



This is a repository copy of *Review: Microglia in motor neuron disease*.

White Rose Research Online URL for this paper:
<http://eprints.whiterose.ac.uk/163599/>

Version: Published Version

Article:

Ashford, B.A. orcid.org/0000-0002-9247-3125, Boche, D., Cooper-Knock, J. et al. (3 more authors) (2020) *Review: Microglia in motor neuron disease*. *Neuropathology and Applied Neurobiology*. ISSN 0305-1846

<https://doi.org/10.1111/nan.12640>

Reuse

This article is distributed under the terms of the Creative Commons Attribution (CC BY) licence. This licence allows you to distribute, remix, tweak, and build upon the work, even commercially, as long as you credit the authors for the original work. More information and the full terms of the licence here:
<https://creativecommons.org/licenses/>

Takedown







If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.



eprints@whiterose.ac.uk
<https://eprints.whiterose.ac.uk/>



Review: Microglia in motor neuron disease

B. A. Ashford* , D. Boche† , J. Cooper-Knock* , P. R. Heath* , J. E. Simpson*  and J. R. Highley* 

*University of Sheffield, Sheffield, UK and †University of Southampton, Southampton, UK

B. A. Ashford, D. Boche, J. Cooper-Knock, P. R. Heath, J. E. Simpson and J. R. Highley (2020) *Neuropathology and Applied Neurobiology* **Review: Microglia in motor neuron disease**

Motor Neuron Disease (MND) is a fatal neurodegenerative condition, which is characterized by the selective loss of the upper and lower motor neurons. At the sites of motor neuron injury, accumulation of activated microglia, the primary immune cells of the central nervous system, is commonly observed in both human *post mortem* studies and animal models of MND. Microglial activation has been found to correlate with many clinical features and importantly, the speed of disease progression in humans. Both anti-inflammatory and pro-inflammatory microglial responses have been shown to influence disease progression in humans and models of MND. As such, microglia could both contribute to and

protect against inflammatory mechanisms of pathogenesis in MND. While murine models have characterized the microglial response to MND, these studies have painted a complex and often contradictory picture, indicating a need for further characterization in humans. This review examines the potential role microglia play in MND in human and animal studies. Both the pro-inflammatory and anti-inflammatory responses will be addressed, throughout the course of disease, followed by the potential of microglia as a target in the development of disease-modifying treatments for MND.

Keywords: humans, inflammation, microglia, motor neuron disease

Motor neuron disease

Motor Neuron Disease (MND) is a fatal neurodegenerative condition characterized by the progressive degeneration of both upper motor neurons in the motor cortex, and the lower motor neurons in the brain stem and spinal cord [1]. MND has a number of subtypes, of which Amyotrophic Lateral Sclerosis (ALS) is the most common and affects around two in every 100 000 people [2]. Throughout much of the literature, ALS and MND are used synonymously; here MND will be used to refer to all subclasses of the disease.

Typically, MND presents with progressive muscle weakness in the extremities, muscular atrophy,

fasciculations, cramping, spasticity and fatigue [1]. Diagnosis of MND normally occurs around 55–60 years of age. While survival time varies from just a few months to decades [3], 80% of patients survive only two to five years after diagnosis, with death usually resulting from paralysis and respiratory failure [1]. Currently there is no cure, and the only US Food and Drug Administration-approved treatments, Riluzole and Edaravone, have modest effects, extending survival by mere months [1].

MND is pathogenically complicated, and the cause of MND in most cases is unknown: 90–95% of cases are sporadic (sMND), while the remaining 5–10% are familial (fMND), generally with autosomal dominant inheritance [4]. More than forty pathogenic mutations have been associated with MND, including mutations in *SOD1* (*Superoxide dismutase*) [5], *TARDBP* which encodes Transactive Response DNA-binding protein 43 kD (TDP-43) [6], *FUS* (*RNA Binding Protein Fused in*

Correspondence: Bridget A. Ashford, Sheffield Institute of Translational Neuroscience, University of Sheffield, 385a Glossop Road, Sheffield S10 2HQ, UK.

Tel: +44 (0)114 222 2230; Fax: +44 (0)114 222 2290; E-mail: baashford1@sheffield.ac.uk

Sarcoma/Translocated in Sarcoma) [7,8], and the most common, *C9orf72* (*chromosome 9 open reading frame 72*) [9]. Interestingly, a number of MND-associated genes have a direct role in immunity, such as *TBK1* [10], *OPTN* [11], *VCP* [12] or pathways that are important for immune function, such as the autophagy and lysosomal pathways (e.g. *SQSTM1* [13], *UBQLN2* [14]).

Neuropathology of MND

There are many pathological changes associated with MND including depletion of giant pyramidal (Betz) cells and atrophy of the precentral gyrus, thinning of the spinal ventral roots, sclerosis and pallor in the corticospinal tracts and loss of lower motor neurons [15].

The hallmark microscopic pathology of MND is the presence of protein aggregates in the remaining motor neurons. In the majority of cases, these aggregates are composed of ubiquitylated TDP-43 [6]. However, aggregates may also include or be composed of other proteins including FUS and SOD1 [16]. TDP-43-positive inclusions are primarily seen in the motor structures of the spinal cord, corticospinal tract and the motor cortex [17]. These inclusions are also observed to a lesser and more variable extent in extramotor regions including the anterior frontal and temporal cortices [18]. MND is considered to be on a disease spectrum with Frontotemporal Dementia which is characterized by changes in personality and behaviour, loss of language skills and cognitive decline [19]. Along this continuum cases show pathology purely in motor regions, ranging to FTD cases with purely nonmotor region pathology [18]. This spectrum is reflected clinically with some MND patients displaying purely motor symptoms, some patients having both motor and cognitive or behavioural decline and some FTD patients without motor symptoms.

Glial involvement in MND

In models of MND, motor neurons show dysfunction in many cellular processes including axonal transport and microtubule function [20,21], RNA processing [22], endoplasmic reticular stress response [23] and endosomal trafficking [24]. However, MND is not a purely neuron-autonomous disease; glial cells are highly involved and also display inclusion pathology both in

human MND and in animal models [25,26]. Microgliosis and astrogliosis, the emergence of activated microglia and astrocytes respectively, can be seen in human *post mortem* tissue and animal models [27,28]. Further, in a chimeric mutant SOD1^{G93A} mouse model, mutant SOD1 (mSOD1) expressing motor neurons showed improved survival when surrounded by wild-type astrocytes and microglia, and in contrast, wild-type motor neurons showed MND pathology when surrounded by mSOD1 glia [29], indicating that the glial environment influences neuronal pathology in MND. While infiltrating immune cells, such as mast cells, T cells, dendritic cells and macrophages, are also a feature of MND [27,30,31], the remainder of this review will focus on the complex role microglia play in the progression of MND.

Microglia – the primary immune cells of the central nervous system

Microglia are the resident immune cells of monocytic lineage and are the primary form of immune defence within the central nervous system (CNS). These highly active cells have several functions in development, homeostasis and innate immunity [32].

Under homeostatic conditions, microglia have a ramified morphology with small somata and short, fine processes that constantly extend, retract and reform in order to survey the local microenvironment [33]. Under pathological conditions, microglia have functions in pathogen destruction, the resolution of inflammation and the promotion of healing and cell growth. Microglia react to signals from potential pathogens (Pathogen-Associated Molecular Patterns; PAMPs), and signals expressed by damaged endogenous cells (Damage-Associated Molecular Patterns; DAMPS), via the activation of pattern recognition receptors [34]. In response, microglial cytoplasmic processes swell and shorten, becoming less ramified, and at the extreme end of the spectrum, the cell can adopt a large, 'amoeboid' morphology (Figure 1). This activation is associated with alterations in the molecules that they express. Once the activating stimulus has been resolved, microglia may revert to their homeostatic ramified state.

However, with ageing and chronic activation microglia have been observed to adopt another phenotype, often referred to as the dystrophic phenotype [35,36].

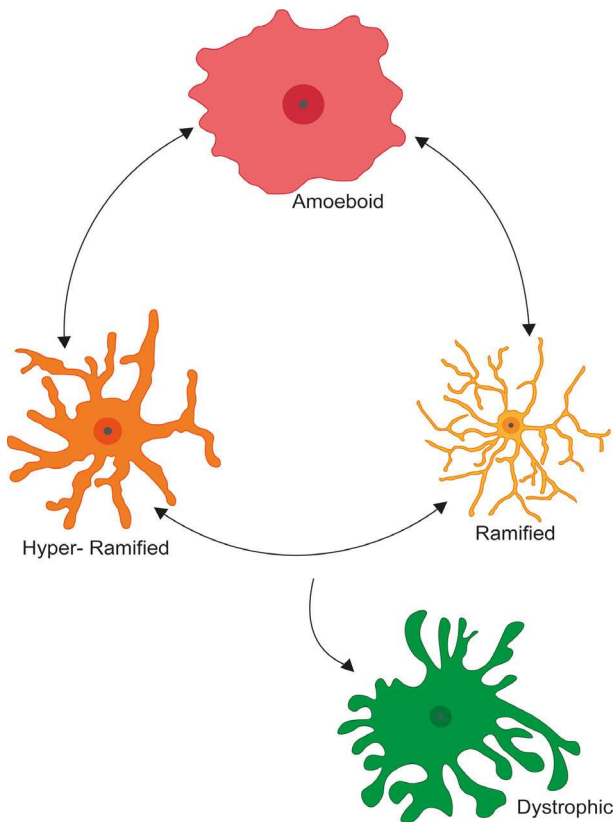


Figure 1. Microglial morphology. In their resting state microglia have a ramified morphology. Once activated, their cytoplasmic processes swell such that the cell ultimately adopts a large amoeboid morphology. Upon resolution of the activating stimulus, microglia revert to their ramified state. With aging microglia can adopt a dystrophic phenotype characterised by loss and beading of the processes and fragmentation of the cytoplasm.

These cells show reduced surveillance and dendritic branching [37], a redistribution of Iba1- particularly in the processes [38] and reduced motility and migration [39]. Furthermore, these cells show changes in gene expression including expression of pro-inflammatory cytokines in homeostatic conditions [40] and more sustained responses to damage [39], as well as storage of iron as ferrate [41]. As this phenotype is most commonly observed in the ageing brain, this dysfunctional phenotype may represent microglial senescence.

Microglia present a multifaceted inflammatory response. This includes the release of pro-inflammatory cytokines and reactive oxygen and nitrogen species to aid the destruction of pathogens and damaged cells. Chemokines may be released to further activate and recruit other glial and immune cells to the site of injury

[32]. Microglia also have functions relating to inflammation mediation and resolution, debris clearance, tissue healing and growth. This is achieved firstly, via the release of anti-inflammatory cytokines and trophic factors, which promote healing, repair and tissue remodelling. Secondly, they may increase the number of surface scavenger receptors to promote pathogen and debris phagocytosis [42].

Historically, activated microglia have been classified as pro-inflammatory (M1) or anti-inflammatory (M2). However, this binary system has received criticism. It has been suggested that activated microglia may fall on a spectrum, with some cells being purely M1 through to some cells which show a purely M2 phenotype [43,44]. However, transcriptomic profiling of *ex vivo* and *in vivo* microglia isolated from mouse models of various neurodegenerative conditions, failed to find evidence of an M1/M2 spectrum, with many microglia exhibiting disease-specific profiles [45,46]. Furthermore, single-cell-based analyses of microglia from both mouse and human cortex have revealed several transcriptionally distinct phenotypes which differ from the M1/M2 phenotypes [47,48]. This said, a lot of the literature still follows this type of characterization and uses classic inflammatory markers to determine microglial function. As such, in this review, we will focus on microglial functions and will use the terms pro-inflammatory and anti-inflammatory to broadly describe microglial activation. However, these terms are used with caution and the reader is respectfully advised to bear in mind that this is done as a 'shorthand' to ease discussion. We do not wish to imply that we endorse a simple binary classification.

A brief summary of some of the main molecules associated with microglia is given in Table 1. Microglial phenotype is affected by a number of complex factors including location within the CNS [49,50], microglial age [51], activating stimuli and disease [47], as well as differences between human and murine microglia [51]. Furthermore, microglia may express molecules with pro-inflammatory and anti-inflammatory functions simultaneously. Therefore, it should be noted that Table 1 is not a comprehensive list and is focused more on findings related to neurodegenerative disease, particularly MND.

Microglial activation is effective in the short-term removal of damaged cells and pathogens and is necessary for the protection of the CNS. However, where

Table 1. Summarization of microglial phenotypes, their activating stimuli and typical markers for their identification

<i>Associated functions</i>	<i>Homeostatic state and pathogen detection</i>	<i>Pathogen destruction- associated with inflammation</i>	<i>Inflammation suppression and phagocytosis</i>	<i>Tissue remodelling and extracellular matrix deposition</i>
Upregulated cytokines		TNF- α , IL-1 β , IL-1 α , IL-6, IL-12a, IL-12b, IL-15, IL-18, IL-23, IL-33, IFN- γ	IL-4, IL-10, IL-13 ARG1*, TGF-β, FIZZ1*	IL-10
Chemokines		CCL1, CCL2 (MCP-1), CCL3, CCL4, CCL5, CCL8, CCL12 (MCP-5), CXCL2, CXCL4, CXCL9, CXCL10, CXCL13	CXCL16, CCR2 (CD192),	CXCL13
Enzymes	HEXB+,	NOX2 (CYBB), NOS2, iNOS, CASP1 (ICE), COX2		MMP-12, ARG1*?, YM1*?
Receptors and Transmembrane Proteins	TGFB β 1+, P2RY12+, P2RY13+, FCRLS*, TMEM119+, GPR34+, SIGLEC-H+, CD11b (ITGAM), Iba1(AIF1*), CXCR3R1, CSF1R	MHC II receptors including HLA-DR, IFNar1, IFNar2, CD45, CD119 (IFNgr1), CD14, TLR-2, CCR8,	CD163, CD204, IFNAR1, IFNAR2, IL-10RA, CD68, IFITM3, CD16 (FcyRIII), CD32 (FcyRII), CD33, CD64, TREM2*?	MHCII, CCR8
Growth factors			IGF1	BDNF
Transcription and signalling factors	SALL1+, PU.1,	IRF1, STAT3		
Other	OLFML3+, GLUT5, ROS	S100A8, S100A9, IRAK1	OPTN, DAP12 (TYROBP), GRN,	
References	[47,142–145]	[32,47,145–151]	[47,145–147,151]	[32,47,146,151–153]

Key: mouse specific*; Human expression uncertain*?; **Result from In-vitro study;** Microglial Marker/Signature Gene+

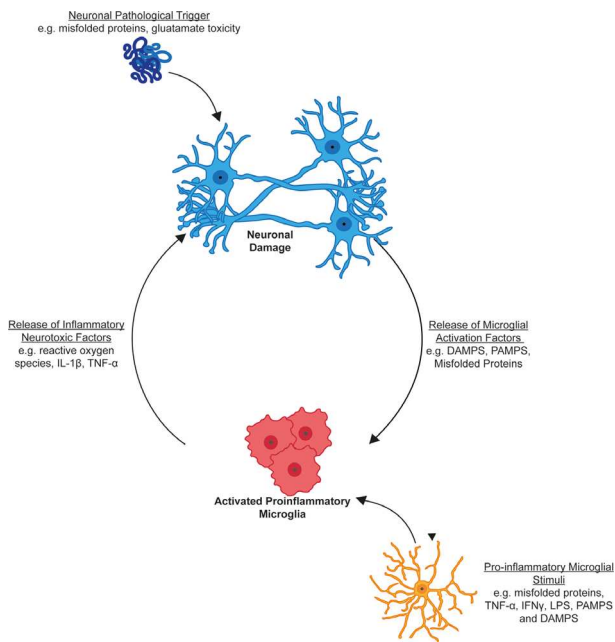


Figure 2. The cycle of microglial/ neuronal neurotoxicity. The toxicity cycle can be started in two ways. First, an external stimulus directly activates microglia, which produce cytotoxic factors that cause neuronal damage. Secondly, neuronal damage can be initiated by another pathological trigger, which results in the release of DAMPs, which in turn activates microglia, resulting in a toxic environment for other neurons (reactive microgliosis). This results in a self-perpetuating cycle of microglial over-activation and neuronal damage.

the initial trigger is not resolved, a destructive cycle of microglial activation and neuron death is initiated (Figure 2). Briefly, this cycle can be triggered by PAMPs causing microglial cytotoxicity leading to neuronal death. Alternatively, if neuron death is triggered by processes such as protein misfolding and aggregation, or excitotoxicity, DAMP release can cause cytotoxic microglial activation with increased inflammation and neuronal death [52]. This process is seen as a driver of progression in neurodegeneration [52,53].

Microglial activation in human MND

Human studies have demonstrated the importance of microglia in MND. *Post mortem* spinal cord from MND patients has been found to contain raised levels of Monocyte Chemoattractant Protein 1 (MCP-1; more commonly known as C-C Motif Chemokine Ligand 2 or

CCL2), a key recruiter of microglia [31]. At sites of neuronal loss, immunohistochemistry for key markers of microglia/macrophages, Cluster of Differentiation (CD) 68 and Ionized calcium binding adaptor molecule 1 (Iba1), reveals microglia that have transitioned from their ramified morphology to the 'activated' morphology described above [31,54,55].

A population of microglia, which are immunopositive for the haemopoietic transmembrane phosphoglycoprotein, CD34, have been observed in the ventral horn of sMND spinal cord [56]. In control cases, immunoreactivity for CD34 is restricted to the blood vessels and capillaries. However, in MND cases, CD34+ cells were found interspersed throughout the ventral horn forming clusters around motor neurons. These cells showed a rounded morphology, with a small subset staining positively for the microglial/macrophage marker Iba1. These cells were also Ki67+, indicating that these cells may represent an alternative, proliferative microglial phenotype.

The presence of activated microglia is associated with a number of key pathological and clinical features of MND. Increased microglial activation has been observed in the key sites of neuronal degeneration, as indicated by increased expression of CD68 and Iba1 in *post mortem* motor cortex and spinal cord, compared to control [54]. This has also been observed to a lesser extent in nonmotor regions including the amygdala, hippocampus and the middle frontal and superior temporal gyri [54]. Furthermore, the presence of Iba1⁺ microglia correlated with the extent of neuronal loss in the motor cortex, spinal cord and middle frontal gyrus, while increased microglial activation in the middle frontal cortex and superior temporal gyrus was associated with impaired executive function [54].

Additionally, increased microglial activation, as illustrated by CD68 and Iba1 immunostaining, in the corticospinal tract has been significantly associated with more rapid disease progression [55]. Familial cases associated with *C9orf72* mutations tend to show more extensive microglial pathology within the corticospinal tract [55]. Reactive microgliosis has also been described in MND cases associated with other disease-causing mutations such as *TBK1* [57], *SOD1* [58], *FUS* [59], and *ANG* [60]. Furthermore, higher scores on a clinical upper motor neuron assessment scale, based on the Ashworth Spasticity Scale, are associated with

increased microglial pathology in the upper corticospinal tract. [55]

While such autopsy studies represent end-stage disease, these findings are supported by *in vivo* Positron Emission Tomography imaging, where neuroinflammation in MND was observed using the 18-kDa translocator protein (TSPO) PK11195 tracer ligand [61]. The binding of this ligand is highly associated with activated microglia but has also been observed in other cell types including astrocytes, and is associated with neuroinflammation. In comparison with neurologically healthy individuals, MND patients showed increased signal in both motor regions and extramotor regions. A further PET study showed neuroinflammation in MND to be associated with cortical thinning and a worse disease phenotype as indexed by the ALS Functional Rating Scale [62]. Caution is required in interpretation of these results due to the lack of specificity of the marker.

Studies of a microglial regulatory protein, Triggering Receptor Expressed on Myeloid cells 2 (TREM2) have also implicated microglia in MND. TREM2 is a membrane-bound protein, which is expressed by multiple myeloid cell populations including microglia, and promotes myeloid activation and phagocytosis, and down-regulates the transcription of pro-inflammatory cytokines [63]. Soluble TREM2, a cleaved form of the protein, was found to be upregulated in human MND patient spinal cord and associated with improved patient survival [64,65]. Furthermore, soluble TREM2 mRNA has been found to be highly expressed in cerebrospinal fluid (CSF) in the early stages of MND and diminished in later stage disease [65]. In the late stages of disease, *TREM2* expression correlated with survival time in MND patients, perhaps reflecting a continued neuroprotective response [65]. A rare *TREM2* missense variant (p.R47H) that dampens microglial neurotoxicity has been identified as risk factor for neurodegenerative conditions including Alzheimer's disease (AD) [66] and potentially MND, although results have varied [64,67].

A recent study by Zhou *et al.* [68] has shown that *TREM2* has a key role in regulating disease-associated microglial gene expression in both human AD and in mouse models of AD using single nuclear RNA sequencing, supporting the role of *TREM2* in microglial gene expression regulation. However, microglial gene expression differed greatly in humans compared to

mouse models of AD, indicating a species-specific role for *TREM2* in microglial regulation. Furthermore, while *TREM2* is highly associated with microglial activation and regulation, the expression of *TREM2* by human microglia has recently been questioned: *TREM2* immunohistochemistry on human cortical tissue showed that *TREM2* was overwhelmingly expressed by infiltrating monocytes, while little expression was observed in native microglia [69]. Such species-specificity stresses the need for human as well as animal research.

Cerebrospinal fluid from MND patients has shown high levels of inflammatory cytokines including TNF- α , IL-1 β and IFN- γ [70–72]. Furthermore, microarray transcriptomic analysis of human spinal cord grey matter has shown an increase in inflammatory signalling in MND, characterized by various interleukins and cathepsins [73].

In summary, human pathology, imaging and genetic studies have supported a role for microglia in MND. The small amount of published research that attempts to determine specifically which microglial functions are relevant in human MND favours an inflammatory process.

Microglial function in animal models of MND

Attempts to determine the molecular behaviours of microglia in MND with greater specificity have predominantly utilized transgenic animal models. The first made, and most widely utilized and characterized MND model was generated in mice using the familial G93A mutation in the *SOD1* gene [74]. Further mSOD1 models have been developed using other patient variants [75–77]. The mSOD1 models rely on high transgene copy numbers, which produces a widespread overexpression of mutant protein, resulting in a reliable but severe disease phenotype [78]. While each variant differs in terms of the age of disease onset, rate of disease progression and symptom presentation, generally the mSOD1 models show extensive MN loss, aggregates of misfolded protein, progressive paralysis and shortened life span [79].

Following this, models have been created which contain transgenic mutations in *TARDBP*, *FUS*, *C9orf72*, *PFN1* and *UBQLN2* (summarized by Lutz [79]), as well as in other species including rats [80]

and zebrafish [81–83]. The MND models replicate many of the key features of disease and have provided a mechanism to study the progression of MND pathology. However, these models often show more aggressive disease progression compared to the human disease, and as it is the case with all disease models, no one model is able to replicate all disease features [78].

Microglia in early phase disease in animal models.

Research to ascertain whether microglial changes are involved in the initial stages of pathology in murine models of MND have reported conflicting findings. mSOD1^{G93A} mice show evidence of peripheral nerve and motor neuron dysfunction early, before the appearance of CD68⁺ macrophages and microglia in the muscle and spinal cord respectively [84]. In contrast, decreased levels of Iba1⁺ microglia in mSOD1^{G93A} mice spinal cord, prior to symptom onset have also been reported [85]. Furthermore, no change in microglial number was observed in mSOD1^{G93A} rat spinal cord isolated at the equivalent presymptomatic stage [28]. However, a significant increase in the number of microglia was observed immediately postsymptom onset [28]. These mixed findings make it difficult to determine whether microglia are actively involved in the initial motor neuron pathology, or whether neuroinflammation is secondary to motor neuron pathology. Given the microglial functions associated with CD68 (phagocytosis) and Iba1 (motility), these data could suggest that microglia may be recruited to the sites of neuronal pathology before the appearance of symptoms, whereas the CD68⁺ microglia may reflect microglia responding to the damage already present. Additional phenotypic investigation of microglia in MND is required to characterize their role in humans. In the later disease stages, animal models have tended to focus on the trophic and anti-inflammatory, versus neurotoxic or pro-inflammatory effects of microglia. These roles will now be considered in turn.

Anti-inflammatory and trophic microglia in animal models of MND

In the early stages of most diseases, microglia are activated by signals from damaged or stressed neurons, and potential pathogens [86]. In MND, these signals

are likely to include various chemokines and DAMPS released by motor neurons, and the uptake of misfolded protein aggregates by microglia [87,88]. Transcriptomic analysis of microglia isolated from 11-week-old mSOD1^{G93A} mice has shown high levels of *Ym1*, an anti-inflammatory macrophage marker, as well as Brain-derived Neurotrophic Factor (BDNF), a neurotrophin which promotes neuronal survival [44]. Interestingly, these microglia were also found to express increased mRNA transcripts for CD163, a haptoglobin-haemoglobin scavenger receptor predominantly expressed by perivascular macrophages and by microglia, following breakdown of the blood-brain barrier [89], indicating this process could occur early in disease. Coculture of these microglia with wild-type motor neurons promotes neuronal survival, but only in the presence of astrocytes, potentially indicating a neuroprotective microglial response and a possible interdependence with astrocytes – however further evidence is needed to validate the microglial/astrocytic interaction.

A further study of microglia from presymptomatic mSOD1^{G93A} mice has demonstrated a 16-fold increase in IL-10 production, a cytokine responsible for inflammatory cytokine downregulation [90]. Conversely, targeting IL-10 overexpression in the spine resulted in delayed symptom onset and extended survival, while blocking IL-10 receptors using the mIL-10R1 antibody results in rapid precipitation of symptoms. Increased IL-10 expression is associated with reduced TLR-2 activity, which promotes microglial inflammatory cytokine production [91].

Together, these results indicate that microglia, in the presymptomatic stages of MND may counteract or prevent the inflammatory response associated with later disease stages potentially delaying symptom onset.

Pro-inflammatory microglia in animal models of MND

Transcriptional analysis of 11-week-old mSOD1^{G93A} mouse spinal cord homogenates has demonstrated a significant upregulation of inflammatory genes including cathepsin D and TNF- α [92]. This was thought to represent an inflammatory response in the presymptomatic stage. However, symptom onset has been observed at as early as 8.5 weeks of age in this model [85], implying that the inflammatory microglial phenotype observed actually occurs in the early,

postsymptom-onset stage of disease. Furthermore, these results contradict other findings of increased anti-inflammatory cytokine expression at the same age in the same mSOD1^{G93A} model [44].

The latter study went on to examine late-stage mSOD1^{G93A} mice and found a shift towards diminished anti-inflammatory markers and high levels of nitric oxide, a toxic immune mediator. Coculture of these late-stage microglia with wild-type motor neurons induced neuronal death [44], likely by increasing motor neuron sensitivity to glutamate, potentially resulting in excitotoxicity [93], a known feature of motor neuron pathobiology in MND. These microglia also appear to inhibit the protective uptake of glutamate by astrocytes in culture [93].

Microglia expressing mSOD1 appear to be more neurotoxic than wild-type microglia. Upon stimulation with the neurotoxicity-inducing PAMP, Lipopolysaccharide (LPS), microglia overexpressing mSOD1^{G93A} released more NO and superoxidase, than both microglia overexpressing wild-type *SOD1*, and wild-type microglia [94,95]. These mSOD1 microglia induced more neuronal death in coculture with motor neurons than their wild-type counterparts.

Interestingly, Weydt and colleagues [96] performed a similar experiment, but compared primary microglia from 3-day-old mice and 60-day-old mice (early symptomatic). Upon stimulation with LPS, only the adult mSOD1^{G93A} microglia showed a significant difference from the wild-type control, with increased *TNF- α* production and decreased *IL-6* expression. This suggests that adult and not perinatal mSOD1-expressing microglia are more neurotoxic than wild-type microglia.

This heightened response by aged mSOD1 microglia may reflect microglial priming, where microglia display an exaggerated response to stimuli after prior activation, such as exposure to mSOD1 [97]. Microglial priming is a feature of ageing and other neurodegenerative conditions such as Alzheimer's disease [98]: these microglia are less responsive to anti-inflammatory agents, promoting chronic inflammation [98].

Furthermore, transplantation of wild-type microglia into mSOD1^{G93A} mice, via bone marrow transplant, results in reduced MN loss and extended survival [95], indicating that in mSOD1 mice, treatment with wild-type microglia is sufficient to benefit motor neurons and consequentially mouse survival.

Further evidence of microglial toxicity in MND murine models comes from experiments with *CX3CR1* gene manipulations. *CX3CR1* encodes the receptor for the chemokine fractalkine. Together, this ligand and receptor mediate neuron-microglial interactions and protect neurons by preventing pro-inflammatory microglial activation [99]. The V249I variant of *CX3CR1* is associated with faster progression and shorter survival in human sMND [100]. Transgenic mSOD1^{G93A} bred with *CX3CR1*^{-/-} mice produced a worsened MND phenotype with enhanced inflammatory microglial activation and decreased neuronal density compared to *mSOD1/CX3CR1*^{+/-}, mSOD1 mice and wild-type mice [101].

Interaction of microglia and MND-associated proteins

A number of the proteins associated with misfolding in MND appear to influence microglia. Phagocytosis of extracellular mSOD1 has been shown to cause pro-inflammatory activation of microglia [102]. On incubation with mSOD1 aggregates, primary mouse microglia adopted the amoeboid morphology. Real-time quantitative PCR (RT-qPCR) revealed increased expression of the inflammatory factors *TNF- α* , *IL-1 β* , *NADPH oxidase 2 (NOX2)* and *superoxidase*, and a concomitant decreased expression of the repair factor *IGF-1*. When cocultured with primary mSOD1^{G93A} mouse motor neurons, these primed microglia proved to be neurotoxic, compared to un-primed mSOD1^{G93A} microglia. However, incubation of mSOD1 aggregates with motor neurons alone had no significant effect on motor neuron survival, indicating that extracellular mSOD1 is not directly toxic to motor neurons alone, and activation of microglia to a pro-inflammatory phenotype is required for neurodegeneration. Motor neuron death could be attenuated, by using antibodies to block TLR-2 and 4, and CD14, indicating a potential TLR-CD14-mediated pathway by which mSOD1 is able to induce the neurotoxic microglial phenotype.

Similarly, mutant TDP-43 (*mTDP-43*) has been shown to induce an exaggerated pro-inflammatory microglial phenotype characterized by *TNF- α* , *IL-1 β* and *NOX2* in primary mouse microglia, compared with wild-type treated with TDP-43 or nontreatment [103]. Furthermore, the expression of mTDP-43 by microglia produces a more pro-inflammatory response to LPS stimulation compared to wild-type microglia *in vitro*

[104]. mTDP-43 appears to activate microglia through the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) pathway [103]. The NF- κ B pathway is a master regulator of inflammation, and is highly upregulated in MND patient spinal cord, and mSOD1 mice [104,105]. When activated, the NF- κ B complex relocates to the nucleus, inducing further pro-inflammatory cytokine and chemokine expression [106]. The NF- κ B pathway can be induced directly via pathologically truncated wild-type TDP-43 and even more potently by mTDP-43 in primary mouse microglia [103]. This results in the production of pro-inflammatory cytokines [103,105]. Additionally, NF- κ B-activated mSOD1 microglia have been shown to attack healthy synapses *in vitro*, causing axonal damage, a feature not observed in wild-type microglia [105].

Thus, the accumulation of pathological, misfolded SOD1 and TDP-43 may contribute to a switch in microglial phenotype from an initially anti-inflammatory response to a pro-inflammatory phenotype.

Interestingly, recent research has indicated that dysfunctional microglia may contribute to the pathological translocation of TDP-43 from the nucleus to the cytoplasm. Using a transgenic zebrafish model, which co-expresses green fluorescent protein-tagged TDP-43 and microglia which express mCherry, Svahn *et al.* [107] visualized the nucleocytoplasmic transmission of TDP-43 and microglial dynamics *in vivo*. After initiating neuronal death by ultraviolet light, microglia rapidly approached and phagocytosed damaged neuronal somata as well as TDP-43 granules in the nucleus. Microglia were then depleted using a morpholino targeted at the macrophage transcription factor, *PU.1*. Ultraviolet light treatment in the absence of functional microglia resulted in the translocation of TDP-43 from the nucleus to the cytoplasm, a key feature of MND pathobiology. This quite artificial model of neuronal death indicates that dysfunctional microglial phagocytosis may contribute to the typical pathological translocation of TDP-43.

Finally, as noted above, hexanucleotide expansions in *C9orf72*, the most common genetic cause of MND, are associated with reduced expression of the *C9orf72* protein. RNA sequencing and mass spectrometry have led to the suggestion that microglia have the highest *C9orf72* levels of any cell type in mice [108,109]. In contrast, another study suggested that there are lower *C9orf72* levels in microglia than neurons. This study

used a mouse strain which carried a *LacZ* reporter in the *C9orf72* orthologue, which was detected by β galactosidase staining. However, as β galactosidase is also a senescence marker, this result could reflect high neuronal senescence rather than low microglial *C9orf72*. Surprisingly, mouse models in which the *C9orf72* orthologue has been knocked down, generally do not display motor neuron pathology as expected. Instead, they have autoimmune-like conditions with microglial and macrophage dysfunction [110,111] together with increased inflammatory cytokines in serum [111] and spinal cord [110]. Furthermore, *C9orf72* knockdown microglia and macrophages appear to display lysosomal dysfunction, which may result in a reduced ability of microglia to clear cellular debris. Transcriptomic characterization of these microglia revealed a predominantly inflammatory phenotype, with increased expression of TNF α and IL-1 β , in knockdown mice compared to controls [110]. Together, these results imply that the reduced levels of *C9orf72* would be associated with an inflammatory microglial response and lysosomal dysfunction in macrophages, features commonly observed in MND models [112].

The presence of discrete microglial phenotypes

Older research (predominantly in transgenic mouse models with MND-causing *SOD1* mutations) has raised the possibility of two phases of microglial activation: an earlier phase prior to symptom onset in which microglia express more genes historically associated with anti-inflammatory processes, followed by a later phase associated more so with expression of pro-inflammatory factors with the disease progression [44,113–116].

However, transcriptional studies using the same model have had conflicting results, with microglia expressing molecules classically associated with both anti- and pro-inflammatory functions throughout disease. Flow cytometry and RNA sequencing of microglia from mSOD1^{G93A} mouse spinal cord over different disease time points have revealed simultaneous upregulation of pro-inflammatory genes such as TNF- α , IL-1 β , and receptors for IFNs, and NOX2, as well anti-inflammatory genes such as *Insulin Growth Factor-1*, *Progranulin* and *DAP12 / TYROBP* [45]. These microglia significantly differed in phenotype to those WT microglia stimulated with LPS, indicating a disease-specific profile.

Table 2. Summary of transcriptomic studies of microglia in mSOD1^{G93A} models

Type of model (treatments conditions)	Method of microglial isolation	Location	Stage of disease			Method	References
			Presymptomatic stage	Symptom Onset – Early stage	Late/end stage		
mSOD1 ^{G93A} mouse spinal cord homogenate	Not Applicable	lumbar spinal cord [L1-L5]	No change	↑Caspase-1, ↑TNF-α, ↑JAK3, ↑CD68, ↑Retinoid X receptor α, ↑Homeobox proteins, ↑GABAA-R	↑↑Vimentin, ↑↑CD147, ↑Caspase-1, ↑↑Bcl-xL ↑↑Cathepsin-D, ↑↑TNF-α, ↑↑JAK3, ↑↑CD68, ↑GABAA-R	cDNA microarray, macroarray and RT-qPCR	[92]
mSOD1 ^{G93A} mouse microglia	Flow Cytometry (CD11b and CD16/CD32)	Lumbar Spinal Cord	Not measured	↑NOX2	↑↑NOX2, ↓BDNF, ↓Ym1, ↓↓CD163	RT-qPCR	[44]
mSOD1 ^{G93A} mouse microglia	Sequential Percoll gradient centrifugation and magnetic bead isolation. Antibodies conjugated to CD11b	Total spinal cord	↑MMP-12, ↑IGF, ↑Optineurin, ↑NOX2	↑↑IGF, ↑↑Optineurin, ↑↑NOX2, ↑TNF-α, ↑IL-1B, ↑TREM2, ↑Progranulin, ↑MMP-12	↑↑IGF, ↑↑↑Optineurin, ↑↑↑NOX2, ↑↑MMP-12, ↑↑Progranulin, ↑↑TNF-α, ↑↑IL-1B, ↑IL-α, ↑TREM2, ↑DAP12, ↑IL-10, ↑ApoE, ↑Tau, ↑Osteopontin	Microarray and RNAseq [over 1000 genes were differentially expressed therefore only given a sample]	[45]
mSOD1 ^{G93A} Rat microglia	Magnetic Bead Column Purification Antibodies Conjugated to CD11b/c	Cortex Brain Stem Spinal cord: Cervical [C1-C7] Spinal Cord: Thoracic [T2-10] Spinal Cord: Lumbar [L1-L5]	No change No change ↑MCP-1 No change No change	↑Osteopontin, ↑Galectin ↓TNF-α, ↑MCP-1, ↑Osteopontin, ↑VEGF, ↑Galectin-3 ↓TNF-α, ↑Osteopontin, ↑Galectin, ↑VEGF ↓TNF-α, ↑Osteopontin, ↓IL-10, ↑VEGF, ↑Galectin	↓TNF-α, ↓IL-6, ↑↑Osteopontin, ↓IL-10, ↑VEGF, ↑↑Galectin ↓IL-6, ↓↓TNF-α, ↑MCP-1, ↑↑Osteopontin, ↓Arg1, ↑↑VEGF, ↑↑Galectin ↓IL-6, ↓↓TNF-α, ↑↑Osteopontin, ↓↓Arg1, ↑↑Galectin, ↑↑VEGF ↓↓TNF-α, ↓IL-6, ↑↑Osteopontin, ↓↓Arg1, ↓↓IL-10, ↓BDNF, ↑↑VEGF, ↑↑Galectin	RT-qPCR	[28]
mSOD1 ^{G93A} mouse microglia cultures (LPS Treated Adult microglia)	Not Given - States microglia were isolated, purity checked using CD11b immunostaining	Whole brain	(Foetal Microglia) No change	↑TNF-α, ↓IL-6	Not measured	Enzyme-Linked Immunosorbent Assay (ELISA)	[96]

Key: Inflammatory, Anti-inflammatory, and Other. **Note:** Arrows represent change in expression since last measured point and do not reflect the magnitude of change. All changes compared to control littermates apart from Nikodemova *et al.*²⁸, which compared to wild-type mice.

In comparison, RT-qPCR characterization of microglia from mSOD1^{G93A} rat spinal cord did not find microglial activation in the presymptomatic stages of disease [28]. However, at disease onset and late-stage disease, mSOD1 microglia showed a simultaneous upregulation of *galectin* and *osteopontin*, factors involved in macrophage activation and chemotaxis, which have been observed as being both neurotoxic and trophic, and *Vascular Endothelial Growth Factor (VEGF)* a pro-survival factor. This was accompanied by the concomitant downregulation of the classic pro-inflammatory marker *TNF- α* , and anti-inflammatory/ trophic markers *BDNF*, *IL-6* and *arginase-1*.

The same study showed microglial function to vary according to location [28]: microglia from the cortex, brain stem and the cervical, thoracic and lumbar spinal cord regions were compared. The above differences were only seen in the spinal cord, but not in cortical regions. However, the cortical region where microglia were sampled was not described. It is thus possible that either unaffected cortices (such as occipital cortex) rather than the motor cortex were sampled, or total brain which may 'dilute' subtle changes. These differences in microglial transcriptome indicate that microglial function may vary with both anatomical region and disease stage. This and other studies [49,50] establish that the inflammatory environment, and the inflammatory response to disease differs between regions. It is thus important that the anatomical location is clearly defined when performing such studies in MND. It is also useful to consider more than one CNS region in such research.

The most relevant mSOD1 murine studies of microglial transcriptome have been summarized in Table 2, highlighting the discrepancies in results. It is of interest to note, even studies comparing the same regions of spinal cord in mSOD1 mice at the same stage of disease have shown differing results.

The inconsistency in these results likely reflects a number of differences in the methodology, including varying methods of gene quantification and the sample isolation protocol e.g. isolated microglia versus tissue homogenate versus cultured microglia. Microglial phenotype is highly dependent on their environment, therefore removing these cells from their *in vivo* environment will alter their transcriptional profile. Furthermore, microglia cell lines and cultures have been found

to be both functionally and transcriptionally different to *ex vivo* microglia [117,118].

Variability in the genetic background could also cause variation in the results. In the mSOD1^{G93A} mouse model, four copies of the transgene, which results in mSOD1 expression equivalent to those expression levels seen in human MND, is insufficient to produce symptoms in mice [74]. However, increasing the transgene copy number not only results in earlier symptom onset but also produces a more rapid disease progression [74,119]. The genetic background of the mice used for breeding has also been found to alter the life span and rate of disease progression, despite containing the same mutation and expressing the same quantity of mutated protein [120–122]. This potentially reflects the variability observed in humans, with patients carrying G93C *SOD1* mutations varying in survival time from approximately 5 to 20 years [123]. Moreover, the sex of the mice appears to affect the rate of disease progression with various reports indicating increased survival time and delayed symptom onset in female mice compared to males [121,124,125], as well as sex differences in response to treatment [125–127].

Microglia as a therapeutic target

With their clear involvement in MND, microglia have been a target in the development of therapeutic options; the predominant aim being to reduce inflammation³⁴. The administration of anti-inflammatory drugs has had mixed results in murine models and has not translated well to humans. Minocycline, a tetracycline antibiotic with broad spectrum anti-inflammatory activity, has been found to cross the blood-brain barrier, and reduce activity of inflammatory and pro-apoptotic enzymes [128]. In early trials, administration of minocycline was found to delay symptom onset and improve survival in mSOD1^{G93A} and mSOD1^{G37R} mice, if administered before symptom onset [129–131]. However, administration after disease onset accelerates disease progression and increases microgliosis [132,133], supporting a beneficial role for microglia in the earliest stage of the disease. In human trials, minocycline was found to increase the rate of decline, significantly reducing lifespan [134]. Similarly, trials of ceftriaxone (antibiotic), celecoxib (anti-inflammatory),

and thalidomide (immunomodulatory drug) showed promise in mSOD1 mice, but were ineffective in humans [135,136]. Criticisms about the original animal trials have been raised including whether adequate sample powering was employed, unaccounted confounding variables and the efficacy of administering a drug before disease onset [137].

Another drug Clemastine, which reduces inflammation while increasing the expression of the anti-inflammatory cytokine arginase-1, has also failed to improve disease in mSOD1^{G93A} mice, unless administered within the presymptomatic phase [138,139].

While attempts to dampen neuroinflammation have given mixed results, targeting specific inflammatory pathways has shown greater success. Inhibition of the previously described NF- κ B pathway *in vitro* in mSOD1^{G93A} microglia rescued motor neuron axon length and survival time to levels seen with wild-type microglia [105]. This was accompanied by reduced expression of TNF- α and nitric oxide in mSOD1^{G93A} microglia, to levels observed in wild-type microglia [105]. Transgenic NF- κ B inhibition in mSOD1^{G93A} mice resulted in a 47% increase in life expectancy compared to nontreated mSOD1^{G93A} mice [105], emphasizing the effectiveness of targeting this pathway. Similar manipulations have not yet been tried in humans.

Furthermore, there has been some success utilizing treatments which aim to mimic the supportive microglial functions in mSOD1 mouse models. The administration of the trophic factors, VEGF and IGF-1, at disease onset using viral vectors, has improved the motor symptoms and extended survival time [140,141].

Microglia show potential as a therapeutic target in MND treatment. However, it is unsurprising that to date, attempts in humans have been unsuccessful given the conflicting findings observed in animal models of MND, and our limited understanding of which microglial functions are relevant to human MND.

Future directions

Research using animal models has shown that microglia contribute to MND pathology with both toxic and trophic functions. However, attempts to subclassify microglial functions, based on the compounds expressed by these cells, have been inconsistent. Therefore, more work is required using human *post mortem*

CNS tissue to characterize the microglial phenotype over the course of the disease.

Despite the inherent challenges that come with using *post mortem* tissue, it has many benefits over mSOD1 mouse models of MND. These models significantly differ from human sporadic MND pathology. Transgenic mSOD1 models greatly overexpress mSOD1 resulting in a severe phenotype that progresses much faster than human MND [79] and could mask more subtle pathological changes. Furthermore, mutations in *SOD1* are observed in only ~2.5% of all MND cases [4], and represent a very specific form of MND. Indeed, *SOD1* fMND cases do not develop the hallmark ubiquitinated TDP-43⁺ inclusions seen in sporadic human MND and most forms of fMND, including *C9orf72*, *Angiogenin*, *Ataxin 2*, and *TANK Binding Kinase 1* cases. Consequentially, mSOD1 models may not be representative of the majority of human MND. Models have been developed using other familial mutations, such as *C9orf72*, *PFN1* and *TARDBP* [79]. Some of these models do show characteristic TDP-43 pathology. However, they also rely on high transgene copy numbers to produce a phenotype, and in some models (namely models utilizing mutations in *C9orf72*) the disease phenotype can be unpredictable, with a rapid disease progression and a survival time of less than 6 months [79].

Furthermore, transcriptional single-cell sorting of immune cells from the human cortex has demonstrated several uncharacterized microglial phenotypes, with some of them unique to humans [47]. This further supports the importance of transcriptional profiling in humans as opposed to experimental models. Characterization of microglia isolated from different regions of human brain and spinal cord using multiplexed single-cell mass spectrometry has confirmed the presence of distinct microglial populations in different locations [48], in a similar manner to observations made in mSOD1 murine models [28]. Therefore, it is important to examine microglial transcripts separately across regions of the brain and spinal cord, to better characterize the different populations of microglia. It may also be essential to characterize microglia in seemingly unaffected areas, such as occipital cortex to determine if there are microglial functions, which make these regions less susceptible to MND pathology.

In summary, microglial activation is a clear feature of MND; both in humans and mouse models, and thus

modulation of the microglial phenotype in MND could help to promote neuronal survival. However, we need first to determine and identify which microglial functions are important to human disease with TDP-43 proteinopathy.

Acknowledgments

The authors would like to thank the Pathological Society of Great Britain for funding this work as PhD studentship which supports the first author.

Author contributions

Conception of review: Bridget A. Ashford, Delphine Boche, Julie E. Simpson and J. Robin Highley; Literature searching, draft of paper, writing, figure design: Bridget A. Ashford; Scientific input and comments on draft: Bridget A. Ashford, Delphine Boche, Johnathan; Cooper-Knock, Paul R. Heath, Julie E. Simpson, J. Robin Highley.

Data availability statement

Data sharing is not applicable to this article as no new data were created or analysed in this study.

References

- 1 Foster LA, Salajegheh MK. Motor neuron disease: pathophysiology, diagnosis, and management. *Am J Med* 2019; **132**: 32–7
- 2 Chiò A, Logroscino G, Traynor BJ, Collins J, Simeone JC, Goldstein LA, et al. Global epidemiology of amyotrophic lateral sclerosis: A systematic review of the published literature. *Neuroepidemiology* 2013; **41**: 118–30
- 3 Westeneng HJ, Debray TPA, Visser AE, van Eijk RPA, Rooney JPK, Calvo A, et al. Prognosis for patients with amyotrophic lateral sclerosis: development and validation of a personalised prediction model. *Lancet Neurol* 2018; **17**: 423–33
- 4 Renton AE, Chiò A, Traynor BJ. State of play in amyotrophic lateral sclerosis genetics. *Nat Neurosci* 2014; **17**: 17–23
- 5 Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, et al. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 1993; **362**: 59–62
- 6 Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* (80-.) 2006; **314**, 130–3.
- 7 Kwiatkowski TJ, Bosco DA, LeClerc AI, Tamrazian E, Vanderburg CR, Russ C, et al. Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science* 2009; **323**: 1205–8
- 8 Vance C, Rogelj B, Hortobagyi T, De Vos KJ, Nishimura AL, Sreedharan J, et al. Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science* 2009; **323**: 1208–11
- 9 DeJesus-Hernandez M, Mackenzie IR, Boeve BF, Boxer AL, Baker M, Rutherford NJ, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* 2011; **72**: 245–56
- 10 Freischmidt A, Wieland T, Richter B, Ruf W, Schaefer V, Müller K, et al. Haploinsufficiency of TBK1 causes familial ALS and fronto-temporal dementia. *Nat Neurosci* 2015; **18**: 631–6
- 11 Maruyama H, Morino H, Ito H, Izumi Y, Kato H, Watanabe Y, et al. Mutations of optineurin in amyotrophic lateral sclerosis. *Nature* 2010; **465**: 223–6
- 12 Johnson JO, Mandrioli J, Benatar M, Abramzon Y, Van Deerlin VM, Trojanowski JQ, et al. Exome sequencing reveals VCP mutations as a cause of familial ALS. *Neuron* 2010; **68**: 857–64
- 13 Fecto F, Yan J, Vemula SP, Liu E, Yang Y, Chen W, et al. SQSTM1 mutations in familial and sporadic amyotrophic lateral sclerosis. *Arch Neurol* 2011; **68**: 1440–6
- 14 Deng H-X, Chen W, Hong S-T, Boycott KM, Gorrie GH, Siddique N, et al. Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. *Nature* 2011; **477**: 211–5
- 15 Greenfield JG. (Joseph G., Love, S., Louis, D. N. & Ellison, D.) David W. Greenfield's. *Neuropathology* 2015
- 16 Blokhuis AM, Groen EJM, Koppers M, van den Berg LH, Pasterkamp RJ, et al. Protein aggregation in amyotrophic lateral sclerosis. *Acta Neuropathologica* 2013; **125**(6): 777–94. <https://doi.org/10.1007/s00401-013-1125-6>
- 17 Brettschneider J, Arai K, Tredici KD, Toledo JB, Robinson JL, Lee EB, et al. TDP-43 pathology and neuronal loss in amyotrophic lateral sclerosis spinal cord. *Acta Neuropathol* 2014; **128**: 423–37
- 18 Arai T, Hasegawa M, Akiyama H, Ikeda K, Nonaka T, Mori H, et al. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem Biophys Res Commun* 2006; **351**: 602–11
- 19 Chare L, Hodges JR, Leyton CE, McGinley C, Tan RH, Kril JJ, et al. New criteria for frontotemporal dementia syndromes: clinical and pathological diagnostic implications. *J Neurol Neurosurg Psychiatry* 2014; **85**: 865–70

- 20 Bilsland LG, Sahai E, Kelly G, Golding M, Greensmith L, Schiavo G. Deficits in axonal transport precede ALS symptoms in vivo. *Proc Natl Acad Sci* 2010; **107**: 20523–8
- 21 Collard J-F, Côté F, Julien J-P. Defective axonal transport in a transgenic mouse model of amyotrophic lateral sclerosis. *Nature* 1995; **375**: 61–4
- 22 Polymenidou M, Lagier-Tourenne C, Hutt KR, Bennett CF, Cleveland DW, Yeo GW. Misregulated RNA processing in amyotrophic lateral sclerosis. *Brain Res* 2012; **1462**: 3–15
- 23 Sasaki S. Endoplasmic reticulum stress in motor neurons of the spinal cord in sporadic amyotrophic lateral sclerosis. *J Neuropathol Exp Neurol* 2010; **69**: 346–55
- 24 Schwenk BM, Hartmann H, Serdaroglu A, Schludi MH, Hornburg D, Meissner F, et al. TDP-43 loss of function inhibits endosomal trafficking and alters trophic signaling in neurons. *EMBO J* 2016; **35**: 2350–70
- 25 Zhang H, Tan C-F, Mori F, Tanji K, Kakita A, Takahashi H, et al. TDP-43-immunoreactive neuronal and glial inclusions in the neostriatum in amyotrophic lateral sclerosis with and without dementia. *Acta Neuropathol* 2007; **115**: 115–22
- 26 Ince PG, Highley JR, Kirby J, Wharton SB, Takahashi H, Strong MJ, et al. Molecular pathology and genetic advances in amyotrophic lateral sclerosis: an emerging molecular pathway and the significance of glial pathology. *Acta Neuropathol* 2011; **122**: 657–71
- 27 Kawamata T, Akiyama H, Yamada T, McGeer PL. Immunologic reactions in amyotrophic lateral sclerosis brain and spinal cord tissue. *Am J Pathol* 1992; **140**: 691–707
- 28 Nikodemova M, Small AL, Smith SMC, Mitchell GS, Watters JJ. Spinal but not cortical microglia acquire an atypical phenotype with high VEGF, galectin-3 and osteopontin, and blunted inflammatory responses in ALS rats. *Neurobiol Dis* 2014; **69**: 43–53
- 29 Clement AM, Nguyen MD, Roberts EA, Garcia ML, Boillée S, Rule M, et al. Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. *Science* (80-.). 2003; **302**, 113–7).
- 30 Graves MC, Fiala M, Dinglasan LAV, Liu NQ, Sayre J, Chiappelli F, et al. Inflammation in amyotrophic lateral sclerosis spinal cord and brain is mediated by activated macrophages, mast cells and T cells. *Amyotroph. Lateral Scler. Other Motor Neuron Disord* 2004; **5**: 213–9
- 31 Henkel JS, Engelhardt JI, Siklós L, Simpson EP, Kim SH, Pan T, et al. Presence of dendritic cells, MCP-1, and activated microglia/macrophages in amyotrophic lateral sclerosis spinal cord tissue. *Ann Neurol* 2004; **55**: 221–35
- 32 Boche D, Perry VH, Nicoll JAR. Review: Activation patterns of microglia and their identification in the human brain. *Neuropathol Appl Neurobiol* 2013; **39**: 3–18
- 33 Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science* 2005; **308**: 1314–8
- 34 Geloso MCMC, Corvino V, Marchese E, Serrano A, Michetti F, D'Ambrosi N. The dual role of microglia in ALS: mechanisms and therapeutic approaches. *Frontiers in Aging Neuroscience* 2017; **9**: 242.
- 35 Streit WJ, Sammons NW, Kuhns AJ, Sparks DL. Dystrophic microglia in the aging human brain. *Glia* 2004; **45**: 208–12
- 36 Streit WJ. Microglial senescence: does the brain's immune system have an expiration date? *Trends Neurosci* 2006; **29**: 506–10
- 37 Davies DS, Ma J, Jegathees T, Goldsbury C. Microglia show altered morphology and reduced arborization in human brain during aging and Alzheimer's disease. *Brain Pathol* 2017; **27**: 795–808
- 38 Tischer J, Krueger M, Mueller W, Staszewski O, Prinz M, Streit WJ et al. Inhomogeneous distribution of Iba-1 characterizes microglial pathology in Alzheimer's disease. *Glia* 2016; **64**: 1562–72
- 39 Damani MR, Zhao L, Fontainhas AM, Amaral J, Fariss RN, Wong WT, et al. Age-related alterations in the dynamic behavior of microglia. *Aging Cell* 2011; **10**: 263–76
- 40 Sparkman NL, Johnson RW. Neuroinflammation associated with aging sensitizes the brain to the effects of infection or stress. *NeuroImmunoModulation* 2008; **15**: 323–30
- 41 Lopes KO, Sparks DL, Streit WJ. Microglial dystrophy in the aged and Alzheimer's disease brain is associated with ferritin immunoreactivity. *Glia* 2008; **56**: 1048–60
- 42 Mueller TD, Zhang J-L, Sebald W, Duschl A. Structure, binding, and antagonists in the IL-4/IL-13 receptor system. *Biochim Biophys Acta - Mol Cell Res* 2002; **1592**: 237–50
- 43 Wang G, Zhang J, Hu X, Zhang L, Mao L, Jiang X, et al. Microglia/macrophage polarization dynamics in white matter after traumatic brain injury. *J. Cereb. Blood Flow Metab* 2013; **33**: 1864–74
- 44 Liao B, Zhao W, Beers DRDR, Henkel JSJS, Appel SHSHS. Transformation from a neuroprotective to a neurotoxic microglial phenotype in a mouse model of ALS. *Exp Neurol* 2012; **237**: 147–52
- 45 Chiu IM, Morimoto ETA, Goodarzi H, Liao JT, O'Keefe S, Phatnani HP, et al. A neurodegeneration-specific gene-expression signature of acutely isolated microglia from an amyotrophic lateral sclerosis mouse model. *Cell Rep.* 2013; **4**: 385–401
- 46 Morganti JM, Riparip L-K, Rosi S. Call off the Dog(ma): M1/M2 polarization is concurrent following traumatic brain injury. *PLoS One* 2016; **11**: e0148001

- 47 Friedman BA, Srinivasan K, Ayalon G, Meilandt WJ, Lin H, Huntley MA, et al. Diverse brain myeloid expression profiles reveal distinct microglial activation states and aspects of alzheimer's disease not evident in mouse models. *Cell Rep* 2018; **22**: 832–47
- 48 Böttcher C, Schlickeiser S, Sneuboer MAM, Kunkel D, Knop A, Paza E, et al. Human microglia regional heterogeneity and phenotypes determined by multiplexed single-cell mass cytometry. *Nat Neurosci* 2019; **22**: 78–90
- 49 Lawson LJ, Perry VH, Dri P, Gordon S. Heterogeneity in the distribution and morphology of microglia in the normal adult mouse brain. *Neuroscience* 1990; **39**: 151–70
- 50 Grabert K, Michoel T, Karavolos MH, Clohisey S, Bailie JK, Stevens MP, et al. Microglial brain region-dependent diversity and selective regional sensitivities to aging. *Nat Neurosci* 2016; **19**: 504–16
- 51 Galatro TF, Holtman IR, Lerario AM, Vainchtein ID, Brouwer N, Sola PR, et al. Transcriptomic analysis of purified human cortical microglia reveals age-associated changes. *Nat Neurosci* 2017; **20**: 1162–71
- 52 Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: Uncovering the molecular mechanisms. *Nat Rev Neurosci* 2007; **8**: 57–69
- 53 Howell OW, Rundle JL, Garg A, Komada M, Brophy PJ, Reynolds R. Activated microglia mediate axoglial disruption that contributes to axonal injury in multiple sclerosis. *J Neuropathol Exp Neurol* 2010; **69**: 1017–33
- 54 Bretschneider J, Libon DJ, Toledo JB, Xie SX, McCluskey L, Elman L, et al. Microglial activation and TDP-43 pathology correlate with executive dysfunction in amyotrophic lateral sclerosis. *Acta Neuropathol*. 2012; **123**: 395–407
- 55 Bretschneider J, Toledo JB, Van Deerlin VM, Elman L, McCluskey L, Lee VM-Y, et al. Microglial activation correlates with disease progression and upper motor neuron clinical symptoms in amyotrophic lateral sclerosis. *PLoS One* 2012; **7**: e39216
- 56 Kovacs M, Trias E, Varela V, Ibarburu S, Beckman JS, Moura IC, et al. CD34 identifies a subset of proliferating microglial cells associated with degenerating motor neurons in ALS. *Int J Mol Sci* 2019; **20**: 3880
- 57 Weinreich M, Shephard SR, Verber N, Wyles M, Heath PR, Highley JR, et al. Neuropathological characterization of a novel TANK binding kinase (TBK1) gene loss of function mutation associated with amyotrophic lateral sclerosis. *Neuropathol Appl Neurobiol* 2019; **46**: 279–91. <https://doi.org/10.1111/nan.12578>
- 58 Ince PG, Tomkins J, Slade JY, Thatcher NM, Shaw PJ. Amyotrophic lateral sclerosis associated with genetic abnormalities in the gene encoding Cu/Zn superoxide dismutase: Molecular pathology of five new cases, and comparison with previous reports and 73 sporadic cases of ALS. *J Neuropathol Exp Neurol* 1998; **57**: 895–904
- 59 Hewitt C, Kirby J, Highley JR, Hartley JA, Hibberd R, Hollinger HC, et al. Novel FUS/TLS mutations and pathology in familial and sporadic amyotrophic lateral sclerosis. *Arch Neurol* 2010; **67**: 455–61
- 60 Kirby J, Highley JR, Cox L, Goodall EF, Hewitt C, Hartley JA, et al. Lack of unique neuropathology in amyotrophic lateral sclerosis associated with p. K54E angiogenin (ANG) mutation. *Neuropathol Appl Neurobiol* 2013; **39**: 562–71
- 61 Turner MR, Cagnin A, Turkheimer FE, Miller CCJ, Shaw CE, Brooks DJ, et al. Evidence of widespread cerebral microglial activation in amyotrophic lateral sclerosis: An [¹¹C](R)-PK11195 positron emission tomography study. *Neurobiol Dis* 2004; **15**: 601–09
- 62 Alshikho MJ, Zürcher NR, Loggia ML, Cernasov P, Chonde DB, Izquierdo Garcia D, et al. Glial activation colocalizes with structural abnormalities in amyotrophic lateral sclerosis. *Neurology* 2016; **87**: 2554–61
- 63 Yeh FL, Hansen DV, Sheng M. TREM2, Microglia, and neurodegenerative diseases. *Trends Mol Med* 2017; **23**: 512–33
- 64 Cady J, Koval ED, Benitez BA, Zaidman C, Jockel-Balsarotti J, Allred P, et al. TREM2 variant p. R47H as a risk factor for sporadic amyotrophic lateral sclerosis. *JAMA Neurol* 2014; **71**: 449–53
- 65 Cooper-Knock J, Green C, Altschuler G, Wei W, Bury JJ, Heath PR, et al. A data-driven approach links microglia to pathology and prognosis in amyotrophic lateral sclerosis. *Acta Neuropathol Commun* 2017; **5**: 23
- 66 Jonsson T, Stefansson H, Steinberg S, Jonsdottir I, Jonsson PV, Snaedal J, et al. Variant of TREM2 associated with the risk of alzheimer's disease. *N Engl J Med* 2013; **368**: 107–16
- 67 Lill CM, Rengmark A, Pihlström L, Fogh I, Shatunov A, Sleiman PM, et al. The role of TREM2 R47H as a risk factor for Alzheimer's disease, frontotemporal lobar degeneration, amyotrophic lateral sclerosis, and Parkinson's disease. *Alzheimer's Dement*. 2015; **11**: 1407–16
- 68 Zhou Y, Song WM, Andhey PS, Swain A, Levy T, Miller KR, et al. Human and mouse single-nucleus transcriptomics reveal TREM2-dependent and TREM2-independent cellular responses in Alzheimer's disease. *Nat Med* 2020; **26**: 131–42
- 69 Fahrenhold M, Rakic S, Classey J, Brayne C, Ince PG, Nicoll JAR et al. TREM2 expression in the human brain: a marker of monocyte recruitment? *Brain Pathol* 2018; **28**: 595–602
- 70 Babu GN, Kumar A, Chandra R, Puri SK, Kalita J, Misra UK, et al. Elevated inflammatory markers in a group of amyotrophic lateral sclerosis patients from northern India. *Neurochem Res* 2008; **33**: 1145–9
- 71 Mitchell RM, Freeman WM, Randazzo WT, Stephens HE, Beard JL, Simmons Z, et al. A CSF biomarker panel for identification of patients with amyotrophic lateral sclerosis. *Neurology* 2009; **72**: 14–9

- 72 Zhang R, Miller RG, Gascon R, Champion S, Katz J, Lancero M, et al. Circulating endotoxin and systemic immune activation in sporadic amyotrophic lateral sclerosis (sALS). *J Neuroimmunol* 2009; **206**: 121–4
- 73 Dangond F, Hwang D, Camelo S, Pasinelli P, Frosch MP, Stephanopoulos G, et al. Molecular signature of late-stage human ALS revealed by expression profiling of postmortem spinal cord gray matter. *Physiol. Genomics* 2004; **16**: 229–39
- 74 Gurney ME, Pu H, Chiu A, Dal Canto M, Polchow C, Alexander D, et al. Motor neuron degeneration in mice that express a human Cu, Zn superoxide dismutase mutation. *Science* 1994; **264**: 1772–5
- 75 Wong PC, Pardo CA, Borchelt DR, Lee MK, Copeland NG, Jenkins NA, et al. An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria. *Neuron* 1995; **14**: 1105–16
- 76 Bruijn LI, Becher MW, Lee MK, Anderson KL, Jenkins NA, Copeland NG, et al. ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. *Neuron* 1997; **18**: 327–38
- 77 Ripps ME, Huntley GW, Hof PR, Morrison JH, Gordon JW. Transgenic mice expressing an altered murine superoxide dismutase gene provide an animal model of amyotrophic lateral sclerosis. *Proc Natl Acad Sci USA* 1995; **92**: 689–93
- 78 Zwiegers P, Lee G, Shaw CA. Reduction in hSOD1 copy number significantly impacts ALS phenotype presentation in G37R (line 29) mice: implications for the assessment of putative therapeutic agents. *J Negat Results Biomed* 2014; **13**: 14
- 79 Lutz C. Mouse models of ALS: Past, present and future. *Brain Res* 2018; **1693**: 1–10
- 80 Philips T, Rothstein JD. Rodent models of amyotrophic lateral sclerosis. *Current Protocols in Pharmacology* 2015; **69**(1): 1–5.
- 81 Ramesh TM, Shaw PJ, McDearmid J. A zebrafish model exemplifies the long preclinical period of motor neuron disease. *J Neurol Neurosurg Psychiatry* 2014; **85**: 1288–9
- 82 Shaw MP, Higginbottom A, McGown A, Castelli LM, James E, Hautbergue GM, et al. Stable transgenic C9orf72 zebrafish model key aspects of the ALS/FTD phenotype and reveal novel pathological features. *Acta Neuropathol Commun* 2018; **6**: 125
- 83 Da Costa MMJ, Allen CE, Higginbottom A, Ramesh T, Shaw PJ, et al. A new zebrafish model produced by TILLING of SOD1-related amyotrophic lateral sclerosis replicates key features of the disease and represents a tool for in vivo therapeutic screening. *Dis Model Mech* 2014; **7**: 73–81
- 84 Kano O, Beers DR, Henkel JS, Appel SH. Peripheral nerve inflammation in ALS mice: cause or consequence. *Neurology* 2012; **78**: 833–5
- 85 Gerber YN, Sabourin J-CCJC, Rabano M, Vivanco MDM, Perrin FE. Early functional deficit and microglial disturbances in a mouse model of amyotrophic lateral sclerosis. *PLoS ONE* 2012; **7**(4):e36000.
- 86 Glass CK, Saijo K, Winner B, Marchetto MC, Gage FH. Mechanisms underlying inflammation in neurodegeneration. *Cell* 2010; **140**: 918–34
- 87 Zhao W, Beers DR, Appel SH. Immune-mediated mechanisms in the pathoproduction of amyotrophic lateral sclerosis. *J Neuroimmune Pharmacol* 2013; **8**: 888–99
- 88 Roberts K, Zeineddine Rafaa, Corcoran L, Li W, Campbell IL, Yerbury JJ, et al. Extracellular aggregated Cu/Zn superoxide dismutase activates microglia to give a cytotoxic phenotype. *Glia* 2013; **61**: 409–19
- 89 Borda JT, Alvarez X, Mohan M, Hasegawa A, Bernardino A, Jean S, et al. CD163, a marker of perivascular macrophages, is up-regulated by microglia in simian immunodeficiency virus encephalitis after hapto-globin-hemoglobin complex stimulation and is suggestive of breakdown of the blood-brain barrier. *Am J Pathol* 2008; **172**: 725–37
- 90 Gravel M, Béland L-C, Soucy G, Abdelhamid E, Rahimian R, Gravel C, et al. IL-10 controls early microglial phenotypes and disease onset in ALS caused by misfolded superoxide dismutase 1. *The Journal of Neuroscience* 2016; **36**(3): 1031–48. <https://doi.org/10.1523/JNEUROSCI.0854-15.2016>
- 91 Henkel JS, Beers DR, Zhao W, Appel SH. Microglia in ALS: The good, the bad, and the resting. *J Neurol Neurosurg Psychiatry* 2009; **4**: 389–98
- 92 Yoshihara T, Ishigaki S, Yamamoto M, Liang Y, Niwa J-I, Takeuchi H, et al. Differential expression of inflammation- and apoptosis-related genes in spinal cords of a mutant SOD1 transgenic mouse model of familial amyotrophic lateral sclerosis. *J Neurochem* 2002; **80**: 158–67
- 93 Zhao W, Xie W, Le W, Beers DR, He Y, Henkel JS, et al. Activated microglia initiate motor neuron injury by a nitric oxide and glutamate-mediated mechanism. *J Neuropathol Exp Neurol* 2004; **63**: 964–77
- 94 Xiao Q, Zhao W, Beers DR, Yen AA, Xie W, Henkel JS, et al. Mutant SOD1 G93A microglia are more neurotoxic relative to wild-type microglia. *J Neurochem* 2007; **102**: 2008–19
- 95 Beers DR, Henkel JS, Xiao Q, Zhao W, Wang J, Yen AA, et al. Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis. *Proc Natl Acad Sci* 2006; **103**: 16021–6
- 96 Weydt P, Yuen EC, Ransom BR, Möller T. Increased cytotoxic potential of microglia from ALS-transgenic mice. *Glia* 2004; **48**: 179–82
- 97 Perry VH, Holmes C. Microglial priming in neurodegenerative disease. *Nat Rev Neurol* 2014; **10**: 217–24
- 98 Norden DM, Godbout JP. Review: Microglia of the aged brain: Primed to be activated and resistant to

- regulation. *Neuropathol Appl Neurobiol* 2013; **39**: 19–34
- 99 Arnoux I, Audinat E. Fractalkine signaling and microglia functions in the developing brain. *Neural Plast* 2015; **2015**: 1–8
- 100 Lopez-Lopez A, Gamez J, Syriani E, Morales M, Salvado M, Rodríguez MJ, et al. CX3CR1 is a modifying gene of survival and progression in amyotrophic lateral sclerosis. *PLoS One* 2014; **9**: e96528
- 101 Cardona AE, Pioro EP, Sasse ME, Kostenko V, Cardona SM, Dijkstra IM, et al. Control of microglial neurotoxicity by the fractalkine receptor. *Nat Neurosci* 2006; **9**: 917–24
- 102 Zhao W, Beers DR, Henkel JS, Zhang W, Urushitani M, Julien J-P, et al. Extracellular mutant SOD1 induces microglial-mediated motoneuron injury. *Glia* 2010; **58**: 231–43
- 103 Zhao W, Beers DR, Bell S, Wang J, Wen S, Baloh RH, et al. TDP-43 activates microglia through NF- κ B and NLRP3 inflammasome. *Exp Neurol* 2015; **273**: 24–35
- 104 Swarup V, Phaneuf D, Dupré N, Petri S, Strong M, Kriz J, Julien J-P. Deregulation of TDP-43 in amyotrophic lateral sclerosis triggers nuclear factor κ B-mediated pathogenic pathways. *The Journal of Experimental Medicine*. 2011; **208**: 2429–2447. <http://dx.doi.org/10.1084/jem.20111313>
- 105 Frakes AE, Ferraiuolo L, Haidet-Phillips AM, Schmelzer L, Braun L, Miranda CJ, et al. Microglia induce motor neuron death via the classical NF- κ B pathway in amyotrophic lateral sclerosis. *Neuron* 2014; **81**: 1009–23
- 106 Ghosh S, Karin M. Missing pieces in the NF- κ B puzzle. *Cell* 2002; **109**: S81–S96
- 107 Svahn AJ, Don EK, Badrock AP, Cole NJ, Graeber MB, Yerbury JY, et al. Nucleo-cytoplasmic transport of TDP-43 studied in real time: impaired microglia function leads to axonal spreading of TDP-43 in degenerating motor neurons. *Acta Neuropathol*. 2018; **136**: 445–59
- 108 Zhang Y, Chen K, Sloan SA, Bennett ML, Scholze AR, Keefe SO, et al. An RNA-sequencing transcriptome and splicing database of glia, neurons, and vascular cells of the cerebral cortex. *J Neurosci* 2014; **34**: 11929–47
- 109 Sharma K, Schmitt S, Bergner CG, Tyanova S, Kannaiyan N, Manrique-Hoyos N, et al. Cell type- and brain region-resolved mouse brain proteome. *Nat Neurosci* 2015; **18**: 1819–31
- 110 O'Rourke JG, Bogdanik L, Yáñez A, Lall D, Wolf AJ, Muhammad AKMG, et al. C9orf72 is required for proper macrophage and microglial function in mice. *Science (80-.)*. 2016; **351**: 1324–9.
- 111 Atanasio A, Decman V, White D, Ramos M, Ikiz B, Lee H-C, et al. C9orf72 ablation causes immune dysregulation characterized by leukocyte expansion, autoantibody production and glomerulonephropathy in mice. *Sci. Rep.* 2016; **6**: 23204
- 112 Ferraiuolo Laura, Kirby Janine, Grierson Andrew J., Sendtner Michael, Shaw Pamela J.. Molecular pathways of motor neuron injury in amyotrophic lateral sclerosis. *Nature Reviews Neurology*. 2011; **7**: 11:616–630. <http://dx.doi.org/10.1038/nrneurol.2011.152>.
- 113 Beers DR, Henkel JS, Zhao W, Wang J, Appel SH. CD4+ T cells support glial neuroprotection, slow disease progression, and modify glial morphology in an animal model of inherited ALS. *Proc Natl Acad Sci* 2008; **105**: 15558–63
- 114 Henkel JS, Beers DR, Siklós L, Appel SH. The chemokine MCP-1 and the dendritic and myeloid cells it attracts are increased in the mSOD1 mouse model of ALS. *Mol. Cell. Neurosci.* 2006; **31**: 427–37
- 115 Hensley K, Floyd RA, Gordon B, Mou S, Pye QN, Stewart C, et al. Temporal patterns of cytokine and apoptosis-related gene expression in spinal cords of the G93A-SOD1 mouse model of amyotrophic lateral sclerosis. *J Neurochem* 2002; **82**: 365–74
- 116 Alexianu ME, Kozovska M, Appel SH. Immune reactivity in a mouse model of familial ALS correlates with disease progression. *Neurology* 2001; **57**: 1282–9
- 117 Melief J, Sneboer MAM, Litjens M, Ormel PR, Palmén SJMC, Huitinga I, et al. Characterizing primary human microglia: A comparative study with myeloid subsets and culture models. *Glia* 2016; **64**: 1857–68
- 118 Das A, Kim SH, Arifuzzaman S, Yoon T, Chai JC, Lee YS, et al. Transcriptome sequencing reveals that LPS-triggered transcriptional responses in established microglia BV2 cell lines are poorly representative of primary microglia. *J Neuroinflammation* 2016; **13**: 1–18
- 119 Turner BJ, Talbot K. Transgenics, toxicity and therapeutics in rodent models of mutant SOD1-mediated familial ALS. *Prog Neurobiol* 2008; **85**: 94–134
- 120 Heiman-Patterson TD, Sher RB, Blankenhorn EA, Alexander G, Deitch JS, Kunst CB, et al. Effect of genetic background on phenotype variability in transgenic mouse models of amyotrophic lateral sclerosis: A window of opportunity in the search for genetic modifiers. *Amyotroph. Lateral Scler.* 2011; **12**: 79–86
- 121 Pfohl SR, Halicek MT, Mitchell CS. Characterization of the contribution of genetic background and gender to disease progression in the SOD1 G93A mouse model of amyotrophic lateral sclerosis: a meta-analysis. *J Neuromuscul Dis* 2015; **2**: 137–50
- 122 Mancuso R, Oliván S, Mancera P, Pastén-Zamorano A, Manzano R, Casas C, et al. Effect of genetic background on onset and disease progression in the SOD1-G93A model of amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler.* 2012; **13**: 302–10
- 123 Régál L, Vanopdenbosch L, Tilkin P, van den Bosch L, Thijs V, Sciot R et al. The G93C mutation in

- superoxide dismutase 1: Clinicopathologic phenotype and prognosis. *Arch Neurol* 2006; **63**: 262–67
- 124 Heiman-Patterson TD, Deitch JS, Blankenhorn EP, Erwin KL, Perreault MJ, Alexander BK, et al. Background and gender effects on survival in the TgN (SOD1-G93A)1Gur mouse model of ALS. *J Neurol Sci* 2005; **236**: 1–7
- 125 Veldink JH, Bär PR, Joosten EAJ, Otten M, Wokke JHJ, van den Berg LH, et al. Sexual differences in onset of disease and response to exercise in a transgenic model of ALS. *Neuromuscul Disord* 2003; **13**: 737–43
- 126 Bame M, Pentiak PA, Needleman R, Brusilow WSA. Effect of sex on lifespan, disease progression, and the response to methionine sulfoximine in the SOD1 G93A mouse model for ALS. *Genet. Med.* 2012; **9**: 524–35
- 127 Stam NC, Nithianantharajah J, Howard ML, Atkin JD, Cheema SS, Hannan AJ, et al. Sex-specific behavioural effects of environmental enrichment in a transgenic mouse model of amyotrophic lateral sclerosis. *Eur. J. Neurosci.* 2008; **28**: 717–23
- 128 Kobayashi K, Imagama S, Ohgomori T, Hirano K, Uchimura K, Sakamoto K, et al. Minocycline selectively inhibits M1 polarization of microglia. *Cell Death & Disease.* 2013; **4**: e525–e525. <http://dx.doi.org/10.1038/cddis.2013.54>
- 129 Kriz J, Nguyen MD, Julien J-P. Minocycline slows disease progression in a mouse model of amyotrophic lateral sclerosis. *Neurobiology of Disease* 2002; **10**: 268–78. <https://doi.org/10.1006/nbdi.2002.0487>
- 130 Van Den Bosch L, Tilkin P, Lemmens G, Robberecht W. Minocycline delays disease onset and mortality in a transgenic model of ALS. *NeuroReport* 2002; **13**: 1067–70
- 131 Zhu S, Stavrovskaya IG, Drozda M, Kim BYS, Ona V, Li M, et al. Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. *Nature* 2002; **417**: 74–8
- 132 Keller AF, Gravel M, Kriz J. Treatment with minocycline after disease onset alters astrocyte reactivity and increases microgliosis in SOD1 mutant mice. *Experimental Neurology* 2011; **228**(1): 69–79.
- 133 Pinkernelle J, Fansa H, Ebmeyer U, Keilhoff G. Prolonged minocycline treatment impairs motor neuronal survival and glial function in organotypic rat spinal cord cultures. *PLoS One* 2013; **8**
- 134 Gordon PH, Moore DH, Miller RG, Florence JM, Verheijde JL, Doorish C et al. Efficacy of minocycline in patients with amyotrophic lateral sclerosis: a phase III randomised trial. *Lancet Neurol.* 2007; **6**: 1045–53
- 135 Cudkovicz ME, Shefner JM, Schoenfeld DA, Zhang H, Andreasson KI, Rothstein JD, et al. Trial of celecoxib in amyotrophic lateral sclerosis. *Ann Neurol* 2006; **60**: 22–31
- 136 Goyal NA, Mozaffar T. Experimental trials in amyotrophic lateral sclerosis: a review of recently completed, ongoing and planned trials using existing and novel drugs. *Expert Opin Investig Drugs* 2014; **23**: 1541–51
- 137 Scott S, Kranz JE, Cole J, Lincecum JM, Thompson K, Kelly N, et al. Design, power, and interpretation of studies in the standard murine model of ALS. *Amyotroph. Lateral Scler.* 2008; **9**: 4–15
- 138 Apolloni S, Fabbriozio P, Amadio S, Volonté C. Actions of the antihistaminergic clemastine on presymptomatic SOD1-G93A mice ameliorate ALS disease progression. *J Neuroinflammation* 2016; **13**: 191
- 139 Apolloni S, Fabbriozio P, Parisi C, Amadio S, Volonté C. Clemastine confers neuroprotection and induces an anti-inflammatory phenotype in SOD1G93A mouse model of amyotrophic lateral sclerosis. *Mol Neurobiol* 2016; **53**: 518–31
- 140 Dodge JC, Treleaven CM, Fidler JA, Hester M, Haidet A, et al. AAV4-mediated expression of IGF-1 and VEGF within cellular components of the ventricular system improves survival outcome in familial ALS mice. *Mol Ther* 2010; **18**: 2075–84
- 141 Wang Y, Duan W, Wang W, Wen D, Liu Y, Liu Y, et al. scAAV9-VEGF prolongs the survival of transgenic ALS mice by promoting activation of M2 microglia and the PI3K/Akt pathway. *Brain Res* 2016; **1648**: 1–10
- 142 Butovsky O, Jedrychowski MP, Moore CS, Cialic R, Lanser AJ, Gabriely G, et al. Identification of a unique TGF- β -dependent molecular and functional signature in microglia. *Nat Neurosci* 2014; **17**: 131–43
- 143 Bennett ML, et al. New tools for studying microglia in the mouse and human CNS. *Proc Natl Acad Sci* 2016; **113**: E1738–46
- 144 Gautier EL, Shay T, Miller J, Greter M, Jakubzick C, Ivanov S, et al. Gene-expression profiles and transcriptional regulatory pathways that underlie the identity and diversity of mouse tissue macrophages. *Nat Immunol* 2012; **13**: 1118–28
- 145 Hickman S, Izzy S, Sen P, Morsett L, El Khoury J. Microglia in neurodegeneration. *Nat Neurosci* 2018; **21**: 1359–69
- 146 Chiu IM, Morimoto ETA, Goodarzi H, Liao JT, O’Keeffe S, Phatnani HP et al. A neurodegeneration-specific gene-expression signature of acutely isolated microglia from an amyotrophic lateral sclerosis mouse model. *Cell Rep* 2013; **4**: 385–401
- 147 Michelucci A, Heurtaux T, Grandbarbe L, Morga E, Heuschling P. Characterization of the microglial phenotype under specific pro-inflammatory and anti-inflammatory conditions: Effects of oligomeric and fibrillar amyloid- β . *J Neuroimmunol* 2009; **210**: 3–12

- 148 Wang J, Wang J, Wang J, Yang B, Weng Q, He Q, et al. Targeting microglia and macrophages: A potential treatment strategy for multiple sclerosis. *Front Pharmacol* 2019; **10**: 286
- 149 Kawamura MF, Yamasaki R, Kawamura N, Tateishi T, Nagara Y, Matsushita T, et al. Impaired recruitment of neuroprotective microglia and T cells during acute neuronal injury coincides with increased neuronal vulnerability in an amyotrophic lateral sclerosis model. *Exp Neurol* 2012; **234**: 437–45
- 150 Fernando MS, Simpson JE, Matthews F, Brayne C, Lewis CE, Barber R, et al. White matter lesions in an unselected cohort of the elderly: Molecular pathology suggests origin from chronic hypoperfusion injury. *Stroke* 2006; **37**: 1391–8
- 151 Walker DG, Lue LF. Immune phenotypes of microglia in human neurodegenerative disease: challenges to detecting microglial polarization in human brains. *Alzheimer's Research & Therapy*. 2015; **7**: 1. <http://dx.doi.org/10.1186/s13195-015-0139-9>
- 152 Hickman SE, Kingery ND, Ohsumi TK, Borowsky ML, Wang L-C, Means TK, et al. The microglial sensome revealed by direct RNA sequencing. *Nat Neurosci* 2013; **16**: 1896–905
- 153 Krumbholz M, Theil D, Cepok S, Hemmer B, Kivisäkk P, Ransohoff RM, et al. Chemokines in multiple sclerosis: CXCL12 and CXCL13 up-regulation is differentially linked to CNS immune cell recruitment. *Brain* 2006; **129**: 200–11

Received 27 January 2020

Accepted after revision 14 June 2020

Published online Article Accepted on 28 June 2020