upon injury (Myer et al. 2006; Neumann et al. 2009), while other non-cell-autonomous mechanisms such as propagation of misfolded proteins may play a key role in TBI pathogenesis (Hawkins et al. 2013). Phosphorylated, oligo-

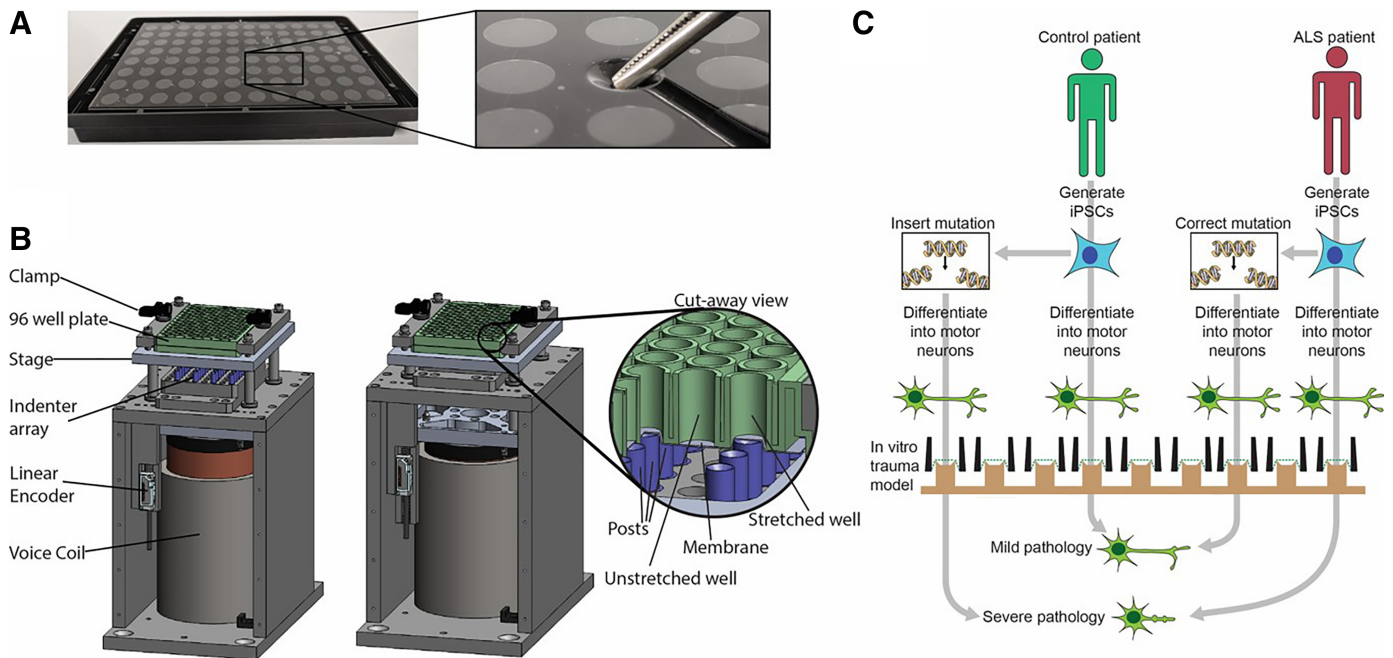


Fig. 2. Understanding the relationship between amyotrophic lateral sclerosis (ALS) and neurotrauma with patient-specific induced pluripotent stem cells (iPSCs)-based neurons. Induced pluripotent stem cell-derived neurons could be used in combination with an in vitro model of neurotrauma to understand gene-trauma interaction in ALS as follows. *A*: stretchable 96-well plates are fabricated by bonding a layer of flexible, transparent silicone to bottomless 96-well plates. The *inset* shows tweezers gently depressing one of the well bottoms to illustrate the flexibility of the growth substrate. *B*: a custom-built device is used to apply a rapid, repeatable, equibiaxial stretch to the bottom of the wells. Stretch is produced by pressing the plate rapidly down against an array of lubricated, Teflon-coated, cylindrical indenters. The cut-away view shows an example of a stretched well alongside a well that is not stretched because the corresponding post is not present. *C*: schematic of an experimental design to study gene-trauma interactions in ALS using this human in vitro model. A mutation associated with ALS can be engineered into an iPSC line derived from a healthy control individual or alternatively can be corrected in an iPSC line derived from a patient with a known disease-causing genetic variant. Both lines are then subjected to identical trauma in vitro, and subsequent pathology is quantified and compared with test the hypothesis that the ALS-associated mutation amplifies the pathology of trauma.

meric tau protein has been shown to accumulate in the brains of rats exposed to a fluid percussion model of TBI (Hawkins et al. 2013), while the capacity of certain misfolded tau species to then propagate through prion-like mechanisms can influence the long-term functional deficits observed after TBI (Ahmed et al. 2014; Gerson et al. 2016; Kfoury et al. 2012).

In some ways, these potential limitations can be of experimental value. The use of isolated neuronal models of TBI allows for the detection of neuron-specific, cell-autonomous mechanisms of neurotoxicity in TBI and/or ALS. In addition, rapid advances in organoid, 3D cell culture systems provide increasing levels of cellular diversity and structural organization (Arlotta and Pasca 2019; Lancaster and Knoblich 2014). Furthermore, various insult mechanisms, such as stretch or fluid percussion, can effectively isolate pathologies resulting from single aspects of TBI, which otherwise entails a highly complex array of tissue damage mechanisms.

The major hurdle that these models do face, however, is the relative immaturity of the neurons in culture (Ho et al. 2016). Both TBI (Sendroy-Terrill et al. 2010) and ALS (Hardiman et al. 2017) are strongly influenced by aging. In the case of TBI, both age at injury and time postinjury negatively predict patient outcomes (Sendroy-Terrill et al. 2010). By nature, iPSCs effectively revert back to an embryonic state after reprogramming (Takahashi and Yamanaka 2006), largely irrespective of donor age, despite some genetic signatures (Lo Sardo et al. 2017; Miller et al. 2013). Although methods of iPSC-derived cell aging exist (Miller et al. 2013), it is unlikely that they faithfully recapitulate all mechanisms of human aging. Recent

developments in direct neuronal transdifferentiation from adult somatic cell types appear to preserve aspects of cellular age (Abernathy et al. 2017; Huh et al. 2016; Victor et al. 2018; Yoo et al. 2011) and may facilitate the development of more accurate in vitro models of TBI pathogenesis.

THE COMMON PATHOPHYSIOLOGY IN TBI AND ALS

Due to the limited number of studies designed to directly examine the link between TBI and ALS in a controlled, laboratory setting, the pathophysiological link between these two disease states remains largely unclear. Any proposed link is therefore highly speculative. TDP-43 and stress-granule dysregulation may be a potential molecular overlap between the two disorders, but this link must be examined more directly (Anderson et al. 2018). Recent evidence directly links stress-granule formation to disruptions on nucleocytoplasmic transport (Zhang et al. 2018), which is a hallmark of *C9orf72* ALS (Chou et al. 2018; Zhang et al. 2015), further suggesting that this may be an important overlap between TBI and ALS. TDP-43 has also been demonstrated to be particularly vulnerable to protease degradation in various neurotoxic states, including TBI, which may worsen loss-of-function effects in affected cells (Yang et al. 2014). We therefore hypothesize that TBI may contribute to the disruption of proteostasis seen in ALS patients, thereby leading to insurmountable proteotoxic stress.

TBI appears to focally induce several pathological processes that may overlap with ALS. Most immediate among these

appears to be excitotoxic firing, due to transient ion dysregulation near the site of injury (Palmer et al. 1993; Wagner et al. 2004). Notably, dysregulation of the glial excitatory amino acid transporter 2 (EAAT2) has been observed in both ALS patients (Rothstein et al. 1995) and rodent models of ALS (Howland et al. 2002), and this loss appears to be specific to regions typically lost in ALS. Homeostatic regulation of the GluA2 subunit of AMPA receptors on MNs is also lost in mutant *SOD1* models of ALS, leading to an increased vulnerability to excitotoxicity (Taylor et al. 2016; Van Damme et al. 2007). Spinal MNs may therefore be particularly vulnerable to excitotoxic damage in ALS, and TBI-induced excitotoxic signaling may ultimately tip the scales in favor of cell death.

Oxidative stress also appears to be a common pathological signature between ALS and TBI (Ansari et al. 2008; Evans et al. 2015; Readnower et al. 2010; Turner and Talbot 2008). How this may preferentially lead to MN damage remains unclear, but the combined toxicity in both disease states may hasten cytotoxicity (Barber and Shaw 2010). Similarly, sustained, diffuse neuroinflammation has been well documented in both TBI (Acosta et al. 2013; Johnson et al. 2013) and ALS (Hall et al. 1998; Keizman et al. 2009). While this may initially be a protective response to neurotoxic damage, sustained inflammation may lead to reactive gliosis and further contribute to neuronal damage through pathological alterations to the extracellular milieu. Necroptosis, or programmed necrotic cell death with autophagic induction (Degterev et al. 2005; Vandenberghe et al. 2010), has recently been identified as a major mechanism of cell death in both ALS (Ito et al. 2016; Re et al. 2014) and TBI (Liu et al. 2016; Wang et al. 2012; You et al. 2008). Because necroptosis is activated, in part, by cell non-autonomous mechanisms such as inflammation (Vandenberghe et al. 2010), it is possible that TBI may increase necroptotic signaling above threshold for MNs to remain viable in some individuals (Fig. 1).

Another potential pathological overlap between the two disease states is cytoskeletal damage and dysregulation. Mutations in genes associated with cytoskeletal homeostasis, including profilin-1 (*PFN1*) (Wu et al. 2012) and tubulin alpha-4A (*TUBA4A*) (Smith et al. 2014), have been shown to cause ALS. Other cytoskeletal gene mutations such as neurofilament heavy chain (Al-Chalabi et al. 1999) and dynactin (Münch et al. 2004) have been associated with increased susceptibility to ALS. Moreover, markers of cytoskeletal damage have been observed in the cerebrospinal fluid (CSF) of sporadic ALS patients (Brettschneider et al. 2006), while aggregation of neurofilament and reduced expression of neurofilament, light-chain have also been described in iPSC-based models of *SOD1*-related ALS (Chen et al. 2014). The accumulation of neurofilament polypeptides (both light and heavy), has also been observed in the blood and CSF of patients with TBI (Zetterberg et al. 2013). Diffuse axonal injury in TBI has been shown to induce localized cytoskeletal damage (Kilinc et al. 2008), which in turn can induce neurofilament compaction and mislocalization (Povlishock and Pettus 1996). Thus, dysregulation of cytoskeletal homeostasis in ALS patients may render them less able to overcome cytoskeletal damage after mild TBI, leading to worsened clinical outcomes.

CONCLUSIONS

There is growing evidence that TBI, particularly of a repetitive nature with mild severity (McKee et al. 2013; Pupillo et al. 2017), might be a risk factor for developing ALS. If true, this would be of special significance to those individuals who engage in high-risk activities such as collision sports. The facts that mutations in *TDP-43* can cause ALS and that both sporadic ALS and CTE cases are frequently characterized by TDP-43 proteinopathy (Mackenzie et al. 2007; McKee et al. 2013) imply a potentially shared mechanism of neurodegeneration.

Animal models have been extremely useful for modeling ALS disease mechanisms and addressing the potential interaction with TBI but may have critical limitations ranging from their inability to capture the genetic complexity of human patients to fundamentally different corticomotor function and connectivity. Patient-specific iPSC technologies have been rapidly improving over the last decade and have become another component of our preclinical tool set for understanding neurodegenerative disease (Ichida and Kiskinis 2015). At the same time, the development of instrumentation that allows for controlled delivery of biofidelic trauma to human neurons in culture (Sherman et al. 2016) enables an additional platform that can be used to address the conflicting clinical observations and animal studies on the effects of TBI on ALS incidence and progression. There is no doubt that these human preclinical model systems will help us understand aspects of ALS that may be unique to patients or patient subgroups, but they will not ever fully reproduce the in vivo context of the human CNS. Therefore, corroboration of key findings between multiple preclinical models (e.g., human and rodent, in vitro and in vivo, etc.), along with careful clinical correlation to bedside and histopathological data should be the path to a better understanding of disease mechanisms and the development of effective treatments for ALS.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

C.K.F. conceived and designed research; C.K.F. and J.D.F. prepared figures; C.K.F., D.J., E.L.D., R.A.G., K.D., A.L., J.D.F., and E.K. drafted

manuscript; C.K.F., D.J., E.L.D., R.A.G., K.D., A.L., J.D.F., and E.K. edited and revised manuscript; C.K.F., D.J., E.L.D., R.A.G., K.D., A.L., J.D.F., and E.K. approved final version of manuscript.

REFERENCES

- Abernathy DG, Kim WK, McCoy MJ, Lake AM, Ouwenga R, Lee SW, Xing X, Li D, Lee HJ, Heuckeroth RO, Dougherty JD, Wang T, Yoo AS. MicroRNAs induce a permissive chromatin environment that enables neuronal subtype-specific reprogramming of adult human fibroblasts. *Cell Stem Cell* 21: 332–348.e9, 2017. doi:10.1016/j.stem.2017.08.002.
- Acosta SA, Tajiri N, Shinozuka K, Ishikawa H, Grimmig B, Diamond DM, Sanberg PR, Bickford PC, Kaneko Y, Borlongan CV. Long-term upregulation of inflammation and suppression of cell proliferation in the brain of adult rats exposed to traumatic brain injury using the controlled cortical impact model. *PLoS One* 8: e53376, 2013. [Erratum in *PLoS One* 8: 10.1371/annotation/a04a7468-d105-42f3-ba47-263ea2864681.] doi:10.1371/journal.pone.0053376.
- Ahmadzadeh H, Smith DH, Shenoy VB. Viscoelasticity of tau proteins leads to strain rate-dependent breaking of microtubules during axonal stretch injury: predictions from a mathematical model. *Biophys J* 106: 1123–1133, 2014. doi:10.1016/j.bpj.2014.01.024.
- Ahmed SM, Rzigalinski BA, Willoughby KA, Sitterding HA, Ellis EF. Stretch-induced injury alters mitochondrial membrane potential and cellular ATP in cultured astrocytes and neurons. *J Neurochem* 74: 1951–1960, 2000. doi:10.1046/j.1471-4159.2000.0741951.x.
- Ahmed Z, Cooper J, Murray TK, Garn K, McNaughton E, Clarke H, Parhizkar S, Ward MA, Cavallini A, Jackson S, Bose S, Clavaguera F, Tolnay M, Lavenir I, Goedert M, Hutton ML, O'Neill MJ. A novel in vivo model of tau propagation with rapid and progressive neurofibrillary tangle pathology: the pattern of spread is determined by connectivity, not proximity. *Acta Neuropathol* 127: 667–683, 2014. doi:10.1007/s00401-014-1254-6.
- Al-Chalabi A, Andersen PM, Nilsson P, Chioza B, Andersson JL, Russ C, Shaw CE, Powell JF, Leigh PN. Deletions of the heavy neurofilament subunit tail in amyotrophic lateral sclerosis. *Hum Mol Genet* 8: 157–164, 1999. doi:10.1093/hmg/8.2.157.
- Al-Chalabi A, Lewis CM. Modelling the effects of penetrance and family size on rates of sporadic and familial disease. *Hum Hered* 71: 281–288, 2011. doi:10.1159/000330167.
- Anderson EN, Gochenaur L, Singh A, Grant R, Patel K, Watkins S, Wu JY, Pandey UB. Traumatic injury induces stress granule formation and enhances motor dysfunctions in ALS/FTD models. *Hum Mol Genet* 27: 1366–1381, 2018. doi:10.1093/hmg/ddy047.
- Ansari MA, Roberts KN, Scheff SW. Oxidative stress and modification of synaptic proteins in hippocampus after traumatic brain injury. *Free Radic Biol Med* 45: 443–452, 2008. doi:10.1016/j.freeradbiomed.2008.04.038.
- Arlotta P, Paçca SP. Cell diversity in the human cerebral cortex: from the embryo to brain organoids. *Curr Opin Neurobiol* 56: 194–198, 2019. doi:10.1016/j.conb.2019.03.001.
- Armon C, Nelson LM. Is head trauma a risk factor for amyotrophic lateral sclerosis? An evidence based review. *Amyotroph Lateral Scler* 13: 351–356, 2012. doi:10.3109/17482968.2012.660954.
- Bar-Kochba E, Scimone MT, Estrada JB, Franck C. Strain and rate-dependent neuronal injury in a 3D in vitro compression model of traumatic brain injury. *Sci Rep* 6: 30550, 2016. doi:10.1038/srep30550.
- Barber SC, Shaw PJ. Oxidative stress in ALS: key role in motor neuron injury and therapeutic target. *Free Radic Biol Med* 48: 629–641, 2010. doi:10.1016/j.freeradbiomed.2009.11.018.
- Barekat A, Gonzalez A, Mautz RE, Kotzebue RW, Molina B, El-Mecharriffe N, Conner CJ, Garza S, Melkani GC, Joiner WJ, Lipinski MM, Finley KD, Ratliff EP. Using *Drosophila* as an integrated model to study mild repetitive traumatic brain injury. *Sci Rep* 6: 25252, 2016. doi:10.1038/srep25252.
- Barmada SJ, Serio A, Arjun A, Bilican B, Daub A, Ando DM, Tsvetkov A, Pleiss M, Li X, Peisach D, Shaw C, Chandran S, Finkbeiner S. Autophagy induction enhances TDP43 turnover and survival in neuronal ALS models. *Nat Chem Biol* 10: 677–685, 2014. doi:10.1038/nchembio.1563.
- Bilican B, Serio A, Barmada SJ, Nishimura AL, Sullivan GJ, Carrasco M, Phatnani HP, Puddifoot CA, Story D, Fletcher J, Park IH, Friedman BA, Daley GQ, Wyllie DJ, Hardingham GE, Wilmut I, Finkbeiner S, Maniatis T, Shaw CE, Chandran S. Mutant induced pluripotent stem cell lines recapitulate aspects of TDP-43 proteinopathies and reveal cell-specific vulnerability. *Proc Natl Acad Sci USA* 109: 5803–5808, 2012. doi:10.1073/pnas.1202922109.
- Brettschneider J, Petzold A, Süßmuth SD, Ludolph AC, Tumani H. Axonal damage markers in cerebrospinal fluid are increased in ALS. *Neurology* 66: 852–856, 2006. doi:10.1212/01.wnl.0000203120.85850.54.
- Chen H, Qian K, Du Z, Cao J, Petersen A, Liu H, Blackburn LW IV, Huang CL, Errigo A, Yin Y, Lu J, Ayala M, Zhang SC. Modeling ALS with iPSCs reveals that mutant SOD1 misregulates neurofilament balance in motor neurons. *Cell Stem Cell* 14: 796–809, 2014. doi:10.1016/j.stem.2014.02.004.
- Chen H, Richard M, Sandler DP, Umbach DM, Kamel F. Head injury and amyotrophic lateral sclerosis. *Am J Epidemiol* 166: 810–816, 2007. doi:10.1093/aje/kwm153.
- Chou CC, Zhang Y, Umoh ME, Vaughan SW, Lorenzini I, Liu F, Sayegh M, Donlin-Asp PG, Chen YH, Duong DM, Seyfried NT, Powers MA, Kukar T, Hales CM, Gearing M, Cairns NJ, Boylan KB, Dickson DW, Rademakers R, Zhang YJ, Petrucelli L, Sattler R, Zarnescu DC, Glass JD, Rossoll W. TDP-43 pathology disrupts nuclear pore complexes and nucleocytoplasmic transport in ALS/FTD. *Nat Neurosci* 21: 228–239, 2018. doi:10.1038/s41593-017-0047-3.
- Cirulli ET, Lasseigne BN, Petrovski S, Sapp PC, Dion PA, Leblond CS, Couthouis J, Lu YF, Wang Q, Krueger BJ, Ren Z, Keebler J, Han Y, Levy SE, Boone BE, Wimbish JR, Waite LL, Jones AL, Carulli JP, Day-Williams AG, Staropoli JF, Xin WW, Chesni A, Raphael AR, McKenna-Yasek D, Cady J, Vianney de Jong JM, Kenna KP, Smith BN, Topp S, Miller J, Gkazi A, Al-Chalabi A, van den Berg LH, Veldink J, Silani V, Ticozzi N, Shaw CE, Baloh RH, Appel S, Simpson E, Lagier-Tourenne C, Pulst SM, Gibson S, Trojanowski JQ, Elman L, McCluskey L, Grossman M, Shneider NA, Chung WK, Ravits JM, Glass JD, Sims KB, Van Deerlin VM, Maniatis T, Hayes SD, Oudreau A, Swarup S, Landers J, Baas F, Allen AS, Bedlack RS, Harper JW, Gitler AD, Rouleau GA, Brown R, Harms MB, Cooper GM, Harris T, Myers RM, Goldstein DB; FALS Sequencing Consortium. Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. *Science* 347: 1436–1441, 2015. doi:10.1126/science.aaa3650.
- Corcia P, Couratier P, Blasco H, Andres CR, Beltran S, Meininger V, Vourc'h P. Genetics of amyotrophic lateral sclerosis. *Rev Neurol (Paris)* 173: 254–262, 2017. doi:10.1016/j.neurol.2017.03.030.
- Cullen DK, Simon CM, LaPlaca MC. Strain rate-dependent induction of reactive astrogliosis and cell death in three-dimensional neuronal-astrocytic co-cultures. *Brain Res* 1158: 103–115, 2007. doi:10.1016/j.brainres.2007.04.070.
- Daube JR, Rubin DI. Needle electromyography. *Muscle Nerve* 39: 244–270, 2009. doi:10.1002/mus.21180.
- De Giorgio F, Maduro C, Fisher EMC, Acevedo-Arozena A. Transgenic and physiological mouse models give insights into different aspects of amyotrophic lateral sclerosis. *Dis Model Mech* 12: dmm037424, 2019. doi:10.1242/dmm.037424.
- Degterev A, Huang Z, Boyce M, Li Y, Jagtap P, Mizushima N, Cuny GD, Mitchison TJ, Moskowitz MA, Yuan J. Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. *Nat Chem Biol* 1: 112–119, 2005. [Erratum in *Nat Chem Biol* 1: 234, 2005. Addendum in *Nat Chem Biol* 9: 192, 2013.] doi:10.1038/nchembio711.
- DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, Nicholson AM, Finch NA, Flynn H, Adamson J, Kouri N, Wojtas A, Sengdy P, Hsiung GY, Karydas A, Seeley WW, Josephs KA, Coppola G, Geschwind DH, Wszolek ZK, Feldman H, Knopman DS, Petersen RC, Miller BL, Dickson DW, Boylan KB, Graff-Radford NR, Rademakers R. Expanded GGGGCC hexanucleotide repeat in non-coding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* 72: 245–256, 2011. doi:10.1016/j.neuron.2011.09.011.
- Deng HX, Zhai H, Bigio EH, Yan J, Fecto F, Ajroud K, Mishra M, Ajroud-Driss S, Heller S, Sufit R, Siddique N, Mugnaini E, Siddique T. FUS-immunoreactive inclusions are a common feature in sporadic and non-SOD1 familial amyotrophic lateral sclerosis. *Ann Neurol* 67: 739–748, 2010. doi:10.1002/ana.22051.
- Devlin AC, Burr K, Borooah S, Foster JD, Cleary EM, Geti I, Vallier L, Shaw CE, Chandran S, Miles GB. Human iPSC-derived motoneurons harbouring TARDBP or C9ORF72 ALS mutations are dysfunctional despite maintaining viability. *Nat Commun* 6: 5999, 2015. doi:10.1038/ncomms6999.
- Dixon KJ. Pathophysiology of traumatic brain injury. *Phys Med Rehabil Clin N Am* 28: 215–225, 2017. doi:10.1016/j.pmr.2016.12.001.

- Donnelly CJ, Zhang PW, Pham JT, Haeusler AR, Mistry NA, Vidensky S, Daley EL, Poth EM, Hoover B, Fines DM, Maragakis N, Tienari PJ, Petrucelli L, Traynor BJ, Wang J, Rigo F, Bennett CF, Blackshaw S, Sattler R, Rothstein JD. RNA toxicity from the ALS/FTD C9orf72 expansion is mitigated by antisense intervention. *Neuron* 80: 415–428, 2013. doi:10.1016/j.neuron.2013.10.015.
- Egawa N, Kitaoka S, Tsukita K, Naitoh M, Takahashi K, Yamamoto T, Adachi F, Kondo T, Okita K, Asaka I, Aoi T, Watanabe A, Yamada Y, Morizane A, Takahashi J, Ayaki T, Ito H, Yoshikawa K, Yamawaki S, Suzuki S, Watanabe D, Hioki H, Kaneko T, Makioka K, Okamoto K, Takuma H, Tamaoka A, Hasegawa K, Nonaka T, Hasegawa M, Kawata A, Yoshida M, Nakahata T, Takahashi R, Marchetto MC, Gage FH, Yamanaka S, Inoue H. Drug screening for ALS using patient-specific induced pluripotent stem cells. *Sci Transl Med* 4: 145ra104, 2012. doi:10.1126/scitranslmed.3004052.
- Elkin BS, Morrison B III. Region-specific tolerance criteria for the living brain. *Stapp Car Crash J* 51: 127–138, 2007. doi:10.4271/2007-22-0005.
- Ellis EF, McKinney JS, Willoughby KA, Liang S, Povlishock JT. A new model for rapid stretch-induced injury of cells in culture: characterization of the model using astrocytes. *J Neurotrauma* 12: 325–339, 1995. doi:10.1089/neu.1995.12.325.
- Evans TM, Jaramillo CA, Sataranatarajan K, Watts L, Sabia M, Qi W, Van Remmen H. The effect of mild traumatic brain injury on peripheral nervous system pathology in wild-type mice and the G93A mutant mouse model of motor neuron disease. *Neuroscience* 298: 410–423, 2015. doi:10.1016/j.neuroscience.2015.04.041.
- Fournier CN, Gearing M, Upadhyayula SR, Klein M, Glass JD. Head injury does not alter disease progression or neuropathologic outcomes in ALS. *Neurology* 84: 1788–1795, 2015. doi:10.1212/WNL.0000000000001522.
- Franz CK, Quach ET, Krudy CA, Federici T, Kliem MA, Snyder BR, Raore B, Boulis NM. A conditioning lesion provides selective protection in a rat model of amyotrophic lateral sclerosis. *PLoS One* 4: e7357, 2009. doi:10.1371/journal.pone.0007357.
- Freibaum BD, Lu Y, Lopez-Gonzalez R, Kim NC, Almeida S, Lee KH, Badders N, Valentine M, Miller BL, Wong PC, Petrucelli L, Kim HJ, Gao FB, Taylor JP. GGGGCC repeat expansion in C9orf72 compromises nucleocytoplasmic transport. *Nature* 525: 129–133, 2015. doi:10.1038/nature14974.
- Frey D, Schneider C, Xu L, Borg J, Spooren W, Caroni P. Early and selective loss of neuromuscular synapse subtypes with low sprouting competence in motoneuron diseases. *J Neurosci* 20: 2534–2542, 2000. doi:10.1523/JNEUROSCI.20-07-02534.2000.
- Gerson J, Castillo-Carranza DL, Sengupta U, Bodani R, Prough DS, DeWitt DS, Hawkins BE, Kaye R. Tau oligomers derived from traumatic brain injury cause cognitive impairment and accelerate onset of pathology in Htau mice. *J Neurotrauma* 33: 2034–2043, 2016. doi:10.1089/neu.2015.4262.
- Haley RW. Excess incidence of ALS in young Gulf War veterans. *Neurology* 61: 750–756, 2003. doi:10.1212/WNL.61.6.750.
- Hall ED, Oostveen JA, Gurney ME. Relationship of microglial and astrocytic activation to disease onset and progression in a transgenic model of familial ALS. *Glia* 23: 249–256, 1998. doi:10.1002/(SICI)1098-1136(199807)23:3<249:AID-GLIA7>3.0.CO;2-#.
- Hardiman O, Al-Chalabi A, Chio A, Corr EM, Logroscino G, Robberecht W, Shaw PJ, Simmons Z, van den Berg LH. Amyotrophic lateral sclerosis. *Nat Rev Dis Primers* 3: 17071, 2017. [Erratum in *Nat Rev Dis Primers* 3: 17071, 2017.] doi:10.1038/nrdp.2017.71.
- Hardy WN, Mason MJ, Foster CD, Shah CS, Kopacz JM, Yang KH, King AI, Bishop J, Bey M, Anderst W, Tashman S. A study of the response of the human cadaver head to impact. *Stapp Car Crash J* 51: 17–80, 2007. doi:10.4271/2007-22-0002.
- Harwood CA, Westgate K, Gunstone S, Brage S, Wareham NJ, McDermott CJ, Shaw PJ. Long-term physical activity: an exogenous risk factor for sporadic amyotrophic lateral sclerosis? *Amyotroph Lateral Scler Frontotemporal Degener* 17: 377–384, 2016. doi:10.3109/21678421.2016.1154575.
- Hawkins BE, Krishnamurthy S, Castillo-Carranza DL, Sengupta U, Prough DS, Jackson GR, DeWitt DS, Kaye R. Rapid accumulation of endogenous tau oligomers in a rat model of traumatic brain injury: possible link between traumatic brain injury and sporadic tauopathies. *J Biol Chem* 288: 17042–17050, 2013. doi:10.1074/jbc.M113.472746.
- Ho R, Sances S, Gowing G, Amoroso MW, O'Rourke JG, Sahabian A, Wichterle H, Baloh RH, Sareen D, Svendsen CN. ALS disrupts spinal motor neuron maturation and aging pathways within gene co-expression networks. *Nat Neurosci* 19: 1256–1267, 2016. doi:10.1038/nn.4345.
- Holbourn AHS. Mechanics of head injuries. *Lancet* 242: 438–441, 1943. doi:10.1016/S0140-6736(00)87453-X.
- Horner RD, Grambow SC, Coffman CJ, Lindquist JH, Oddone EZ, Allen KD, Kasarskis EJ. Amyotrophic lateral sclerosis among 1991 Gulf War veterans: evidence for a time-limited outbreak. *Neuroepidemiology* 31: 28–32, 2008. doi:10.1159/000136648.
- Howland DS, Liu J, She Y, Goad B, Maragakis NJ, Kim B, Erickson J, Kulik J, DeVito L, Psaltis G, DeGennaro LJ, Cleveland DW, Rothstein JD. Focal loss of the glutamate transporter EAAT2 in a transgenic rat model of SOD1 mutant-mediated amyotrophic lateral sclerosis (ALS). *Proc Natl Acad Sci USA* 99: 1604–1609, 2002. doi:10.1073/pnas.0325392999.
- Huh CJ, Zhang B, Victor MB, Dahiya S, Batista LF, Horvath S, Yoo AS. Maintenance of age in human neurons generated by microRNA-based neuronal conversion of fibroblasts. *eLife* 5: e18648, 2016. doi:10.7554/eLife.18648.
- Hunsberger JG, Efthymiou AG, Malik N, Behl M, Mead IL, Zeng X, Simeonov A, Rao M. Induced pluripotent stem cell models to enable in vitro models for screening in the central nervous system. *Stem Cells Dev* 24: 1852–1864, 2015. doi:10.1089/scd.2014.0531.
- Ichida JK, Kiskinis E. Probing disorders of the nervous system using reprogramming approaches. *EMBO J* 34: 1456–1477, 2015. doi:10.15252/embj.201591267.
- Ito Y, Ofengeim D, Najafav A, Das S, Saberi S, Li Y, Hitomi J, Zhu H, Chen H, Mayo L, Geng J, Amin P, DeWitt JP, Mookhtiar AK, Florez M, Ouchida AT, Fan JB, Pasparakis M, Kelliher MA, Ravits J, Yuan J. RIPK1 mediates axonal degeneration by promoting inflammation and necroptosis in ALS. *Science* 353: 603–608, 2016. doi:10.1126/science.aaf6803.
- Johnson VE, Stewart JE, Begbie FD, Trojanowski JQ, Smith DH, Stewart W. Inflammation and white matter degeneration persist for years after a single traumatic brain injury. *Brain* 136: 28–42, 2013. doi:10.1093/brain/aww322.
- Johnson VE, Stewart W, Smith DH. Widespread τ and amyloid- β pathology many years after a single traumatic brain injury in humans. *Brain Pathol* 22: 142–149, 2012. doi:10.1111/j.1750-3639.2011.00513.x.
- Johnson VE, Stewart W, Trojanowski JQ, Smith DH. Acute and chronically increased immunoreactivity to phosphorylation-independent but not pathological TDP-43 after a single traumatic brain injury in humans. *Acta Neuropathol* 122: 715–726, 2011. doi:10.1007/s00401-011-0909-9.
- Jovičić A, Mertens J, Boeynaems S, Bogaert E, Chai N, Yamada SB, Paul JW III, Sun S, Herdy JR, Bieri G, Kramer NJ, Gage FH, Van Den Bosch L, Robberecht W, Gitler AD. Modifiers of C9orf72 dipeptide repeat toxicity connect nucleocytoplasmic transport defects to FTD/ALS. *Nat Neurosci* 18: 1226–1229, 2015. doi:10.1038/nn.4085.
- Katzenberger RJ, Loewen CA, Bockstruck RT, Woods MA, Ganetzky B, Wassarman DA. A method to inflict closed head traumatic brain injury in drosophila. *J Vis Exp* 100: e52905, 2015. doi:10.3791/52905.
- Katzenberger RJ, Loewen CA, Wassarman DR, Petersen AJ, Ganetzky B, Wassarman DA. A *Drosophila* model of closed head traumatic brain injury. *Proc Natl Acad Sci USA* 110: E4152–E4159, 2013. doi:10.1073/pnas.1316895110.
- Keizman D, Rogowski O, Berliner S, Ish-Shalom M, Maimon N, Nefussy B, Artamonov I, Drory VE. Low-grade systemic inflammation in patients with amyotrophic lateral sclerosis. *Acta Neurol Scand* 119: 383–389, 2009. doi:10.1111/j.1600-0404.2008.01112.x.
- Kfoury N, Holmes BB, Jiang H, Holtzman DM, Diamond MI. Transcellular propagation of Tau aggregation by fibrillar species. *J Biol Chem* 287: 19440–19451, 2012. doi:10.1074/jbc.M112.346072.
- Kiernan MC, Vucic S, Cheah BC, Turner MR, Eisen A, Hardiman O, Burrell JR, Zoing MC. Amyotrophic lateral sclerosis. *Lancet* 377: 942–955, 2011. doi:10.1016/S0140-6736(10)61156-7.
- Kilinc D, Gallo G, Barbee KA. Mechanically-induced membrane poration causes axonal beading and localized cytoskeletal damage. *Exp Neurol* 212: 422–430, 2008. doi:10.1016/j.expneurol.2008.04.025.
- Kiskinis E, Kralj JM, Zou P, Weinstein EN, Zhang H, Tsiaras K, Wiskow O, Ortega JA, Eggan K, Cohen AE. All-optical electrophysiology for high-throughput functional characterization of a human iPSC-derived motor neuron model of ALS. *Stem Cell Reports* 10: 1991–2004, 2018. doi:10.1016/j.stemcr.2018.04.020.
- Kiskinis E, Sandoe J, Williams LA, Boulting GL, Moccia R, Wainger BJ, Han S, Peng T, Thams S, Mikkilineni S, Mellin C, Merkle FT, Davis-Dusenbery BN, Ziller M, Oakley D, Ichida J, Di Costanzo S, Atwater N,

- Maeder ML, Goodwin MJ, Nemesh J, Handsaker RE, Paull D, Noggle S, McCarroll SA, Joung JK, Woolf CJ, Brown RH, Eggan K. Pathways disrupted in human ALS motor neurons identified through genetic correction of mutant SOD1. *Cell Stem Cell* 14: 781–795, 2014. doi:10.1016/j.stem.2014.03.004.
- Lacorte E, Ferrigno L, Leoncini E, Corbo M, Boccia S, Vanacore N. Physical activity, and physical activity related to sports, leisure and occupational activity as risk factors for ALS: A systematic review. *Neurosci Biobehav Rev* 66: 61–79, 2016. doi:10.1016/j.neubiorev.2016.04.007.
- Lancaster MA, Knoblich JA. Organogenesis in a dish: modeling development and disease using organoid technologies. *Science* 345: 1247125, 2014. doi:10.1126/science.1247125.
- LaPlaca MC, Cullen DK, McLoughlin JJ, Cargill RS II. High rate shear strain of three-dimensional neural cell cultures: a new in vitro traumatic brain injury model. *J Biomech* 38: 1093–1105, 2005. doi:10.1016/j.jbiomech.2004.05.032.
- Lemon RN. Descending pathways in motor control. *Annu Rev Neurosci* 31: 195–218, 2008. doi:10.1146/annurev.neuro.31.060407.125547.
- Ling SC, Polymenidou M, Cleveland DW. Converging mechanisms in ALS and FTD: disrupted RNA and protein homeostasis. *Neuron* 79: 416–438, 2013. doi:10.1016/j.neuron.2013.07.033.
- Liu T, Zhao DX, Cui H, Chen L, Bao YH, Wang Y, Jiang JY. Therapeutic hypothermia attenuates tissue damage and cytokine expression after traumatic brain injury by inhibiting necroptosis in the rat. *Sci Rep* 6: 24547, 2016. doi:10.1038/srep24547.
- Lo Sardo V, Ferguson W, Erikson GA, Topol EJ, Baldwin KK, Torkamani A. Influence of donor age on induced pluripotent stem cells. *Nat Biotechnol* 35: 69–74, 2017. doi:10.1038/nbt.3749.
- Mackenzie IR, Bigio EH, Ince PG, Geser F, Neumann M, Cairns NJ, Kwong LK, Forman MS, Ravits J, Stewart H, Eisen A, McClusky L, Kretzschmar HA, Monoranu CM, Highley JR, Kirby J, Siddique T, Shaw PJ, Lee VM, Trojanowski JQ. Pathological TDP-43 distinguishes sporadic amyotrophic lateral sclerosis from amyotrophic lateral sclerosis with SOD1 mutations. *Ann Neurol* 61: 427–434, 2007. doi:10.1002/ana.21147.
- Mackenzie IR, Nicholson AM, Sarkar M, Messing J, Purice MD, Pottier C, Annu K, Baker M, Perkerson RB, Kurti A, Matchett BJ, Mittag T, Temirov J, Hsiung GR, Krieger C, Murray ME, Kato M, Fryer JD, Petrucelli L, Zinman L, Weintraub S, Mesulam M, Keith J, Zivkovic SA, Hirsch-Reinshagen V, Roos RP, Züchner S, Graff-Radford NR, Petersen RC, Caselli RJ, Wszolek ZK, Finger E, Lippa C, Lacomis D, Stewart H, Dickson DW, Kim HJ, Rogaeva E, Bigio E, Boylan KB, Taylor JP, Rademakers R. TIA1 mutations in amyotrophic lateral sclerosis and frontotemporal dementia promote phase separation and alter stress granule dynamics. *Neuron* 95: 808–816.e9, 2017. doi:10.1016/j.neuron.2017.07.025.
- Maekawa S, Leigh PN, King A, Jones E, Steele JC, Bodi I, Shaw CE, Hortobagyi T, Al-Sarraj S. TDP-43 is consistently co-localized with ubiquitinated inclusions in sporadic and Guam amyotrophic lateral sclerosis but not in familial amyotrophic lateral sclerosis with and without SOD1 mutations. *Neuropathology* 29: 672–683, 2009. doi:10.1111/j.1440-1789.2009.01029.x.
- Magou GC, Guo Y, Choudhury M, Chen L, Hususan N, Masotti S, Pfister BJ. Engineering a high throughput axon injury system. *J Neurotrauma* 28: 2203–2218, 2011. doi:10.1089/neu.2010.1596.
- Matsumoto A, Okada Y, Nakamichi M, Nakamura M, Toyama Y, Sobue G, Nagai M, Aoki M, Itoyama Y, Okano H. Disease progression of human SOD1 (G93A) transgenic ALS model rats. *J Neurosci Res* 83: 119–133, 2006. doi:10.1002/jnr.20708.
- McKee AC, Cantu RC, Nowinski CJ, Hedley-Whyte ET, Gavett BE, Budson AE, Santini VE, Lee HS, Kubilus CA, Stern RA. Chronic traumatic encephalopathy in athletes: progressive tauopathy after repetitive head injury. *J Neuropathol Exp Neurol* 68: 709–735, 2009. doi:10.1097/NEN.0b013e31819a9d503.
- McKee AC, Gavett BE, Stern RA, Nowinski CJ, Cantu RC, Kowall NW, Perl DP, Hedley-Whyte ET, Price B, Sullivan C, Morin P, Lee HS, Kubilus CA, Daneshvar DH, Wulff M, Budson AE. TDP-43 proteinopathy and motor neuron disease in chronic traumatic encephalopathy. *J Neuropathol Exp Neurol* 69: 918–929, 2010. doi:10.1097/NEN.0b013e3181ee7d85.
- McKee AC, Stern RA, Nowinski CJ, Stein TD, Alvarez VE, Daneshvar DH, Lee HS, Wojtowicz SM, Hall G, Baugh CM, Riley DO, Kubilus CA, Cormier KA, Jacobs MA, Martin BR, Abraham CR, Ikezu T, Reichard RR, Wolozin BL, Budson AE, Goldstein LE, Kowall NW, Cantu RC. The spectrum of disease in chronic traumatic encephalopathy. *Brain* 136: 43–64, 2013. doi:10.1093/brain/aww307.
- McNeish J, Gardner JP, Wainger BJ, Woolf CJ, Eggan K. From dish to bedside: lessons learned while translating findings from a stem cell model of disease to a clinical trial. *Cell Stem Cell* 17: 8–10, 2015. doi:10.1016/j.stem.2015.06.013.
- Miller JD, Ganat YM, Kishinevsky S, Bowman RL, Liu B, Tu EY, Mandal PK, Vera E, Shim JW, Kriks S, Taldone T, Fusaki N, Tomishima MJ, Krainc D, Milner TA, Rossi DJ, Studer L. Human iPSC-based modeling of late-onset disease via progerin-induced aging. *Cell Stem Cell* 13: 691–705, 2013. doi:10.1016/j.stem.2013.11.006.
- Mitne-Neto M, Machado-Costa M, Marchetto MC, Bengtson MH, Joazeiro CA, Tsuda H, Bellen HJ, Silva HC, Oliveira AS, Lazar M, Muotri AR, Zatz M. Downregulation of VAPB expression in motor neurons derived from induced pluripotent stem cells of ALS8 patients. *Hum Mol Genet* 20: 3642–3652, 2011. doi:10.1093/hmg/ddr284.
- Moisse K, Volkening K, Leystra-Lantz C, Welch I, Hill T, Strong MJ. Divergent patterns of cytosolic TDP-43 and neuronal progranulin expression following axotomy: implications for TDP-43 in the physiological response to neuronal injury. *Brain Res* 1249: 202–211, 2009. doi:10.1016/j.brainres.2008.10.021.
- Morrison B III, Cater HL, Benham CD, Sundstrom LE. An in vitro model of traumatic brain injury utilizing two-dimensional stretch of organotypic hippocampal slice cultures. *J Neurosci Methods* 150: 192–201, 2006. doi:10.1016/j.jneumeth.2005.06.014.
- Morrison B III, Cater HL, Wang CC, Thomas FC, Hung CT, Ateshian GA, Sundstrom LE. A tissue level tolerance criterion for living brain developed with an in vitro model of traumatic mechanical loading. *Stapp Car Crash J* 47: 93–105, 2003. doi:10.4271/2003-22-0006.
- Morrison B III, Elkin BS, Dollé JP, Yarmush ML. In vitro models of traumatic brain injury. *Annu Rev Biomed Eng* 13: 91–126, 2011. doi:10.1146/annurev-bioeng-071910-124706.
- Mui S, Rebeck GW, McKenna-Yasek D, Hyman BT, Brown RH Jr. Apolipoprotein E ε4 allele is not associated with earlier age at onset in amyotrophic lateral sclerosis. *Ann Neurol* 38: 460–463, 1995. doi:10.1002/ana.410380318.
- Münch C, Sedlmeier R, Meyer T, Homberg V, Sperfeld AD, Kurt A, Prudlo J, Peraus G, Hanemann CO, Stumm G, Ludolph AC. Point mutations of the p150 subunit of dynactin (DCTN1) gene in ALS. *Neurology* 63: 724–726, 2004. doi:10.1212/01.WNL.0000134608.83927.B1.
- Myer DJ, Gurkoff GG, Lee SM, Hovda DA, Sofroniew MV. Essential protective roles of reactive astrocytes in traumatic brain injury. *Brain* 129: 2761–2772, 2006. doi:10.1093/brain/awl165.
- Nakano T, Nakaso K, Nakashima K, Ohama E. Expression of ubiquitin-binding protein p62 in ubiquitin-immunoreactive intraneuronal inclusions in amyotrophic lateral sclerosis with dementia: analysis of five autopsy cases with broad clinicopathological spectrum. *Acta Neuropathol* 107: 359–364, 2004. doi:10.1007/s00401-004-0821-7.
- Neumann H, Kotter MR, Franklin RJ. Debris clearance by microglia: an essential link between degeneration and regeneration. *Brain* 132: 288–295, 2009. doi:10.1093/brain/awn109.
- Palmer AM, Marion DW, Botscheller ML, Swedlow PE, Styren SD, DeKosky ST. Traumatic brain injury-induced excitotoxicity assessed in a controlled cortical impact model. *J Neurochem* 61: 2015–2024, 1993. doi:10.1111/j.1471-4159.1993.tb07437.x.
- Pfister T, Pfister K, Hagel B, Ghali WA, Ronksley PE. The incidence of concussion in youth sports: a systematic review and meta-analysis. *Br J Sports Med* 50: 292–297, 2016. doi:10.1136/bjsports-2015-094978.
- Povlishock JT, Pettus EH. Traumatically induced axonal damage: evidence for enduring changes in axolemmal permeability with associated cytoskeletal change. *Acta Neurochir Suppl (Wien)* 66: 81–86, 1996. doi:10.1007/978-3-7091-9465-2_15.
- Prien A, Grafe A, Rössler R, Junge A, Verhagen E. Epidemiology of head injuries focusing on concussions in team contact sports: a systematic review. *Sports Med* 48: 953–969, 2018. doi:10.1007/s40279-017-0854-4.
- Progress & Updates: Answer ALS Research. <https://research.answerals.org/progress-updates/>. [25 January 2018].
- Pun S, Santos AF, Saxena S, Xu L, Caroni P. Selective vulnerability and pruning of phasic motoneuron axons in motoneuron disease alleviated by CNTF. *Nat Neurosci* 9: 408–419, 2006. doi:10.1038/nn1653.
- Pupillo E, Poloni M, Bianchi E, Giussani G, Logroscino G, Zoccollella S, Chio A, Calvo A, Corbo M, Lunetta C, Marin B, Mitchell D, Hardiman O, Rooney J, Stevic Z, Bandettini di Poggio M, Filosto M, Cotelli MS, Perini M, Riva N, Tremolizzo L, Vitelli E, Damiani D, Beghi E,

- Consortiumdagger E. Trauma and amyotrophic lateral sclerosis: a european population-based case-control study from the EURALS consortium. *Amyotroph Lateral Scler Frontotemporal Degener* 19: 118–125, 2017. doi:10.1080/21678421.2017.1386687.
- Re DB, Le Verche V, Yu C, Amoroso MW, Politi KA, Phani S, Ikiz B, Hoffmann L, Koolen M, Nagata T, Papadimitriou D, Nagy P, Mitsumoto H, Kariya S, Wichterle H, Henderson CE, Przedborski S. Necroptosis drives motor neuron death in models of both sporadic and familial ALS. *Neuron* 81: 1001–1008, 2014. doi:10.1016/j.neuron.2014.01.011.
- Readnower RD, Chavko M, Adeeb S, Conroy MD, Pauly JR, McCarron RM, Sullivan PG. Increase in blood-brain barrier permeability, oxidative stress, and activated microglia in a rat model of blast-induced traumatic brain injury. *J Neurosci Res* 88: 3530–3539, 2010. doi:10.1002/jnr.22510.
- Renton AE, Chiò A, Traynor BJ. State of play in amyotrophic lateral sclerosis genetics. *Nat Neurosci* 17: 17–23, 2014. doi:10.1038/nn.3584.
- Renton AE, Majounie E, Waite A, Simón-Sánchez J, Rollinson S, Gibbs JR, Schymick JC, Laaksovirta H, van Swieten JC, Myllykangas L, Kalimo H, Paetau A, Abramzon Y, Remes AM, Kaganovich A, Scholz SW, Duckworth J, Ding J, Harmer DW, Hernandez DG, Johnson JO, Mok K, Ryten M, Trabzuni D, Guerreiro RJ, Orrell RW, Neal J, Murray A, Pearson J, Jansen IE, Sondervan D, Seelaar H, Blake D, Young K, Halliwell N, Callister JB, Toulson G, Richardson A, Gerhard A, Snowden J, Mann D, Neary D, Nalls MA, Peuralinna T, Jansson L, Isoviiita VM, Kaivorinne AL, Hölttä-Vuori M, Ikonen E, Sulkava R, Benarab M, Wu J, Chiò A, Restagno G, Borghero G, Sabatelli M, Heckerman D, Rogaeva E, Zinman L, Rothstein JD, Sendtner M, Drepper C, Eichler EE, Alkan C, Abdullaev Z, Pack SD, Dutra A, Pak E, Hardy J, Singleton A, Williams NM, Heutink P, Pickering-Brown S, Morris HR, Tienari PJ, Traynor BJ; ITALSGEN Consortium. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* 72: 257–268, 2011. doi:10.1016/j.neuron.2011.09.010.
- Rosenbohm A, Kassubek J, Weydt P, Marroquin N, Volk AE, Kubisch C, Huppertz HJ, Weber M, Andersen PM, Weishaupt JH, Ludolph AC; ALS Schwaben Register Group. Can lesions to the motor cortex induce amyotrophic lateral sclerosis? *J Neurol* 261: 283–290, 2014. doi:10.1007/s00415-013-7185-7.
- Rothstein JD, Van Kammen M, Levey AI, Martin LJ, Kuncel RW. Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. *Ann Neurol* 38: 73–84, 1995. doi:10.1002/ana.410380114.
- Sareen D, O'Rourke JG, Meera P, Muhammad AK, Grant S, Simpkinson M, Bell S, Carmona S, Ornelas L, Sahabian A, Gendron T, Petrucelli L, Baughn M, Ravits J, Harms MB, Rigo F, Bennett CF, Otis TS, Svendsen CN, Baloh RH. Targeting RNA foci in iPSC-derived motor neurons from ALS patients with a C9ORF72 repeat expansion. *Sci Transl Med* 5: 208ra149, 2013. doi:10.1126/scitranslmed.3007529.
- Scarmeas N, Shih T, Stern Y, Ottman R, Rowland LP. Premorbid weight, body mass, and varsity athletics in ALS. *Neurology* 59: 773–775, 2002. doi:10.1212/WNL.59.5.773.
- Schmidt S, Kwee LC, Allen KD, Oddone EZ. Association of ALS with head injury, cigarette smoking and APOE genotypes. *J Neurol Sci* 291: 22–29, 2010. doi:10.1016/j.jns.2010.01.011.
- Seeger TA, Kirton A, Esser KM, Gallagher C, Dunn J, Zewdie E, Damji O, Ciechanski P, Barlow MJ. Cortical excitability after pediatric mild traumatic brain injury. *Brain Stimul* 10: 305–314, 2017. doi:10.1016/j.brs.2016.11.011.
- Sendroy-Terrill M, Whitenack GG, Brooks CA. Aging with traumatic brain injury: cross-sectional follow-up of people receiving inpatient rehabilitation over more than 3 decades. *Arch Phys Med Rehabil* 91: 489–497, 2010. doi:10.1016/j.apmr.2009.11.011.
- Serio A, Bilcan B, Barmada SJ, Ando DM, Zhao C, Siller R, Burr K, Haghi G, Story D, Nishimura AL, Carrasco MA, Phatnani HP, Shum C, Wilmut I, Maniatis T, Shaw CE, Finkbeiner S, Chandran S. Astrocyte pathology and the absence of non-cell autonomy in an induced pluripotent stem cell model of TDP-43 proteinopathy. *Proc Natl Acad Sci USA* 110: 4697–4702, 2013. doi:10.1073/pnas.1300398110.
- Sherman SA, Phillips JK, Costa JT, Cho FS, Oungoulian SR, Finan JD. Stretch injury of human induced pluripotent stem cell derived neurons in a 96 well format. *Sci Rep* 6: 34097, 2016. doi:10.1038/srep34097.
- Shibata N, Hirano A, Kobayashi M, Siddique T, Deng HX, Hung WY, Kato T, Asayama K. Intense superoxide dismutase-1 immunoreactivity in intracytoplasmic hyaline inclusions of familial amyotrophic lateral sclerosis with posterior column involvement. *J Neuropathol Exp Neurol* 55: 481–490, 1996. doi:10.1097/00005072-199604000-00011.
- Siddique T, Pericak-Vance MA, Caliendo J, Hong ST, Hung WY, Kaplan J, McKenna-Yasek D, Rimmler JB, Sapp P, Saunders AM, Scott WK, Siddique N, Haines JL, Brown RH. Lack of association between apolipoprotein E genotype and sporadic amyotrophic lateral sclerosis. *Neurogenetics* 1: 213–216, 1998. doi:10.1007/s100480050031.
- Smith BN, Ticozzi N, Fallini C, Gkazi AS, Topp S, Kenna KP, Scotter EL, Kost J, Keagle P, Miller JW, Calini D, Vance C, Danielson EW, Troakes C, Tiloca C, Al-Sarraj S, Lewis EA, King A, Colombrita C, Pensato V, Castellotti B, de Bellerocche J, Baas F, ten Asbroek AL, Sapp PC, McKenna-Yasek D, McLaughlin RL, Polak M, Ares S, Esteban-Pérez J, Muñoz-Blanco JL, Simpson M, van Rheenen W, Diekstra FP, Lauria G, Duga S, Corti S, Cereda C, Corrado L, Sorarù G, Morrison KE, Williams KL, Nicholson GA, Blair IP, Dion PA, Leblond CS, Rouleau GA, Hardiman O, Veldink JH, van den Berg LH, Al-Chalabi A, Pall H, Shaw PJ, Turner MR, Talbot K, Taroni F, García-Redondo A, Wu Z, Glass JD, Gellera C, Ratti A, Brown RH Jr, Silani V, Shaw CE, Landers JE; SLAGEN Consortium. Exome-wide rare variant analysis identifies TUBA4A mutations associated with familial ALS. *Neuron* 84: 324–331, 2014. doi:10.1016/j.neuron.2014.09.027.
- Smith DH, Wolf JA, Lusardi TA, Lee VM, Meaney DF. High tolerance and delayed elastic response of cultured axons to dynamic stretch injury. *J Neurosci* 19: 4263–4269, 1999. doi:10.1523/JNEUROSCI.19-11-04263.1999.
- Sreedharan J, Blair IP, Tripathi VB, Hu X, Vance C, Rogelj B, Ackerley S, Durnall JC, Williams KL, Buratti E, Baralle F, de Bellerocche J, Mitchell JD, Leigh PN, Al-Chalabi A, Miller CC, Nicholson G, Shaw CE. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science* 319: 1668–1672, 2008. doi:10.1126/science.1154584.
- Strong MJ, Yang W, Strong WL, Leystra-Lantz C, Jaffe H, Pant HC. Tau protein hyperphosphorylation in sporadic ALS with cognitive impairment. *Neurology* 66: 1770–1771, 2006. doi:10.1212/01.wnl.0000218161.15834.db.
- Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126: 663–676, 2006. doi:10.1016/j.cell.2006.07.024.
- Taylor JP, Brown RH Jr, Cleveland DW. Decoding ALS: from genes to mechanism. *Nature* 539: 197–206, 2016. doi:10.1038/nature20413.
- Thomsen GM, Ma AM, Ko A, Harada MY, Wyss L, Haro PS, Vit JP, Shelest O, Rhee P, Svendsen CN, Ley EJ. A model of recurrent concussion that leads to long-term motor deficits, CTE-like tauopathy and exacerbation of an ALS phenotype. *J Trauma Acute Care Surg* 81: 1070–1079, 2016. doi:10.1097/TA.0000000000001248.
- Thomsen GM, Vit JP, Lamb A, Gowing G, Shelest O, Alkaslasi M, Ley EJ, Svendsen CN. Acute traumatic brain injury does not exacerbate amyotrophic lateral sclerosis in the SOD1 (G93A) rat model(1,2,3). *eNeuro* 2: ENEURO.0059-14.2015, 2015. doi:10.1523/ENEURO.0059-14.2015.
- Turner BJ, Talbot K. Transgenics, toxicity and therapeutics in rodent models of mutant SOD1-mediated familial ALS. *Prog Neurobiol* 85: 94–134, 2008. doi:10.1016/j.pneurobio.2008.01.001.
- Turner MR, Abisgold J, Yeates DG, Talbot K, Goldacre MJ. Head and other physical trauma requiring hospitalisation is not a significant risk factor in the development of ALS. *J Neurol Sci* 288: 45–48, 2010. doi:10.1016/j.jns.2009.10.010.
- Van Damme P, Bogaert E, Dewil M, Hersmus N, Kiraly D, Scheveneels W, Bockx I, Braeken D, Verpoorten N, Verhoeven K, Timmerman V, Herijgers P, Callewaert G, Carmeliet P, Van Den Bosch L, Robberecht W. Astrocytes regulate GluR2 expression in motor neurons and their vulnerability to excitotoxicity. *Proc Natl Acad Sci USA* 104: 14825–14830, 2007. doi:10.1073/pnas.0705046104.
- Van Damme P, Robberecht W, Van Den Bosch L. Modelling amyotrophic lateral sclerosis: progress and possibilities. *Dis Model Mech* 10: 537–549, 2017. doi:10.1242/dmm.029058.
- Vandenabeele P, Galluzzi L, Vanden Berghe T, Kroemer G. Molecular mechanisms of necroptosis: an ordered cellular explosion. *Nat Rev Mol Cell Biol* 11: 700–714, 2010. doi:10.1038/nrm2970.
- Victor MB, Richner M, Olsen HE, Lee SW, Monteyes AM, Ma C, Huh CJ, Zhang B, Davidson BL, Yang XW, Yoo AS. Striatal neurons directly converted from Huntington's disease patient fibroblasts recapitulate age-associated disease phenotypes. *Nat Neurosci* 21: 341–352, 2018. doi:10.1038/s41593-018-0075-7.
- Wagner AK, Bayir H, Ren D, Puccio A, Zafonte RD, Kochanek PM. Relationships between cerebrospinal fluid markers of excitotoxicity, ischemia, and oxidative damage after severe TBI: the impact of gender,

- age, and hypothermia. *J Neurotrauma* 21: 125–136, 2004. doi:10.1089/089771504322778596.
- Wainger BJ, Kiskinis E, Mellin C, Wiskow O, Han SS, Sandoe J, Perez NP, Williams LA, Lee S, Boulting G, Berry JD, Brown RH Jr, Cudkovic ME, Bean BP, Eggan K, Woolf CJ. Intrinsic membrane hyperexcitability of amyotrophic lateral sclerosis patient-derived motor neurons. *Cell Reports* 7: 1–11, 2014. doi:10.1016/j.celrep.2014.03.019.
- Wang YQ, Wang L, Zhang MY, Wang T, Bao HJ, Liu WL, Dai DK, Zhang L, Chang P, Dong WW, Chen XP, Tao LY. Necrostatin-1 suppresses autophagy and apoptosis in mice traumatic brain injury model. *Neurochem Res* 37: 1849–1858, 2012. doi:10.1007/s11064-012-0791-4.
- Weaver SM, Chau A, Portelli JN, Grafman J. Genetic polymorphisms influence recovery from traumatic brain injury. *Neuroscientist* 18: 631–644, 2012. doi:10.1177/1073858411435706.
- Woods AH. Trauma as a cause of amyotrophic lateral sclerosis. *JAMA* LVI: 1876–1877, 1911. doi:10.1001/jama.1911.02560250012006.
- Wu CH, Fallini C, Ticozzi N, Keagle PJ, Sapp PC, Piotrowska K, Lowe P, Koppers M, McKenna-Yasek D, Baron DM, Kost JE, Gonzalez-Perez P, Fox AD, Adams J, Taroni F, Tiloca C, Leclerc AL, Chafe SC, Mangroo D, Moore MJ, Zitzewitz JA, Xu ZS, van den Berg LH, Glass JD, Siciliano G, Cirulli ET, Goldstein DB, Salachas F, Meininger V, Rossoll W, Ratti A, Gellera C, Bosco DA, Bassell GJ, Silani V, Drory VE, Brown RH Jr, Landers JE. Mutations in the profilin 1 gene cause familial amyotrophic lateral sclerosis. *Nature* 488: 499–503, 2012. doi:10.1038/nature11280.
- Xing C, Hayakawa K, Lok J, Arai K, Lo EH. Injury and repair in the neurovascular unit. *Neurol Res* 34: 325–330, 2012. doi:10.1179/1743132812Y.0000000019.
- Yang W, Sopper MM, Leystra-Lantz C, Strong MJ. Microtubule-associated tau protein positive neuronal and glial inclusions in ALS. *Neurology* 61: 1766–1773, 2003. doi:10.1212/01.WNL.0000099372.75786.F8.
- Yang YM, Gupta SK, Kim KJ, Powers BE, Cerqueira A, Wainger BJ, Ngo HD, Rosowski KA, Schein PA, Ackeifi CA, Arvanites AC, Davidow LS, Woolf CJ, Rubin LL. A small molecule screen in stem-cell-derived motor neurons identifies a kinase inhibitor as a candidate therapeutic for ALS. *Cell Stem Cell* 12: 713–726, 2013. doi:10.1016/j.stem.2013.04.003.
- Yang Z, Lin F, Robertson CS, Wang KK. Dual vulnerability of TDP-43 to calpain and caspase-3 proteolysis after neurotoxic conditions and traumatic brain injury. *J Cereb Blood Flow Metab* 34: 1444–1452, 2014. doi:10.1038/jebfm.2014.105.
- Yoo AS, Sun AX, Li L, Shcheglovitov A, Portmann T, Li Y, Lee-Messer C, Dolmetsch RE, Tsien RW, Crabtree GR. MicroRNA-mediated conversion of human fibroblasts to neurons. *Nature* 476: 228–231, 2011. doi:10.1038/nature10323.
- You Z, Savitz SI, Yang J, Degterev A, Yuan J, Cuny GD, Moskowitz MA, Whalen MJ. Necrostatin-1 reduces histopathology and improves functional outcome after controlled cortical impact in mice. *J Cereb Blood Flow Metab* 28: 1564–1573, 2008. doi:10.1038/jebfm.2008.44.
- Zetterberg H, Smith DH, Blennow K. Biomarkers of mild traumatic brain injury in cerebrospinal fluid and blood. *Nat Rev Neurol* 9: 201–210, 2013. doi:10.1038/nrneurol.2013.9.
- Zhang K, Daigle JG, Cunningham KM, Coyne AN, Ruan K, Grima JC, Bowen KE, Wadhwa H, Yang P, Rigo F, Taylor JP, Gitler AD, Rothstein JD, Lloyd TE. Stress granule assembly disrupts nucleocytoplasmic transport. *Cell* 173: 958–971.e17, 2018. doi:10.1016/j.cell.2018.03.025.
- Zhang K, Donnelly CJ, Haeusler AR, Grima JC, Machamer JB, Steinwald P, Daley EL, Miller SJ, Cunningham KM, Vidensky S, Gupta S, Thomas MA, Hong I, Chiu SL, Haganir RL, Ostrow LW, Matunis MJ, Wang J, Sattler R, Lloyd TE, Rothstein JD. The C9orf72 repeat expansion disrupts nucleocytoplasmic transport. *Nature* 525: 56–61, 2015. doi:10.1038/nature14973.

