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1 [Au: We really need to reduce the number of references to ~250; accordingly, I have suggested a few
2 places in the manuscript where the number of references could be reduced. I have reduced this to
3 256]

4 Amyotrophic lateral sclerosis

5 Orla Hardiman¹, Ammar Al-Chalabi², Adriano Chio³, Emma M Corr¹, Giancarlo Logroscino⁴, Wim
6 Robberecht⁵, Pamela J Shaw⁶, Zachary Simmons⁷ and Leonard H van den Berg⁸.

7

- 8 1. Academic Unit of Neurology, Room 5.41 Trinity Biomedical Science Institute, Trinity College
9 Dublin, Pearse Street, Dublin 2, Ireland.
- 10 2. Department of Basic and Clinical Neuroscience, Maurice Wohl Clinical Neuroscience
11 Institute, Institute of Psychiatry, Psychology and Neuroscience, King's College London,
12 London, UK.
- 13 3. Rita Levi Montalcini Department of Neurosciences, University of Turin, Turin, Italy
- 14 4. Department of Neuroscience, University of Bari, Bari, Italy.
- 15 5. KU Leuven - University of Leuven, University Hospitals Leuven, Department of Neurology, B-3000
16 Leuven, Belgium.
- 17 6. Sheffield Institute for Translational Neuroscience, University of Sheffield, Sheffield, United
18 Kingdom.
- 19 7. Department of Neurology, Milton S. Hershey Medical Center, Penn State Health, Hershey,
20 Pennsylvania, USA.
- 21 8. Department of Neurology, Rudolf Magnus Institute of Neuroscience, University Medical
22 Center Utrecht, Utrecht, The Netherlands.

23

24 **Competing interests** [Au: Please note I've restructured the order of the CIs here so they follow the
25 same order as the author list]

26 O.H. declares grants from the Health Research Board and Science Foundation Ireland, and receives
27 funding through the EU Joint Programme in Neurodegenerative Disease Research (JPND) [Au: I presume
28 the highlighted are non-profit associations? If so, they do not need to be declared here, only for-profit
29 companies - we can move this into the Acknowledgements section if you wish? OK], has served on
30 advisory boards for Biogen, Cytokinetics, Orion, Merck and Roche and has consulted for Mitsubishi. She

31 is Editor in Chief of the Journal ALS and the Frontotemporal Degenerations. A.A.C. has consulted for
32 Chronos Therapeutics, OrionPharma, Cytokinetics, Biogen Idec, Mitsubishi Tanabe Pharma and GSK, has
33 received speaking honoraria from Cytokinetics and Lilly, has been the chief or principal investigator of
34 clinical trials for [Au: funded by?] OrionPharma, Cytokinetics, Biogen Idec and GSK and receives royalties
35 for books The Brain (OneWorld Publications) and Genetics of Complex Human Diseases (Cold Spring
36 Harbor Laboratory Press). A.C. has served on scientific advisory boards for Biogen Idec, Cytokinetics,
37 Italfarmaco, Neuraltus and Mitsubishi. G.L. is the Associate Editor of Neuroepidemiology (Karger
38 Publishers) [Au: you can omit this if this is not a paid position] . P.J.S. has served on scientific advisory
39 boards for Biogen, Orion Pharma, Sanofi and Treeway and has received research grants from Reneuron,
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44 Development (SOPHIA, STRENGTH, ALS-CarE project), funded through the EU Joint Programme –
45 Neurodegenerative Disease Research, JPND) [Au: I presume the highlighted are non-profit
46 associations? If so, they do not need to be declared here, only for-profit companies - we can move this
47 into the Acknowledgements section if you wish?], has served on the Scientific Advisory Boards of
48 Biogen, Cytokinetics and Orion and has received honoraria for presentations from Baxalta.

49 [Au: Please add the competing interests for Emma Corr and Wim here - if these authors do not have
50 any competing interests, this should be added here. Our competing interest policy can be found here:
51 <http://www.nature.com/authors/policies/competing.html>. Essentially, competing financial interests
52 include honoraria, consultation fees, research grants, stocks, etc. from for-profit companies. Emma
53 Corr and Wim do not have any competing interests] .

54

55 Author contributions

56 Introduction (O.H.); Epidemiology (G.L.); Mechanisms/pathophysiology, (W.R. and P.J.S.);
57 Genetics, Diagnosis, screening and prevention, (O.H and L.H.B.); Management, (A.C.); Quality of
58 life, (Z.S.); Outlook, (A.A.); Overview of Primer, (E.M.C. and O.H.).

59

60 **Abstract** Amyotrophic lateral sclerosis (ALS), also known as Motor Neuron Disease (MND) [? It is
61 **synonymous.**], is characterized by the degeneration of both upper and lower motor neurons, leading to
62 muscle weakness and eventual paralysis. Until recently, ALS was classified primarily within the

63 neuromuscular domain, although new imaging and neuropathological data have indicated the
64 involvement of the non-motor neuraxis in disease pathology. In most patients, the mechanisms
65 underlying development of ALS are poorly understood, although a subset of patients have familial
66 disease and carry mutations in genes that have various roles in neuronal function. Two disease
67 modifying therapies which can slow disease progression, are available for the treatment of ALS, but
68 patient management is largely mediated by the use of symptomatic therapies, such as the use of muscle
69 relaxants for spasticity and speech therapy for dysarthria.

70

71

72 **[H1] Introduction**

73 Amyotrophic lateral sclerosis (ALS) is a heterogeneous neurodegenerative syndrome that is
74 characterized by the degeneration of both upper (that is, neurons that project from the cortex to the
75 brain stem and the spinal cord) and lower (that is, neurons that project from the brainstem or spinal
76 cord to the muscle) motor neurons leading to motor and extra-motor symptoms (Figure 1). The initial
77 presentation of ALS can vary between patients; some present with spinal-onset disease (that is, the
78 onset of muscle weakness of the limbs), but others can present with bulbar-onset disease (characterized
79 by dysarthria – difficulty with speech – and dysphagia – difficulty swallowing. In most patients, the cause
80 of ALS is unknown, although some individuals develop familial forms of the disease, which are
81 associated with mutations in genes that have a wide range of functions, including functions in non-
82 motor cells. In the familial forms of the disease, some of the implicated genes are incompletely
83 penetrant, and with rare exceptions, genotype does not necessarily predict phenotype ¹. Although the
84 primary symptoms of ALS are associated with motor dysfunction (such as muscle weakness, spasticity
85 and dysphagia), up to 50% of patients develop cognitive and/or behavioral impairment during the
86 course of disease and 13% of patients present with concomitant behavioral variant frontotemporal
87 dementia (bv-FTD)²⁻⁴. The high prevalence of cognitive and/or behavioural symptoms, coupled with the
88 finding of a hexanucleotide repeat expansion in *C9orf72* as the major genetic cause of ALS and FTD ^{5,6},
89 have contributed to the re-characterization of ALS as a neurodegenerative, rather than a neuromuscular
90 disorder, and have signposted the direction of research over the coming decade.

91

92 The classification of ALS can vary depending on the criteria used. The traditional definitions of ALS
93 subgroups are based on the extent of upper and lower motor neuron involvement, although other
94 classification systems include different parameters, such as the site of onset (that is, bulbar or spinal

95 onset of disease), the level of certainty of diagnosis according to the revised El Escorial Criteria and
96 heritability (sporadic or familial disease)⁷. To date, none of these classification systems have
97 incorporated the cognitive or behavioural symptoms and within each classification system a range of
98 sub-phenotypes and clinical trajectories can be demonstrated.

99

100 This Primer will review the aspects of ALS that contribute to disease heterogeneity, and will look to the
101 future of new therapeutic trials that incorporate recent advances in our understanding of this disease
102 spectrum. For new therapies, the challenge is to define mechanisms of disease amenable to drug
103 targeting, and to define sub-cohorts of patients that are likely to respond to these new therapeutic
104 agents.

105

106

107 **[H1] Epidemiology**

108

109 **[H2] Descriptive epidemiology**

110 The majority of population based epidemiological studies for ALS have come from high quality European
111 patient Registers ⁸. These European population based Registers have been combined to form the
112 European ALS Epidemiology Consortium (EURALS), which has provided data comparing the incidence of
113 ALS between European countries ⁹. In Europe, the incidence ranges from 2-3 cases per 100,000
114 individuals. Defined geographical areas are ideally suited to estimate the incidence and prevalence, and
115 to support more-detailed studies of risk, clinical trajectory, outcome and utilization of services for ALS⁸.
116 As ALS is a rare disease, a population-based approach with multiple sources of ascertainment is the best
117 way to describe the entire phenotypic spectrum ¹⁰ as population-based registers provide more complete
118 information about the disease than datasets from specialist clinics, which are often biased in favour of
119 younger patients and those with less severe disease [?OK] ¹⁰. Similarly, clinical trial cohorts such as those
120 collected within the US-based pooled resource open-access ALS clinical trials database (?OK] ProACT)
121 dataset also select for patients with ALS who have better prognosis; survival within these cohorts is ~12
122 months longer than that of true population-based cohorts.

123 [OK] [OK] Contrary to earlier assumptions, the incidence of ALS has been shown to differ based on
124 ancestral origin; studies in populations of European origin [] have shown a crude incidence of >3 cases
125 per 100,000 individuals ^{11,12}, but incidence rates are lower in East Asia (around 0.8 per 100,00) and

126 South Asia (0.7 per 100,000). In some regions (such as Guam and the Kii peninsula of Japan) the
127 reported incidence was very high, but dropped substantially over the past 30 years for reasons that
128 remain unclear. In areas where different ancestral populations live in close proximity (as in Northern
129 America), the incidence rates of ALS in indigenous populations is particularly low (0.63 cases per 100,000
130 individuals)¹³, whereas reported incidences in regions of relatively homogeneous populations (such as
131 Ireland, Scotland and the Faroe Islands) are high (2.6 cases per 100, 000 individuals)^{9,14}.

132
133 In addition, variations in the phenotype and natural history of ALS have been reported in different
134 ancestral populations; indeed reported survival of patients with ALS is much shorter in Europe (24
135 months) than in Central Asia (48 months)¹⁵. [OK] In addition, admixed populations (that is, populations
136 of mixed ancestry [OK]) might have lower mortality rates of ALS. In a population-based study in Cuba,
137 ALS mortality rate was 0.55 per 100,000 individuals in a mixed population,[OK] but was about 0.9 per
138 100,000 individuals [Au:OK?OK] in white or black individuals¹⁶, confirming the importance of ancestral
139 origin in disease risk. [ok] In Europe, most men have spinal onset disease, and women have increased
140 propensity for bulbar onset disease⁹. The percentage of individuals with bulbar onset disease is much
141 lower in Asia compared with Europe, but a North to South gradient has been described in Europe, with
142 higher percentage of individuals with spinal onset disease in Southern Europe⁹. Based on available data,
143 the age of diagnosis and first symptoms is higher in Europe compared to Asia and South America. [OK]
144 In Europe, the age of onset peaks at 65⁹. [] The main limitation of global ALS epidemiology is that
145 almost 80% of studies have been conducted in Europe and the US, and mainly comprise patient cohorts
146 of Northern European ancestry. International consortia collecting data in areas with mixed populations
147 and in different continents will be required to fully elucidate the range of clinical presentations, and to
148 understand the roles of ancestry, genetics and environmental exposures in ALS causation.

149

150 [H2] Causes of ALS

151

152 [H3] Genetics. ALS is considered a complex genetic disorder with a Mendelian pattern of inheritance in a
153 proportion of cases, but no discernible family history in the rest. Mathematical models developed using
154 population-based registers have suggested that individuals with ALS are likely to carry a number of 'at
155 risk' variants that interact with environmental factors through a series of at least 6 notional steps
156 leading to disease manifestation. One of these steps is thought to be the genetic risk (from birth), but
157 the interplay of environmental factors that lead to the remaining steps have yet to be defined. In

158 transgenic mice, the genetic background can alter the phenotypic presentation of ALS [OK ?] ^{17, 18},
159 suggesting that human disease phenotypes could also have a genetic basis, and that genomic and
160 epigenomic “fingerprinting” could permit the clustering of different phenotypic manifestations into
161 discrete underlying causes that are amenable to therapeutic intervention.

162
163 Large combined genome-wide association studies (GWAS) of apparently sporadic ALS suggest that the
164 genetic architecture is based primarily on rare variants, in contrast to other diseases, such as
165 schizophrenia, which are associated with large numbers of common variants. GWAS in ALS are also
166 complicated as the rare variants that confer risk might be specific to individuals, families and ancestral
167 populations ¹⁹, rendering GWAS less suited for study of ALS genetics than is schizophrenia. Initiatives
168 such as the Project MinE Consortium (www.projectmine.com), which aims to undertake whole genome
169 sequencing of >16,000 patients with ALS and 6,000 control individuals, are likely to provide greater
170 clarity of the genetic architecture of ALS.

171
172 Of the known genes of major effect for the development of ALS (Table 1 [OK), our current knowledge
173 comes primarily from the study of ancestral European (Europe, USA, Canada and Australia) and East
174 Asian populations; within these populations, OKthe dichotomization of ALS into ‘familial’ and ‘sporadic’
175 subtypes is an over-simplification. Although at least 30 genes are known to confer a major risk for ALS,
176 evidence suggests a role of oligogenic inheritance (in which a phenotypic trait is determined by more
177 than one gene? OK]) and of genetic pleiotropy (in which a single gene [OK] has multiple phenotypic
178 manifestations). Within populations of European extraction, up to 20% of people with ALS have a family
179 history of either ALS or FTD (Familial ALS) , and of these 4 genes account for up to 70% of all cases of
180 familial ALS , namely *C9orf72*, *TARDBP* (also known as *TDP43*), *SOD1* and *FUS* [?OK] ²⁰. However, even in
181 the case of these known Mendelian inherited genes, familial forms of ALS are often characterized by
182 lower than 50% penetrance [and genetic pleiotropy, with evidence of oligogenic and polygenic
183 inheritance in individuals with apparently sporadic disease ^{21, 22}.

184
185 **[H3] Environmental and lifestyle factors. OK]**. Epidemiological case control studies have sought to
186 determine the environmental causes of ALS. Early epidemiological studies from regions with a high
187 incidence of ALS and dementia such as Guam and the Kii peninsula of Japan suggested a role for
188 neurotoxins [Would prefer to retain neurotoxins] contained within cycad seeds, including β-
189 methylamino-L-alanine OK] . Although the role of β-methylamino-L-alanine²³ has not been

190 substantiated, a possible role for related cyanotoxins has been proposed, and exposure to water
191 harbouring cyanobacterial blooms has been suggested to contribute to risk of ALS in susceptible
192 individuals ²⁴.

193
194 ALS has been reported at a higher frequency among groups of athletes compared to the general
195 population although whether physical activity is a risk factor for ALS, or a marker of underlying athletic
196 prowess is unclear. Evidence from a UK study suggests that individuals with ALS had higher rates of pre-
197 morbid [Pre-Morbid is ok – standard use PJS I AGREE] physical activity, but two other European studies
198 suggested either no effect, or a protective effect ²²⁻²⁴. Reasons for this discrepancy [might relate to study
199 design and true population-based differences. However, because ALS is a rare disease, smaller case
200 control studies are often underpowered and are subject to both bias and error in interpretation. To
201 address these problems in study design, a very large case control study OK has been completed as part
202 of the EuroMOTOR project (www.euromotorproject.eu), which has collected >1,500 population-based
203 incident cases and 3,000 matched controls across 3 countries. Analysis is ongoing, although preliminary
204 data suggest that exposure to smoking might increase the risk of developing ALS, but type 2 diabetes
205 mellitus, high levels of circulating lipids and exposure to [EXPOSURE is more accurate female
206 contraceptive hormones seem to be protective ^{25,26} [(YES- OH is the senior author) PENDING] .

208 **[H1] Mechanisms/pathophysiology**

210 **[H2] Histopathology [?OK]**

211 Although the fundamental pathophysiological mechanisms underlying ALS are not well understood, the
212 neuropathological hallmark of disease is the aggregation and accumulation of ubiquitinated
213 proteinaceous inclusions in motor neurons [?YES] . Protein inclusions occur in other neurodegenerative
214 disorders (such as amyloid plaques in Alzheimer Disease and synuclein-containing Lewy Bodies in
215 Parkinson Disease OK]. The biological processes leading to formation of these inclusions OK] has been
216 the subject of intensive research, but is poorly understood ⁴.

217 In most subtypes of ALS the tar DNA-binding protein 43 OK] (TDP-43) is the major constituent of these
218 inclusions, although mutations in *TARDBP* are a rare cause of ALS ^{27,28} OK r] Indeed, approximately 97%
219 of patients with ALS have features of a TDP-43 proteinopathy, with depletion of TDP-43 in the nucleus,
220 but the formation of cytoplasmic aggregates with skein-like or compact morphology in residual motor

221 neurons (Figure 2A). In specific subtypes of ALS, other types of protein aggregates might be seen, such
222 as P62-positive, TDP-43 negative protein inclusions that are caused by dipeptide repeat proteins and
223 might be seen outside the motor system in patients with 'ALS associated with C9ORF72 mutations OK]
224 (Figure 2C) and neurofilamentous hyaline conglomerate inclusions (Figure 2B) and the accumulation of
225 misfolded superoxide dismutase (SOD1) in patients with SOD1-ALS YES] . [Au: green text mvoed here
226 from the 'impaired protein homeostasis' section for flow OK] Although protein aggregates are the
227 hallmark of ALS, the high molecular weight YES] complexes that precede the formation of the
228 aggregates, rather than the aggregates themselves^{29, 30}, might be the toxic species. Shedding of higher
229 molecular protein complexes might mediate cell to cell propagation of disease, linking the progression
230 of ALS to a prion-like mechanism, as has also been suggested for tau and synuclein-mediated diseases³¹,
231 ³².

232
233 The gross pathological features of ALS comprise skeletal muscle atrophy, atrophy of the motor cortex
234 and pallor LEAVE PALLOR -(more accurate in neuropathology)] and sclerosis of the corticospinal and
235 corticobulbar tracts OK]), together with thinning of the hypoglossal nerves (which are involved in the
236 control of the muscles of the tongue) and the ventral roots of the spinal cord. Microscopic examination
237 usually reveals a depletion of at least 50% of spinal motor neurons and diffuse astrocytic gliosis and
238 microglial infiltration in the grey and white matter of the spinal cord (Figure 2D AND 2F). OK OK NOW].
239 Axonal loss, gliosis and myelin pallor are seen in the corticospinal tracts, and astrocytic gliosis is usually
240 observed in the motor cortex, together with variable depletion of upper motor neurons. Skeletal muscle
241 shows features of denervation and reinnervation, with fibre type grouping and clusters of angular
242 atrophic fibres.

245 [H2] Overview of pathophysiology OK

246 Progress has been made in the identification of the genetic causes of ALS^{21, 22} and models in rat, mouse,
247 zebrafish, flies, worms and yeast have been developed to study the mechanisms by which gene
248 mutations cause motor neuron degeneration and to model particular biological processes thought to be
249 important in disease pathobiology. All of these models have limitations and none fully recapitulates
250 human disease, which is partly because most models are based on gene overexpression (with multiple
251 copies of the human variant inserted into the transgenic model) and because the human neuro-axis
252 differs substantially. OK] from that of lower animals. OK] Nevertheless, findings from animal models

253 **OK]** can contribute to our understanding of the cell biology underlying neurodegeneration and can
254 open new avenues towards targeted drug development. In reality, the cellular disruption **?OK]** in ALS is
255 likely the result of many different interacting mechanisms that culminate in larger network disruption,
256 and the separation of different mechanisms is somewhat artificial. **[OK]** This is exemplified by the
257 finding that multiple factors can contribute to neuronal damage in models of *Sod1* **OK MODIFIED BY PJS**
258 **?] mutations** (Table 1). The relative extent by which each of these factors contributes to the overall
259 pathobiology of human disease cannot be fully ascertained, it would be erroneous to assume that all of
260 these factors are involved in all cases of ALS, as human disease is heterogeneous. Notwithstanding, each
261 of the thematic areas should be considered in detail, as they represent our current knowledge base of
262 the pathophysiology of ALS, and are the drivers of current and future therapeutic initiatives (**Figure 3**).

263

264 **[H2] Impaired protein homeostasis**

265 **[] OK**

266 Mutations in some genes **OK]** lead to the translation of proteins that are misfolded, have an abnormal
267 cellular localization or are aberrantly formed, and that can directly or indirectly impair the proteasome
268 or autophagy machinery of the cell, leading to impaired cellular protein turnover. Indeed, genes
269 associated with familial ALS encode proteins that can **[OK]** promote dysfunction of the ubiquitin-
270 proteasome system. For example, mutant SOD1 is associated with reduced expression of ubiquitin-
271 proteasome system components ³³, valosin-containing protein (VCP) and ubiquilin-2 are involved in
272 substrate delivery to the proteasome, and this function is disrupted in the presence of ALS-associated
273 mutations **[n SENTENCE IS OK AS IT STANDS]** ³⁴⁻³⁶. In addition, dysregulation of chaperone proteins has
274 been identified in ALS associated with *SOD1* and *TARDBP* mutations ³⁷⁻⁴⁰. Mutations in *VAPB* (encoding
275 vesicle-associated membrane protein associated protein B **[Au OK:]**) can cause defective activation of
276 the unfolded protein response in disease models ^{41, 42}.

277

278 *C9orf72* **[? PROTEIN YES]** is a key regulator of autophagy initiation ⁴³ and loss of this function might
279 contribute to the presence of ubiquitin and p62 positive, TDP-43 negative inclusions in extra-motor
280 areas of the central nervous system (CNS) in *C9orf72*-related ALS **[YES] . OK]** Sequestosome-1,
281 optineurin and ubiquilin-2 have a role in the early steps of autophagy ⁴⁴⁻⁴⁶, and alsin,
282 polyphosphoinositide phosphatase (FIG4), transitional endoplasmic reticulum ATPase (VCP) and charged
283 multivesicular body protein 2b (CHMP2B) have roles in the maturation of autophagosomes into
284 autophagolysosomes by regulating the fusion of autophagosomes with multivesicular bodies,

285 endosomes and lysosomes lysosomes⁴⁷⁻⁵¹. Mutations in *SQSRM1* [? OK] might disrupt the correct
286 delivery of autophagic substrates to the autophagosome⁵² and mutations in *UBQLN2* and *OPTN* OK ?]
287 (which both encode autophagy receptors) are also associated with ALS. The activities of sequestosome-1
288 and optineurin are regulated by serine/threonine-protein kinase OK] (TBK1) and^{53, 54} haploinsufficiency
289 of *TBK1* [YES is a cause of familial ALS, which supports the hypothesis that reduced substrate delivery to
290 autophagosomes might contribute to motor neuron injury in ALS. Reduced VCP activity YES] has been
291 shown to decrease the maturation of autophagosomes. Other proteins implicated in ALS
292 pathophysiology, including alsin and FIG4 YES ?] , can affect autophagy at the stage of initiation, OK ?]
293 although the mechanism for this is unclear^{47, 55}. Both SOD1 and TDP-43 are known substrates of
294 autophagy, suggesting that defective autophagy could contribute to the toxic accumulation of these
295 proteins in ALS. The formation of dipeptide repeat proteins through repeat-associated non-ATG (RAN)
296 translation from the expanded RNA repeat of the *C9orf72* [Au: this is quite technical - can we edit to
297 this to 'C9orf72 repeat expansions might cause dysproteostasis, but this remains..' for non-experts
298 WOULD PREFER TO KEEP ORIGINAL TEXT IF POSSIBLE ?] gene might also result in dysproteostasis, but
299 this remains to be conclusively demonstrated and the mechanism elucidated.

300

301 [OK

302

303 [H2] Aberrant RNA metabolism

304 Alteration of mRNA processing is a key theme in ALS pathogenesis⁵⁶. [OK] mRNA undergoes a complex
305 system of processing as it transits from the nucleus to cytoplasm, where it is translated into protein. In
306 neurons, mRNAs can be transported to allow local translation in the axonal compartment. Although the
307 functional consequences of RNA dysregulation that lead to age-related and selective degeneration of
308 neuronal populations NO- other neurons also affected] remain poorly understood [Both actually but
309 the latter in this context , analysis of the transcriptome of actively transcribing mRNAs will be essential in
310 elucidating the upstream molecular events contributing to neuronal injury.

311

312 OK The discovery of mutations in *TARDBP* and *FUS* as rare causes of [Au: familial? NO- ok at stands ALS
313 has identified a crucial pathogenetic role for RNA binding proteins that contain low complexity domains
314⁵⁷. Mutant TDP-43 or FUS proteins mislocalize from the nuclear to the cytoplasmic compartment and this
315 is hypothesised to [OK] result in the loss of the normal processing of their target RNAs^{58, 59}. Indeed, up
316 to one third of the transcriptome is altered in models of TARDBP-related ALS [OK ?]⁶⁰, and dysregulation

317 of gene expression has also been observed in relation to mutations in *C9orf72*, *SOD1*, and *FUS* **OK ?** ⁶¹,
318 including transcription, alternative splicing of mRNA, axonal transport of mRNAs and biogenesis of
319 microRNAs ^{62, 63}.

320
321 [
322 The GGGGCC repeat expansion in the noncoding region of *C9orf72* **[YES]** forms stable parallel uni- and
323 multimeric G-quadruplex structures, which avidly interact with RNA processing factors ^{64, 65}. **[OK]** In
324 addition, the repeat expansion gives rise to abnormal RNA species that can be identified as nuclear RNA
325 foci and the *C9orf72* mutation **[? MUTATION OK]** might induce direct RNA toxicity, by, for example,
326 sequestering RNA binding proteins ⁶⁶⁻⁶⁸. Indeed, a large set **[No need to change – large set ok ?]** of
327 proteins that bind to the expanded repeat have been identified ⁶⁹. In addition, repeat expansions could
328 lead to the formation of R-loops **OK]** (that is, DNA-RNA hybrid structures) that increase susceptibility to
329 DNA damage and genome instability ^{70, 71}. Indeed, R-Loops and genome instability due to double strand
330 DNA breaks and defective serine-protein kinase ATM-mediated DNA repair have been identified as
331 important components of neuronal injury due to GGGGCC repeat expansion in *C9orf72* **[OK]** ⁷².

332
333 **OK?OK]** Mutations in *ANG* (encoding angiogenin, which has a role in RNA processing ^{73, 74}) and *SETX*
334 (encoding senataxin, which regulates the transcription of ribosomal RNA ^{75, 76}) **OK]** are associated with
335 ALS, and might lead to disturbances in RNA metabolism. In addition, mutations in **[OK? OK]** *ELP3*
336 (encoding elongator protein 3), *TAF15* (encoding TATA-binding protein-associated factor 2N)**OK]** and
337 *EWSR1* (encoding RNA-binding protein EWS **OK]** ⁷⁷⁻⁷⁹ have also been associated with ALS. These genes
338 encode proteins that are involved in regulation of RNA metabolism; ELP3 contributes to the regulation
339 of transcription elongation, and TAF15 and EWSR1, which are functionally and structurally related to
340 FUS, have a role in the control of transcription and alternative splicing ^{80, 81}.

341
342 Mutations in other genes involved in RNA metabolism **[Au: such as TAF15, EWSR1, hnRNPA1,**
343 **hnRNPA2B1 [Au: This gene doesn't show up on the HUGO database, does this have another name?**
344 **CORRECTED]** and *MATR3* have been implicated in ALS ^{82, 83} **[Au: Changed 'have been found' to**
345 **'implicated in ALS', OK? OK Please cite fewer refs here REFS REDUCED].** The mislocalization of the
346 mutant proteins into the cytoplasm might result in a toxic gain-of-function, and the effect of these
347 proteins on the formation of stress granules is an area of intense research **effort [Au: why specifically**
348 **on stress granules? Do the aforementioned proteins all compose stress granules, for example? PLEASE**

349 LEAVE AS ORIGINAL – STRESS GRANULES ARE IMPORTANT AS MOTOR NEURON INJURY IS OCCURRING

350]⁸⁴⁻⁸⁶ .

351

352 [H2] Nucleocytoplasmic and endosomal transport

353 In addition to altering RNA metabolism [OK] , the GGGGCC repeat expansion in *C9orf72* is believed to

354 alter the intracellular localisation of *C9orf72* mRNA. Dipeptide repeat proteins are generated from the

355 repeat expansion in *C9orf72* and interfere with proper nucleocytoplasmic transport and trigger

356 neurotoxicity via several mechanisms^{87, 88}. [Au: I've deleted the sentence discussing liquid phase

357 separation as this is quite technical. Please restrict the number of reference here to 1-ZONE OF THE

358 REVIEWERS SPECIFICALLY ASKED FOR INCLUSION OF DISCUSSION OF LIQID PHASE SEPARATION. ONE

359 REF REMOVED] OK . For example, arginine-rich dipeptide repeat proteins isolated from [OK] *C9orf72*

360 expansions can induce phase separation of proteins that have a role in RNA and stress granule

361 metabolism, and produce spontaneous stress granule assembly⁸⁹. In addition, increased binding of

362 mRNA export adaptors to expanded *C9orf72* pre-mRNAs might target those pre-mRNAs for nuclear

363 export, which could allow RNA translation to occur with potential toxicity from the expression of

364 abnormal dipeptide repeat protein species YES]^{68, 90}. Indeed, sequestration of the nuclear export

365 adaptor serine/arginine-rich splicing factor 1 (SRSF1) by the repeat expansion region of the [Au:OK? OK]

366 RNA, triggers nuclear RNA export factor 1 [?OK] (NXF1)-dependent nuclear export of *C9orf72* transcripts

367 retaining the hexanucleotide repeats, allowing RAN translation to dipeptide repeats in the cytoplasm

368 [YES] . Depletion of SRSF1 in cellular and *in vivo* models reduces the production of dipeptide repeat

369 proteins and neurotoxicity⁹¹.

370

371 [H2] Endosomal and vesicle transport

372 [OK] TDP-43 is involved in the regulation of endosomal trafficking and TDP-43 loss-of-function

373 [PROBABLY] has been shown to alter dendritic endosomes [, which resulted in reduced signalling of

374 neurotrophins [OK] and detrimental effects on neuronal health⁹². Mutations in *ALS2* (encoding alsin)

375 and *UNC13A* can alter endosomal and vesicle transport . Indeed, alsin is a guanine nucleotide exchange

376 factor for the small GTPase Rab5, and is involved in endosome trafficking and fusion^{55, 93}. UNC-13

377 homolog A [OK encoded by *UNC13A*, which is a risk factor for ALS), is involved in synaptic-vesicle

378 priming and neurotransmitter release⁹⁴.

379

380 [H2] Axon structure and function

381 The finding of *DCTN* (encoding dynactin) [YES] , *PFN1* (encoding profilin 1) and *TUBA4A* (encoding
382 tubulin alpha-4A chain) mutations suggests that abnormalities of proteins that are essential for axonal
383 transport are associated with ALS⁹⁵⁻⁹⁷. In addition, mutations in *NEFH* ([Au: please complete this with
384 the gene name(s) - should this be *NEFH* and *NEFL*? YES *NEFH* This isn't mentioned in figure 3, should
385 this be added here under the 'axonopathy' heading? YES] encoding neurofilament) have also been
386 described in a small number of patients⁹⁸, although whether these mutations are pathogenetic through
387 axonal dysfunction remains to be seen. Rare mutations in *PRPH* encoding peripherin, another
388 cytoskeletal protein, have been suggested to have a role in ALS pathogenesis, possibly through effects
389 on neurofilament housekeeping including protein cargo trafficking [Au: have mutations in *PRPH* been
390 identified in patients with ALS? THE SENTENCE HAS BEEN ALTERED]^{99,100}.

391

392 [H2] DNA repair

393 Impaired DNA repair was suggested to have a role in ALS pathophysiology following the identification of
394 *FUS* mutations, although the exact role of DNA repair failure in ALS remains to be clarified^{101, 102}.
395 Mutations in *NEK1* and *C21orf2* [, both of which encode proteins involved in DNA repair, have recently
396 been identified as causes for ALS¹⁰³⁻¹⁰⁵ although the biological pathways associated with their their
397 causal role awaits confirmation [LEAVE THIS SENTENCE AS MODIFIED HERE

398

399 [H2] Excitotoxicity

400 Motor neurons are very sensitive to toxicity induced by calcium entry following excessive glutamate
401 stimulation as they have a lower calcium buffering capacity than other neuronal subtypes and α -amino-
402 3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors that are more calcium permeable (as
403 they contain less of the GluR2 subunit)¹⁰⁶. In addition, excitatory amino acid transporter 2 (EAAT2), an
404 astroglial [protein that is the main synaptic glutamate re-uptake transporter, is impaired in ALS, which is
405 likely to result in synaptic glutamate abundance and motor neuron toxicity. The loss of EAAT2 has been
406 observed in both rodent models and patients with familial or sporadic ALS. Excitotoxicity is thought to
407 be a mechanism common to all forms of ALS, although the evidence for this remains indirect. One
408 argument is that riluzole, which can attenuate disease progression and is an approved drug for
409 neuroprotection in ALS, can inhibit glutamate release^{107, 108}. However, whether this underlies the
410 therapeutic effect of riluzole remains unclear.

411

412 [H2] Oligodendrocyte degeneration

413 Oligodendrocyte degeneration has been observed in ALS. In the healthy CNS, oligodendrocytes are
414 replaced by the proliferation [Au: and presumably differentiation NOT FULLY DIFFERENTIATED, so BEST
415 TO LEAT TEXT AS IS ?] of oligodendrocyte precursor cells, which are abundantly present ^{109, 110}. At least
416 in animal models of ALS, and for reasons that are now clear [Au: please expand on this in 1-2 sentences;
417 what causes this failure to differentiate?] CAUSES ARE NOT KNOWN, oligodendrocyte precursor cells
418 [Au: I've specified precursor cells here, OK?]OK fail to go through the final stages of differentiation.
419 Oligodendrocytes provide vital metabolic support to axons through the shuttling of lactate through
420 monocarboxylate transporter 2 ^{111, 112}, and accordingly, dysfunction of oligodendrocytes contributes to
421 the motor axonal failure [YES] in ALS. Restoring oligodendrocytic function by transgenically deleting
422 mutant SOD1 from these cells significantly slows disease progression and prolongs their life span ¹¹³. In
423 patients with ALS, abnormalities in oligodendrocytes can occur, but whether these changes contribute
424 to the disease remains to be demonstrated.

425

426 [H2] Neuroinflammation

427 [OK Neuroinflammation can be observed in imaging studies in patients with ALS, human postmortem
428 samples and rodent models of ALS ^{114, 115}. [Au: cite fewer refs here? Refs removed]. Astrocytes and
429 microglial cells release a number of hazardous and possibly neuroprotective factors. Deleting mutant
430 *Sod1* OK] from these cells in a mouse model increases survival and slows disease progression ¹¹⁶,
431 indicating that inflammation is an important factor for amplifying neuronal injury and disease
432 progression in ALS. [OK Microglia have dual activation phenotypes, which can be neuroprotective (the
433 M2 phenotype) or toxic (also known as classically activated, or M1 phenotype); evidence from SOD1-
434 transgenic mice suggests the phenotype of microglia evolves with disease progression, from a
435 neuroprotective phenotype at disease onset to a neurotoxic phenotype, with an altered cytokine release
436 profile, at end-stage disease ¹¹⁷ OK In addition, evidence highlights complex signalling between CNS
437 resident immune cells and peripheral cells, including monocytes and T-lymphocytes.

438 [H2] Mitochondrial dysfunction

439 Mitochondrial function is impaired in ALS and changes in mitochondrial morphology have been shown in
440 some patients, and in the SOD1 mouse model ^{118, 119}. In the SOD1 model, vacuoles containing protein
441 aggregates containing mutant SOD1 can be observed in the mitochondrial inter-membrane space,
442 leading to impairment of protein import ¹²⁰. In addition, oxidative damage to mitochondrial proteins
443 leads to defects in respiratory chain function in patients with ALS and in SOD1 mouse models ¹²¹, and

444 various experimental models of ALS have defects in axonal transport of mitochondria, which could
445 contribute to the axonopathy at the neuromuscular junction^{122, 123}.

446
447 Many of the functions disrupted in ALS are regulated by signalling between the endoplasmic reticulum
448 and mitochondria, underpinned by tight junction associations mediated by the endoplasmic reticulum
449 protein VAPB and the outer mitochondrial protein regulator of microtubule dynamics protein¹²⁴. These
450 associations are perturbed by *TARDBP* and *FUS* mutations^{125, 126}. TDP-43 preferentially binds to mRNAs
451 encoding respiratory chain complex 1 subunits and causes complex 1 disassembly¹²⁷ and accumulates in
452 the mitochondria of patients with ALS and mutations in *TARDBP* increase the mitochondrial localization
453 of TDP-43. Suppression of TDP-43 localization to mitochondria improves mitochondrial dysfunction and
454 reduces neuronal loss in mTDP-43 cell based models. In C9orf72-related ALS models, the dipeptide
455 repeat protein poly(GR) appears to compromise mitochondrial function and causes oxidative stress and
456 DNA damage¹²⁸. *CHCHD10* mutations, which are associated with familial ALS, can promote the loss of
457 mitochondrial cristae junctions, impair mitochondrial genome maintenance and interfere with apoptosis
458 by preventing of cytochrome-C release¹²⁹.

459

460 [H2] Final common pathway

461 The main mechanism involved in the pathogenesis of ALS is probably dependent on the initial cause,
462 although multiple mechanisms appear to explain the toxicity of one mutation and these mechanisms are
463 likely highly interlinked. This is clearly the case for *SOD1* mutations. In the case of C9orf72 repeat
464 expansions, multiple factors likely contribute to neuronal injury including toxic gains-of-function related
465 to RNA foci and the presence of dipeptide repeat proteins, but loss of the normal function of the
466 C9orf72 protein might also have a role.

467

468 Whatever the mechanisms of ALS, the end result is that the motor neuron cannot maintain its axonal
469 projections, leading to axonal retraction and denervation of the target cell. For lower motor neurons,
470 this results in denervation of the muscle, but for upper motor neurons results in the loss of proper
471 control of lower motor neurons, hypertonicity and weakness .. In addition, a loss of important neural
472 networks within motor and extra-motor domains is also apparent¹³⁰. [JOK As many of the proteins
473 encoded by genes that are implicated in ALS are ubiquitously expressed (Table 1), it is unclear why
474 motor neurons are the most susceptible to the hazardous effects of these mutations. The large size of
475 motor neurons, and in particular the need to maintain their long axonal projections, could make these

476 cells more sensitive to metabolic abnormalities than others, but other neuronal subtypes, such as
477 sensory neurons, have even larger axonal projections. Other factors that have been suggested to have a
478 role are the high expression of EphA4 and matrix metalloprotein 9 and the low expression of
479 osteopontin and insulin-like growth factor 2 by motor neurons, which might limit axonal sprouting and
480 repair. Of particular interest is that within the motor neuron pool, neurons that establish the fast
481 fatigable motor units die first in ALS^{131, 132}, but how this relates to the other vulnerability factors needs
482 to be clarified.

483

484 **[H1] Diagnosis, screening and prevention**

485

486 **[H2] Clinical presentations**

487 [OK The clinical hallmark of ALS is the involvement of both upper and lower motor neurons (Figure 1).
488 Patients can present with symptoms of an upper motor neuron predominant onset (that is, spasticity
489 and [Au: muscle] weakness) in whom lower motor neuron involvement only becomes evident at later
490 stages of disease.^{7, 133-136} [Au: cite fewer refs here? Keep these refs if possible]. Conversely, patients
491 can present with symptoms of lower motor neuron dysfunction, which includes fasciculations, cramps
492 and muscle wasting. Approximately one third of patients with ALS present with bulbar-onset disease,
493 which is characterized by progressive dysarthria, followed by difficulty swallowing and often with
494 associated emotional lability. Limb onset disease accounts for 60% of cases, is usually asymmetrical in
495 presentation and can first develop in the upper or lower limb. [Up to 5% of patients present with
496 respiratory problems and are often seen first in cardiology and pulmonology clinics prior to their referral
497 to neurology clinics¹³⁷. In these cases, patients can also present with unexplained weight loss. Evidence
498 suggests that some patients with ALS are hypermetabolic;¹³⁸ although the pathophysiology
499 underpinning this is not well understood. Cardiovascular risk factors (such as hyperlipidemia or obesity)
500 might attenuate risk¹³⁸, but do not alter clinical outcome¹³⁹. Patients can present with a pure motor
501 phenotype of ALS, and have normal cognition and behaviour, but some patients can present with a
502 purely cognitive or behavioural phenotype consistent with frontotemporal dementia(FTD)), or a mixed
503 phenotype with minor changes in executive impairment that progress over time. Frontotemporal
504 dementia is part of the presenting features of 13% of incident cases²⁻⁴ and approximately 30% of all
505 incident patients have some evidence of executive dysfunction at the time of first presentation^{3, 140}.
506 Depending on the population and the extent of cognitive testing performed, most studies have

507 suggested that up to 50% of patients can remain cognitively normal throughout the course of the
508 disease ³ Behavioural changes are common in patients with ALS, with apathy as the most prevalent
509 symptom. Detailed examination of behavioural changes in patients with ALS, using a disease specific
510 behavioural scale (that is, the Beaumont Behavioural Index) suggests that up to 40% of incident cases
511 have new behavioural changes that can be clustered into at least 5 different groups which roughly map
512 to known neuroanatomical networks and pathways ¹⁴¹. Substantial autonomic impairment (such as
513 cardiovascular, gastrointestinal and bladder dysfunction) does not occur in the majority of patients with
514 ALS.

515

516 **[H2] Diagnostic criteria**

517 No definitive test for the diagnosis of ALS is available, and diagnosis is a process of clinical investigation
518 to exclude other possible causes of the presenting symptoms, combined with evidence of disease
519 progression. However, the growing understanding of the extra-motor features of ALS, the presence of
520 phenotypic overlap with other neurodegenerative diseases and the identification of genetic and
521 pathological subtypes of ALS can confound accurate and timely diagnosis ⁷.

522

523 Diagnosing ALS is based on the El Escorial criteria (**Box 2**) ¹⁴². Diagnosis according to these criteria
524 requires a history of progressive weakness spreading within a region or to other regions (such as bulbar
525 regions (speech and swallowing), cervical regions (upper limbs), thoracic regions (chest wall and
526 abdominal muscles) or lumbar regions (lower limbs), with evidence of lower motor neuron (through the
527 presence of specific symptoms or evidence of denervation on electromyography) and upper motor
528 neuron (through the presence of specific symptoms and brisk deep tendon reflexes) involvement. In the
529 original criteria, diagnostic certainty ranged from Suspected ALS, (although this is no longer included in
530 the revised criteria), to Definite ALS (in which three body regions with mixed upper and lower motor
531 neuron findings were observed), which relates to the burden of disease. Neurophysiological findings
532 have been classified using the Awaji Criteria, which can enhance diagnostic and prognostic sensitivity ¹⁴³.
533 Variants of the El Escorial criteria are used in research settings and for the purposes of clinical trial
534 enrolment, but these criteria should not be routinely used in clinical practice for routine patient
535 management, as “possible ALS” described by the criteria is almost always ALS clinically ^{144, 145}. Genetic
536 testing can also be included in patients with a strong family history of ALS ¹⁴⁶ and clinical evidence of
537 disease, although this is not uniformly applied across centres ¹⁴⁷.

538

539 [H2] Cognitive and behavioural deficits

540 Standard diagnostic and stratification parameters for ALS do not yet include cognitive or behavioural
541 status, which is altered in up to 50% of cases (depending on the extent of cognitive and behavioural
542 assessment ²⁻⁴. Various screening tools have been designed to identify patients with ALS and cognitive
543 and behavioural changes in the clinic, such as the Edinburgh Cognitive and Behavioural ALS Screen
544 (ECAS), which is validated in several languages and is widely used, as it has a high degree of sensitivity
545 with lower degrees of specificity ¹⁴⁸. Individuals with abnormal ECAS scores (after adjustment to
546 population-based and educational norms) should be referred for a full neuropsychological evaluation ¹⁴⁹.
547 The detection of cognitive and behavioural changes is important for patients with ALS and their
548 caregivers, as executive impairment is associated with a more-rapid disease trajectory and behavioural
549 changes are associated with higher caregiver burden ¹⁵⁰.

550

551 [H2] Biomarkers

552 As ALS is a clinical syndrome with a heterogeneous phenotypic manifestation (and clinical course,
553 diagnostic and prognostic biomarkers are urgently required for the purposes of stratification. Levels of
554 neurofilament light chain (NfL) and phosphorylated neurofilament heavy chain in the cerebrospinal fluid
555 (CSF) can differentiate patients with ALS from those with mimics including cervical myelopathy,
556 multifocal motor neuropathy and inclusion body myositis, with moderate sensitivity and specificity [Au:
557 could you quote some values here? 'moderate' is quite an unspecific term], and levels have a
558 moderate correlation with disease progression [Au: how do they correlate? Do levels increase with
559 progression?] ¹⁵¹⁻¹⁵³. However, CSF neurofilament levels are not integrated into standard clinical
560 practice. Levels of NfL in serum are sensitive and specific for separating patients with ALS from healthy
561 controls, but data on comparison with ALS mimics are not available.

562

563 MRI studies of patients with ALS have shown corticospinal tract degeneration, with extensive
564 involvement within the frontal and temporal regions and basal ganglia, compared with controls
565 Evidence suggests that selective network vulnerability of structural and functional 'connectomes' could
566 drive the clinical manifestations of ALS, such as vulnerability of the corticospinal, orbitofrontal,
567 orbitotemporal and frontostriatal circuits ¹⁵⁴⁻¹⁵⁶. The presence of network disruption is also supported by
568 findings using spectral electroencephalogram ¹³⁰, and that patients with different degrees of cognitive
569 impairment show significantly different patterns of frontal lobe metabolic impairment on ¹⁸F
570 fluorodeoxyglucose PET imaging ¹⁵⁷. However, neither imaging nor spectral electroencephalogram can

571 provide individualised data that can be used as a reliable biomarker of upper motor neuron dysfunction
572 and of cognitive impairment in patients with ALS.

573

574 **[H2] Differential diagnosis**

575 The differential diagnosis in patients with pure bulbar pure upper motor neuron or pure lower motor
576 neuron presentations includes ALS variants, treatable ALS mimics and disorders with a more benign
577 prognosis^{134, 158}. Other forms of motor neuron disease include progressive muscular atrophy (that is, the
578 exclusive degeneration of lower motor neurons) and primary lateral sclerosis (that is, the exclusive
579 degeneration of upper motor neurons). Some patients with progressive muscular atrophy have
580 mutations in genes associated with ALS¹⁵⁹. Similarly, patients with primary lateral sclerosis may have a
581 family member with ALS and most autopsies of patients with primary lateral sclerosis show subtle signs
582 of ALS pathology in the lower motor neurons within the brain stem and spinal cord^{135, 158}.

583

584 Several conditions have similar initial clinical features as ALS and should be considered in the differential
585 diagnosis¹⁴⁵, including cervical myelopathy, multifocal motor neuropathy, myasthenia gravis, Lambert
586 Eaton myasthenic syndrome and inclusion body myositis. Features that should alert the clinician to a
587 possible mimic syndrome include presentation with of symmetrical findings; prominent extensor plantar
588 responses (which should raise suspicion of a cervical myelopathy) and the presence of sensory findings.
589 Although sensory symptoms are common in ALS, clinical evidence of sensory loss is atypical and should
590 trigger further investigations. In addition, the presence of substantial weakness in the absence of
591 wasting – which is common in multifocal motor neuropathy and myasthenia gravis – and the presence
592 of disproportionate involvement of quadriceps – which is common in inclusion body myositis – may
593 indicate the presence of an ALS mimic syndrome¹⁶⁰. As ALS is a progressive disease, failure of the
594 condition to progress over months should also trigger a re-investigation¹⁶¹.

595

596 **[H2] Staging and prognosis [**

597 Several different staging systems for ALS have been described (Figure 4)¹⁶²⁻¹⁶⁵, including the King's
598 system, which is based on the number of affected regions of the body, and the Milano-Torino system
599 (MITOS), which is based on a clinical scale. The prognosis of ALS is highly variable and prognostic
600 algorithms have been generated from population-based and clinical trial-based datasets^{166, 167}. Negative
601 prognostic indicators include bulbar or respiratory onset disease, the presence of executive impairment
602 or frontotemporal dementia and weight loss. Several biochemical markers of prognosis have been

603 reported including serum urate, serum creatinine, serum chloride, and increased serum and CSF
604 neurofilament levels ^{153, 168-170} [Au: please cite fewer refs here. Keep if possible?] Declining respiratory
605 function, measured by slow vital capacity, forced vital capacity and sniff nasal inspiratory pressure also
606 correlate with short survival ^{166, 167, 171, 172}. [Au: please limit the number of references here to 1-2. Keep
607 if possible?]

608

609 [H2] Clinical genetics and predictive testing

610 Consensus guidelines recommend genetic testing of probands with ALS who have a first or second
611 degree relative with ALS and/or frontotemporal dementia ^{19, 173}. As the genetic risk for ALS depends on
612 ancestral origin, the genetic testing should be contextualized; for example, *C9orf72* variants are rare in
613 Asia, whereas mutations in *OPTN* are more common in Asian than in European populations. Although
614 the potential benefits of genetic testing for patients are clear and could improve knowledge about their
615 disease, family planning and their possible inclusion in clinical trials, individuals also have a right not to
616 know their genetic status. Pre-symptomatic testing of family members of patients with ALS remains
617 controversial. Guidelines for genetic testing in research settings have been published ¹⁷⁴, but most
618 centres do not advocate routine testing outside of specialist centres ¹⁴⁷.

619 [H1] Management

620

621 ALS management is best achieved by a multidisciplinary approach to care, comprising a clinical team
622 with different specialities, including neurologists, psychologists, nutritionists, pulmonologists, physical
623 therapists, speech therapists and specialized nurses^{175, 176}. Multidisciplinary care increases survival ¹⁷⁷⁻¹⁷⁹,
624 reduces the number of hospital admissions and shortens hospital stays ¹⁷⁸ and increases quality of life of
625 patients with ALS ¹⁸⁰. This is likely related to the optimization of pharmacological and non-
626 pharmacological interventions and enhanced adherence to treatment guidelines.

627

628 [H2] Disease-modifying therapies

629 Although > 50 drugs with different mechanisms of action have been studied for the treatment of ALS,
630 only 2 compounds (riluzole and edaravone) have come to market. The negative results of these trials
631 might include clinical and pathogenetic heterogeneity in disease, and faults in trial design ¹⁸¹.

632 Riluzole was the first FDA approved treatment for ALS, and, although the mechanism of action is poorly
633 understood, is speculated to reduce glutamatergic neurotransmission, by blocking voltage-gated sodium

634 channels on presynaptic neurons. . In the original trial, Riluzole, increased 18-month survival of patients
635 by 3 months compared with placebo , but had no significant effect on muscle strength ¹⁸². Riluzole is a
636 relatively safe drug, although the most common adverse effects are an increase in liver enzymes and
637 asthenia (that is, a lack of energy) and some cases of fatal hepatic failure and pancreatitis have been
638 reported. In addition to the traditional tablet form of the drug, an oral suspension has been produced
639 and marketed in some countries for patients who are unable to swallow solid forms of the drug, owing
640 to severe dysphagia ¹⁸³. Edaravone, which is thought to act as an anti-oxidant agent has a beneficial
641 effect on progression in a highly selected cohort of patients with early onset and rapidly progressive
642 disease ¹⁸⁴, and accordingly, has been licensed by the US FDA but not by the European Medicines
643 Agency. Whether edaravone should be provided to all patients of ALS regardless of clinical
644 presentation is a matter of debate ¹⁸⁵

645

646 [H2] Symptomatic treatments

647 Other symptoms of ALS can be treated with pharmacological and non-pharmacological interventions.
648 Nuedexta may improve bulbar function ¹⁸⁶ and is available in the US but not in Europe. However, most
649 of these therapies for the symptoms of ALS have not been tested in randomized controlled trials and
650 are based on management of other diseases.

651

652 [H3] *Spasticity*. Spasticity is present in most patients with ALS, but only a small proportion need
653 treatment. The most commonly used drugs are baclofen and tizanidine (both of which are muscle
654 relaxants) although no randomized controlled trials in patients with ALS have been conducted. When
655 patients have severe, disabling spasticity, baclofen can be administered through an intrathecal pump. .
656 Cannabinoids have been approved for the treatment of spasticity in patients with multiple sclerosis and
657 are also used off-label or as a self-prescribed medication in patients with ALS¹⁸⁷.

658

659 [H3] *Sialorrhoea*. Sialorrhoea (that is hypersalivation), causing drooling and the pooling of saliva within
660 the oral cavity is one of the most disturbing symptoms in patients with ALS, and is more commonly
661 observed in patients with bulbar-onset disease and during late-stages. Sialorrhoea can be treated [with
662 anticholinergic drugs, such as scopolamine, atropine, hyoscine, amitriptyline and glycopyrrolate.
663 Adverse effects associated with the use of anti-cholinergics include blurred vision, mouth dryness and
664 constipation, and these drugs are contraindicated in patients with heart conduction disturbances and
665 prostatic hypertrophy. In patients in whom pharmacological treatments are ineffective or are not

666 indicated, botulinum toxin A or B injections into the salivary glands can used to treat sialorrhoea^{188, 189}.
667 Salivary gland irradiation has been also proposed ¹⁹⁰.

668
669 **[H3] Pain.** Pain is reported in 15–85% of patients with ALS, depending on the duration of the disease
670 and the setting of the study, and is more frequently of nociceptive than of neuropathic origin. ¹⁹¹
671 Depending of the type of pain, pharmacological treatments include gabapentin, pregabalin and tricyclic
672 antidepressants (for neuropathic pain), and NSAIDs, opioids and cannabis for nociceptive pain), but no
673 randomized controlled trials evaluating treatment of pain in patients with ALS are available. Nociceptive
674 pain can be also treated with intra-joint injections of lidocaine or steroids, and physical therapy,
675 including assistive range-of-motion exercises.

676
677 **[H3] Muscle cramps.** Muscle cramps are the main cause of pain in about one-quarter of patients with
678 ALS (mainly patients with the spinal onset disease) and are caused by the instability of motor units ¹⁹².
679 Commonly used treatments for muscle cramps include quinine sulphate, levetiracetam and mexiletine.
680 Indeed, mexiletine has been shown to induce a significant dose-dependent reduction in muscle cramps
681 in a phase 2 randomized controlled trial in patients with ALS ¹⁹³. Of note, the FDA has advised against the
682 use of quinine sulphate for the treatment of cramps because it can cause cardiac arrhythmias,
683 bradycardia and prolongation of Q-T interval.

684
685 **[H3] Dysphagia**
686 Dysphagia is reported by about 60% of patients with spinal onset ALS, within two years from onset and
687 100 % of patients with bulbar-onset disease ¹⁹⁴. Several strategies can be implemented to reduce the
688 effects of dysphagia in patients, including dietary changes such as modification of the consistency of the
689 diet, the use of fluid thickeners and prescription of high-protein and high-caloric supplements,
690 swallowing facilitating manoeuvres and exercises (such as oral and pharyngeal range-of-motion
691 exercises, head postures and the technique of supraglottic swallow). An option for severe difficulties
692 with swallowing is to use enteral nutrition via the insertion of a gastrostomy tube. No established
693 criteria are available for the initiation of enteral nutrition in patients with ALS, but weight loss of >5% or
694 unsafe swallowing are generally considered to be red flags that should prompt intervention. ¹⁷⁵. Several
695 techniques are available for minimally invasive tube insertion and open surgery is not recommended ¹⁹⁵,
696 ¹⁹⁶. Parenteral nutrition provided through a central venous catheter is an alternative to enteral nutrition

697 in patients with ALS who have severe respiratory insufficiency for whom PEG [Au: Percutaneous
698 endoscopic gastrostomy] or RIG [Au: Radiologically Inserted Gastrostomy?] are contraindicated ^{197, 198}.

699
700 **[H3] Dysarthria.** Dysarthria is the presenting symptom in 30% of patients and is found in > 80% of
701 patients during the course of the disease, up to complete anarthria. Speech therapy can delay the
702 progression of dysarthria and augmentative-alternative communication [techniques such as
703 customised software are the treatment of choice and can enhance quality of life in the most advanced
704 phases of ALS ¹⁹⁹. Communication techniques based on brain-computer interfaces (BEST LEAVE THIS IN
705 PLACE] have been developed, but their use in the clinical setting is still very limited as their effectiveness
706 has not been definitely demonstrated ²⁰⁰. Moreover, the use of brain-computer interfaces might be
707 hindered by patients' cognitive dysfunction or old age ²⁰¹.

708
709 **[H3] Deep venous thrombosis.** Patients with ALS have leg weakness and reduced mobility, which can
710 increase the risk of symptomatic and asymptomatic deep venous thrombosis (DVT). The annual
711 incidence of DVT in patients with ALS ranges from 2.7 to 11.2% ^{202, 203}. In the absence of specific studies
712 on the prevention and treatment of DVT in ALS general guidelines should be applied, including the use
713 of compression stockings and anticoagulation therapies

714
715 **[H3] Mood alterations.** Depression is a relatively common symptom in patients with ALS and has been
716 found in up to 50% of patients. Depression is generally treated with selective serotonin reuptake
717 inhibitors (SSRI) or tricyclic antidepressants. Pseudobulbar affect (that is, episodes of uncontrollable
718 crying or laughing) is a distressing symptom that has been reported in up to 50% of patients with ALS ²⁰⁴
719 [and can be treated with SSRIs and tricyclic antidepressants, although this is off-label.
720 Dextromethorphan (a sigma-1-receptor agonist and an uncompetitive NMDA receptor antagonist) and
721 low-dose quinidine were effective in reducing symptoms of pseudobulbar affect by 50% in patients with
722 ALS or those with multiple sclerosis ²⁰⁵.

723
724 **[H3] Cognitive impairment.** Cognitive impairment, in particular frontotemporal dementia, is one of the
725 most disabling symptoms in patients with ALS. No pharmacological therapy is effective for the treatment
726 of frontotemporal dementia, and acetylcholinesterase inhibitors, which are used for Alzheimer disease,
727 are not effective. However, some symptoms of frontotemporal dementia can be pharmacologically
728 treated; evidence suggests SSRIs might help to control the loss of inhibition, overeating and compulsive

729 behaviour, and antipsychotics can be used to reduce restlessness. Education of caregivers about the
730 symptoms of frontotemporal dementia can be useful to help the management of patients at home ²⁰⁶.

731

732 **[H3] Respiratory insufficiency.**

733 The vast majority of patients with ALS die from respiratory failure. Non-invasive ventilation is the
734 symptomatic treatment of choice for respiratory failure, and provides significantly longer survival
735 compared to those who do not use NIV (316 vs 229 days) and improves quality of life ^{207 208}. Accepted
736 criteria for starting non-invasive ventilation are symptoms or signs related to respiratory muscle
737 weakness (such as, dyspnoea, orthopnoea or daytime fatigue), a vital capacity of < 80% of predicted
738 levels, PaCO₂ > 45 mmHg, SaO₂ < 90% during ≥ 5% of sleep time ¹⁷⁶. One distressing symptom that is
739 related to respiratory muscle weakness in patients with ALS is the inability to cough effectively. This can
740 be controlled by the use of cough-assist devices, such as the breath-stacking technique or a mechanical
741 insufflator-exsufflator ²⁰⁹.

742

743 **[H2] End of Life Management**

744 The end of life phase for patients with ALS can be difficult to define, although recent staging systems
745 including KINGS and MITOS [are useful in this regard. The end of life period can be particularly
746 challenging and is characterized by substantial mobility, communication and, in some cases, cognitive
747 difficulties. An early discussion of end of life issues will ensure that patients can communicate their
748 wishes before the onset of substantial communication and cognitive difficulties, can avoid unwanted
749 interventions or procedures, and can provide time for reflection and the integration of choices within
750 the patient's priorities and life plans. In addition, such discussions can alleviate patient's fears, especially
751 around fatally choking. The attitudes, culture and personal values of patients, caregivers and health care
752 providers can influence the timing and content of end of life discussions, decision-making and the
753 patient's acceptance or refusal of interventions and treatment options. Some patients with ALS might
754 choose life-prolonging measures, but others might contemplate life-limiting procedures; the availability
755 and utilization of different interventions and technologies, such as assisted death and tracheostomy,
756 varies across centres and between countries. Advance care directives are recognized as important at
757 end of life in ALS, and provide patients with the option to exercise autonomy regarding preferred end of
758 life management strategies. Formal care at the end of life should aim to maximize quality of life of both
759 the patient and caregiver and, where possible, incorporate appropriate multidisciplinary care including
760 palliative care options.

761

762 **[H1] Quality of Life**

763

764 Much of the effort of physicians and other health care providers is focused on optimizing the quality of
765 life (QOL) of patients with ALS. **[Au: green text moved to here for flow. OK]** The choice of a specific QOL
766 instrument is complex, and has been reviewed ²¹⁰. The perception by individuals with ALS of their QOL
767 takes shape at the time of disclosing the diagnosis, and can be influenced by the manner in which they
768 are informed . Well-recognized systematic approaches are available, such as the SPIKES approach, that
769 can convey the diagnosis in a less distressing manner and can leave the patient feeling hopeful and
770 supported ²¹¹⁻²¹³.

771

772 **[Au: I've deleted the text stating healthy individuals think the QOL of patients with an illness is lower,**
773 **and that HRQOL is not the same as QOL, as this isn't needed here]** Health-related QOL (HRQOL) refers
774 to an individual's perception of their QOL as a function of physical and mental well-being ²¹⁴; measures
775 of HRQOL generally decline as ALS advances ^{210, 215}. In contrast, OQOL **[Au: overall QOL?]** encompasses
776 medical factors and a wide variety of non-medical factors, such as family, friends, occupation, financial
777 well-being, spirituality or religion and existential concerns ²¹⁶. Patients with ALS often view their OQOL
778 as good, which persists despite the progression of physical disability ^{217, 218}. This might be explained by a
779 'response shift' (also called a frame shift or well-being paradox), whereby the individual recalibrates the
780 factors that are deemed meaningful to maintenance of their QOL. Most commonly, this centres around
781 the decreased importance of physical activities and the greater role of interactive and existential factors,
782 such as social relationships and spirituality ²¹⁹⁻²²¹. However, not all patients maintain a high QOL with
783 advancing illness. Many factors can negatively affect QOL in patients with ALS, identifying potential
784 areas for intervention, although other factors can improve QOL (**Figure 5**) ^{180, 207, 214, 222-228} **[Au: please**
785 **restrict the number of references here to 1-2]** .

786

787 Despite good QOL of patients with ALS in aggregate **[Au: '..a good QOL of most patients with ALS?'**
788 **edited for brevity]** , psychological health is, on average, poorer than that of the population as a whole
789 ²²⁹. This has substantial implications as depression, hopelessness and anxiety all associated with a poor
790 QOL. **[Au: I've moved the green text to here from earlier on for flow]** Psychological interventions have

791 been less well studied [Au: than what? please add a comparator here] ²³⁰ and this warrants further
792 attention.

793
794

795 QOL can affect the wishes for care of patients with ALS at the end of their lives. [Au: edited for flow] In a
796 study from the Netherlands, 16.8% of patients with ALS chose physician-assisted death, common
797 reasons for which were hopelessness, loss of dignity, dependency on others and fatigue ²¹⁵. Similarly,
798 the decision for euthanasia in patients with ALS in Washington State was driven by loss of autonomy,
799 participation in enjoyable activities and dignity ²¹⁶. These studies do not prove poor QOL in these
800 individuals, but they do raise this as a concern. The quality of death in patients with ALS has been
801 studied less comprehensively [Au: than QOL?] . Death was perceived as peaceful by 88% to 98% of
802 caregivers in Germany, the United Kingdom, the United States and Canada ^{217, 231}. However, caution must
803 be used in interpreting grouped statistics. Incompletely relieved symptoms such as coughing from [Au:
804 excess?] mucus, restlessness, anxiety and muscle [Au: added muscle here] cramps resulted in moderate
805 to severe suffering in the last 24 hours of life in 8 of 171 patients ²¹⁷.

806

807 **[H1] Outlook**

808

809 The knowledge of ALS and the care of patients with this condition have increased substantially in recent
810 years, and this trend is likely to continue. 25 years ago, riluzole had not been enrolled in a clinical trial,
811 non-invasive ventilation was not in routine use for patients, the pathological basis of ALS as a TDP-43
812 proteinopathy was unknown and no genetic causes for ALS had been identified. In addition, the El
813 Escorial criteria were not developed, no simple ALS functional scale existed, multidisciplinary care was in
814 its infancy and the recognition of cognitive change in patients with ALS was limited, and the link with
815 frontotemporal dementia was not made. What will be different in another 25 years, and how much of
816 what we regard as self-evident now, will be overturned, is tempting to consider.

817

818 **[H2] Epidemiology**

819 We can expect that the numbers of patients with ALS will increase in the future ²¹⁸, and that population
820 differences in incidence and phenotype will be recognized. Better multidisciplinary care and an
821 improved understanding of interventions means that a patient diagnosed with ALS can expect to live

822 longer than previously. In addition, the development of new drugs to improve respiratory function or
823 directly affect the disease process are expected to improve survival.

824

825 **[H2] Pathophysiology**

826 A big barrier to effective ALS treatments is due to our lack of knowledge of the pathological pathways
827 that lead to the disease, and how they affect the overall integrity of brain networks. Our understanding
828 of ALS is improving, including contextualizing the role of TDP-43, the importance of RNA processing for
829 motor neurons, the spread of disease and the molecular cascades that lead to neuronal death. The
830 development of new cellular and animal models of ALS is beginning to lead to improvements in our
831 understanding of the disease , both because the molecular pathways can be dissected more easily, and
832 because the models can be used to more effectively to identify drugs worth enrolling into human trials.
833 These insights are the result of genetic findings, which have led to experiments aiming to understand
834 how loss-of normal protein function and gain-of toxic function cause ALS. As the number of genes
835 implicated in ALS increases and laboratory models improve, we can expect to design new drugs to
836 intervene in those pathways.

837

838 Indeed, our understanding of the genetics of ALS has transformed over the last 25 years, with the
839 finding that both familial and sporadic ALS have a genetic basis and the number of validated involved
840 genes steadily increasing. These findings are in large part due to the willingness of the ALS research
841 community to collaborate, which has generated the huge datasets required for credible gene discovery.
842 The finding that the genetic architecture of ALS includes an important role for rare genetic variation has
843 consequences for the likelihood that gene therapy could be effective in this disease. Indeed, as rare
844 variants are more likely to have a large effect on the risk of disease and can be directly manipulated by
845 gene therapy, we can expect to see precision medicine spearheaded by targeted gene therapies.

846 The relationship between ALS and cognitive, cerebellar, autonomic and other non-motor changes is an
847 area of research that is expected to grow. One consequence of this research is that ALS is probably
848 primarily a disease of neural networks, which is defined by the involvement of upper and lower motor
849 neurons, but that can also affect other cell populations and neuronal networks. We can also expect an
850 increased understanding of the role of inflammation in ALS, both in triggering disease and influencing
851 the rate of progression.

852

853 **[H2] Diagnosis and prognosis**

854 The use of biomarkers for ALS has been investigated for many years, although our understanding has
855 only recently matured for research to yield useful results. Diagnostic biomarkers would be useful for
856 individuals with an atypical or complicated presentation, biomarkers for prognosis would be useful for
857 planning treatment options, and biomarkers of disease progression would be useful for monitoring
858 response to existing therapies or potential new therapies in a clinical trial. New signal analysis based
859 technologies will become available as biomarkers that can image the living human brain ¹³⁹.

860

861 **[H2] Management**

862

863 **[H3] Clinical Trials**

864 The validity of pre-clinical studies should be evaluated rigorously by evidence-based analyses, and
865 translation of new therapies to humans should be undertaken only if findings are robust and
866 reproducible. Moreover, as ALS is a human disease, testing safe candidate compounds without prior
867 testing in animal models could be undertaken. In this instance, careful phase I and 2 studies including
868 detailed pharmacokinetic studies with extensive dose-finding and toxicity studies will be needed. As
869 some previous ALS clinical trials failed due to faulty trial design, a detailed correlative analysis of drug
870 levels in serum and CSF should be undertaken in early phases trials, and all trials should include a
871 biomarker readout to confirm that the drug is reaching its target. **[Au: what do you mean by ‘target
872 engagement’? Please clarify]** .

873

874 The failure of previous clinical trials for ALS could also result from disease heterogeneity. Methods to
875 stratify patients that have a shared pathobiology are urgently required, and in the absence of this, pre-
876 specified, post-hoc analyses should be used to identify potential responder groups. This is exemplified
877 by a recent successful Phase 3 trial of edaravone ¹⁸⁴, as recruitment to this trial was based on a post-hoc
878 analysis to identify possible responders, and stringent recruitment criteria were used to provide a
879 clinically homogeneous population that were likely to respond to treatment.

880

881 **[H3] New Drugs**

882 An extensive pipeline of new therapeutics for ALS is available, and some of these drugs target known
883 mutations and pathogenetic pathways. Symptomatic therapies including tirazemtiv based on
884 improving respiratory function in patients with ALS are currently in Phase 3 trials and exciting Phase I
885 trials assessing the use of antisense oligonucleotides in *SOD1* and *C9orf72* [related ALS are underway. In

886 the future, treatments are likely to be targeted at specific subgroups of patients and biomarkers that are
887 personalized to the individual disease subtype and have been developed from patient subcohorts that
888 have been extensively phenotyped and stratified using genomics, transcriptomics, metabolomics and
889 advanced imaging and signal analysis.

890

891

892

893 **Display items**

894

895 **Box 1. Mechanisms of SOD1 toxicity in cellular and rodent models [Au: Title OK? I have deleted this**
896 **figure as this is very repetitive with figure 3 and made the figure legend into a box. We can illustrate**
897 **the mechanisms included in this figure (prion-like seeding, etc.) in figure 3 if you wish, although I don't**
898 **think this is necessary as this is nicely described in this box] .**

899 Transgenic mice with mutations in *SOD1* (encoding superoxide dismutase, SOD1 [Au: I've added the
900 **gene product here as this is useful to note**]) can be used to study ALS pathophysiology. These mice
901 over-express mutant SOD1 and many have an aggressive disease course over approximately 80-90 days.
902 However, they display quite well clinical and pathological features similar to human ALS.

903 xx [Au: Please add 1-2 sentences here discussing the phenotype of these mice – **DONE do they show**
904 **sensorimotor dysfunction, for example? Reduced bowel and bladder function? I have adapted the**
905 **table that was in figure 1 into continuous prose (highlighted in yellow). I've also added a reference to**
906 **the NRNeuroscience review (ref 242) - please check this carefully OK** . **SOD1 mutations can drive**
907 **neurotoxicity in several ways, including protein misfolding [Au: presumably the misfolded protein here**
908 **is SOD1? BUT ALSO AGGREGATES OF NEUROFILAMENT PROTEINS], proteasome impairment,**
909 **excitotoxicity, oxidative stress, ER stress, impaired axonal transport, axonopathy, inflammation, altered**
910 **RNA processing and mitochondrial dysfunction.** ²³² Other mechanisms of SOD1-related neurotoxicity
911 have recently emerged and have gained interest. SOD1 can acts as a transcription factor for genes
912 involved in **resistance to oxidative stress PLEASE LEAVE AS IS. '?**] and repair of oxidative damage [Au:
913 **DNA repair?**] ²³³. RNA oxidation is emerging as a prominent pathological outcome of generalized
914 oxidative stress in the cell with increasing importance in neurodegeneration [Au: **does RNA oxidation**
915 **occur in SOD1 transgenic mice?YES**] . [Au: **what do you mean by this? Do you mean astrocytes and**
916 **oligodendrocytes with mutations in SOD1?**] Astrocytes and oligodendrocytes reprogrammed from
917 fibroblasts of patient with SOD1 mutations have been shown to induce hyperexcitability and cell death
918 [Au: **cell death of the motor neurons only, or also of astrocytes and oligos?**] in healthy control motor
919 neurons. Glial toxicity is mediated through both contact (lactate independent) and soluble mechanisms
920 and is rescued by SOD1 knockdown using short hairpin RNA in glia derived from patients with AOS1-
921 related familial ALS, but also in glia derived from patients with sporadic ALS without SOD1 mutation ¹¹³.
922 Wild-type and mutant SOD1 proteins form insoluble **intraneuronal** fibrils, which aggregate with
923 increased propensity in the mutant form. A prion-like transmission of mutant SOD1 fibrils can seed wild-
924 type SOD1 protein aggregation in neighbouring neurons and propagate neuronal injury²³⁴.

925 **Box 2. El Escorial criteria [Au: please add these criteria here] .**

926

927

928 [Au: If figures/boxes/tables have been published before, we need you to complete the 'Third party
929 right' table so we can apply for permission with the original publisher on your behalf. Please do note
930 that permission is not always granted, so the sooner we can get this process started, the better.
931 Where possible, please provide original images. If figures have not been published before, but do not
932 belong to you (but for example to a colleague), we need them to complete a license to publish. Please
933 get in touch so that I can send you the required paperwork. Please find more information on the
934 permissions in the accompanying email.]

935 **Figure 1. Clinical manifestations of ALS [Au: Note this has been renumbered as figure 1, so the**
936 **symptoms of ALS are introduced early on in the manuscript] .**

937 Although motor manifestations such as muscle weakness and difficulty swallowing are the main clinical
938 manifestations of amyotrophic lateral sclerosis, up to half of patients have non-motor symptoms, such
939 as cognitive defects.

940 **Figure 2. Histopathology of ALS.**

941 a) [Au: I've edited this for brevity and house style, please check this carefully] Normal localization of
942 TDP-43 in the nucleus (black arrow head), and aberrant localisation in a diseased neuron with loss of
943 nuclear expression and a 'skein-like' inclusion in the cytoplasm (black arrow). b) [Au: I've deleted the
944 H&E image as this isn't needed here OK] Normal motor neuron (black arrow) and a hyaline
945 conglomerate inclusion that stains for SMI31 (black arrow head) in a patient with ALS caused by a *SOD1*
946 mutation. c) TDP-43-negative, p62 positive [OK] dipeptide repeat inclusions with a 'stellate' morphology
947 in the pyramidal cells of CA4 (black arrow) and granule cells of the dentate fascia (black arrow head) in
948 the hippocampus of a patient with ALS caused by a mutation in *C9orf72*. d) The spinal cord ventral horn
949 of a patient with ALS and a [Au: I've deleted normal, healthy is sufficient here] healthy individual (e)
950 showing a depleted numbers of motor neurons in ALS (arrows). F) CD68 (a microglial marker)
951 immunohistochemistry shows marked microglial [Au: I'm not sure what you mean by this, please clarify
952 DONE] reactivity in the lateral tracts (black arrow) and ventral [Au: I've changed anterior to ventral,
953 OK? OK] horns (black arrowhead), with no labelling [Au:OK? OK] in the dorsal columns (white arrow).
954
955

956 **Figure 3. Pathophysiology of ALS [Au: figure title OK?] .**

957 Mutations in several amyotrophic lateral sclerosis (ALS) causative genes [Au: do you mean mutations in
958 these genes? reworded] can exert motor neuronal injury through more than one pathophysiological
959 mechanism, although these mechanisms are often interlinked. *SOD1* is the longest studied gene
960 implicated in ALS and has been linked to the most pathophysiological mechanisms, although the effects
961 of mutations in *ALS3* and *ALS7* are still unknown. Aberrant RNA metabolism and impaired protein
962 homeostasis are predominant factors linking multiple ALS causative genes [Au: what do you mean by
963 'most causative genes'? Please clarify DONE] to neuronal injury. Mitochondrial dysfunction can arise
964 from a mutation in *CHCHD10* and from secondary respiratory chain deficiencies that arise from protein
965 aggregates generated in the presence of other ALS genetic mutations [Au: can we just say 'arise from
966 protein aggregates' here? REWORDED]. Both cases lead to an increase in oxidative stress, which puts
967 further stress on an already impaired protein homeostasis system. Other mechanisms of ALS can directly

968 alter neuronal function (such as nuclear export, impaired DNA repair, dysregulated vesicle transport and
969 axon dysfunction) and the function of non-neuronal glial cells. [Au: I've added in the highlighted text so
970 all pathologies illustrated in the figure are mentioned in the legend, OK? Please feel free to edit this
971 OK] The interplay of mechanisms is indicated by arrows.

972

973 **Figure 4. Staging systems for ALS.**

974 [Au: green text moved here from the main manuscript text for flow, and edited for brevity] The King's
975 staging system is based on the number of body regions affected by ALS and the presence of respiratory
976 or nutritional failure ¹⁶². The Milano-Torino staging [Au: definition of MITOS OK?] (MITOS) system is
977 based on the ALS functional rating scale (ALSFRS-R), a 48 point clinical measurement scale that records
978 changes in bulbar, gross motor, fine motor and respiratory parameters [Au: can we edit this to
979 '...changes in four functional domains: bulbar, gross motor, fine motor and respiratory'? If not, what
980 are the functional domains that are referred to in the figure?] ¹⁶³. These staging systems do not
981 incorporate cognitive or behavioural changes. The King's staging system is sensitive to early changes in
982 ALS, but the sensitivity of the MITOS scale is greater in the later stages of disease ^{164, 165}.

983

984

985 **Figure 5. Factors affecting QOL in patients with ALS.**

986 Several factors that positively or negatively affect overall quality of life (QOL) and health-related QOL
987 (HRQOL) have been identified in patients with amyotrophic lateral sclerosis. These factors include motor
988 symptoms, psychological symptoms and therapeutic interventions. AAC, augmentative and assistive
989 communication; VC, verbal communication.

990

991

992 Table 1. Genes implicated in ALS.

Gene locus	Gene (protein) [Au: I've reformatted this so the gene name is first and the protein name following in brackets]	Inheritance	Implicated disease mechanisms	References
ALS1	<i>SOD1</i> (Superoxide dismutase 1)	AD/AR	Oxidative stress	235, 236
ALS2	<i>ALS2</i> (Alsin)	AR	Endosomal trafficking	237, 238
ALS3	Unknown	AD	Unknown	239
ALS4	<i>SETX</i> (Senataxin)	AD	RNA metabolism	240
ALS5	Unknown	AR	DNA damage repair, axon growth	241
ALS6	<i>FUS/TLS</i> (Fused in sarcoma/translated in liposarcoma)	AD/AR	RNA metabolism	242, 243
ALS7	Unknown	AD	Unknown	244
ALS8	<i>VAPB</i> (Vesicle associated membrane protein (<i>VAMP</i>) – associated protein B)[Au: should this be split up into two rows? Have <i>VAMP</i> and <i>VAPB</i> both been implicated in ALS?]	AD	ER stress	42
ALS9	<i>ANG</i> (Angiogenin)	AD	RNA metabolism	245
ALS10	<i>TARDBP</i> (TAR DNA binding protein)	AD	RNA metabolism	27, 246
ALS11	<i>FIG4</i> (Polyphosphoinositide 5-phosphatase [Au: protein name OK?][CORRECTED])	AD	Endosomal trafficking	247
ALS12	<i>OPTN</i> (Optineurin)	AD/AR	Autophagy	248
ALS13	<i>ATXN2</i> (Ataxin 2)	AD	RNA metabolism	249
ALS14	<i>VCP</i> (Valosin-containing protein)	AD	Autophagy	36
ALS15	<i>UBQLN2</i> (Ubiquilin 2)	XD	UPS, autophagy	34
ALS16	<i>SIGMAR1</i> (Sigma non-opioid intracellular receptor 1)	AD	UPS, autophagy	250, 251
ALS17	<i>CHMP2B</i> (Charged multivesicular body protein 2B)	AD	Endosomal trafficking	252
ALS18	<i>PFN1</i> (Profilin 1)	AD	Cytoskeleton	97
ALS19	<i>ERBB4</i> (V-erb-b2 avian	AD	Neuronal	253

	erythroblastic leukaemia viral oncogene homolog 4)		development	
ALS20	<i>HNRNPA1</i> (Heterogeneous nuclear ribonucleoprotein A1)	AD	RNA metabolism	82
ALS21	<i>MATR3</i> (Matrin 3)	AD	RNA metabolism	83
ALS22	<i>TUBA4A</i> (Tubulin alpha-4A) [Au:protein name OK? corrected])	AD	Cytoskeleton	102
ALS- FTD1	<i>C9orf72</i> (Chromosome 9 open reading frame 72)	AD	RNA metabolism, autophagy	5, 6
ALS- FTD2	<i>CHCHD10</i> (Coiled-coil-helix- coiled-coil-helix domain containing 10)	AD	Mitochondrial maintenance	255
ALS- FTD3	<i>SQSTM1</i> (Sequestosome 1)	AD	Autophagy	256
ALS- FTD4	TBK1 (TANK-binding kinase 1)			53, 54

993 AD, autosomal dominant; AR, autosomal recessive; XD, X-linked dominant

994

995

996

997 References [Au: Please select ~10 references of particular importance and give a single sentence for
998 each stating why the paper is important. Please copy the whole reference (not just the number, since
999 this will inevitably change) to a separate list and provide the justifying sentence after it.]

1000

1001

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