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Purpose of review
Amyotrophic lateral sclerosis (ALS), like other neurodegenerative diseases, remains incurable, but gene mutations linked to ALS are providing clues as to how to target therapies. It is important for researchers to keep abreast of the rapid influx of new data in ALS, and we aim to summarize the major genetic advances made in the field over the past 2 years.

Recent findings
Significant variation in seven genes has recently been found in ALS: TBK1, CCNF, GLE1, MATR3, TUBA4A, CHCHD10 and NEK1. These have mostly been identified through large exome screening studies, though traditional linkage approaches and candidate gene screening remain important. We briefly update C9orf72 research, noting in particular the development of reagents to better understand the normal role of C9orf72 protein.

Summary
Striking advances in our understanding of the genetic heterogeneity of ALS continue to be made, year on year. These implicate proteostasis, RNA export, nuclear transport, the cytoskeleton, mitochondrial function, the cell cycle and DNA repair. Functional studies to integrate these hits are needed. By building a web of knowledge with interlinked genes and mechanisms, it is hoped we can better understand ALS and work toward effective therapies.

Keywords
amyotrophic lateral sclerosis, C9orf72, genetics, RNA processing, TDP-43

INTRODUCTION
Amyotrophic lateral sclerosis (ALS) is the most common motor neuron disease of adults and is invariably fatal, often within 5 years of disease onset. Importantly, ALS is on a clinical, genetic and pathological spectrum with frontotemporal dementia (FTD). Riluzole, a glutamate modulator and the only disease-modifying agent widely used for ALS, has only a modest effect on survival. Knockdown gene therapies hold promise for those individuals with ALS-causing gene mutations in SOD1 and C9orf72, and clinical trials are underway. However, the majority of ALS is not caused by mutations in these or other identified genes. These apparently sporadic amyotrophic lateral sclerosis (sALS) cases account for around 90% of all ALS, whereas familial amyotrophic lateral sclerosis (fALS), usually dominantly inherited, accounts for the remainder.

The distinction between sALS and fALS, though practical, is increasingly recognized to be artificial, but what is important is that sALS and fALS are often indistinguishable. This suggests that ALS-linked genes in rare fALS cases can inform our understanding of sALS. With this in mind, the dizzying pace of gene discovery in ALS in the past 10 years is to be welcomed, and advances in exome and genome sequencing strategies have been invaluable in this task. Identifying genetic risk factors and modifiers that influence ALS risk in sALS is also adding to our understanding of the mechanisms underlying ALS as a whole.

In this review, we will highlight the latest developments in the genetics of ALS focussing on genes identified in the past 2 years. Refer to Table 1 for a
**KEY POINTS**

- The exponential rise in genetic discovery in ALS continues unabated and will continue to do so for some time.
- New genes implicate proteostasis, RNA export, nuclear transport, the cytoskeleton, mitochondrial function, the cell cycle and DNA repair.
- Novel and sophisticated approaches are needed to integrate and model this new knowledge to identify therapeutic targets.

A summary of the genes primarily discussed in this article. We will then highlight recent advances in our understanding of C9orf72, still the hottest topic in the field. Readers are encouraged to refer to the Amyotrophic Lateral Sclerosis Online Genetics Database website (http://alsod.iop.kcl.ac.uk/) for a comprehensive list of other ALS genes or Table 2 for a summary.

**NEW ALS GENES**

**TBK1**

Perhaps the most significant recent genetic discovery in ALS since C9orf72 has been the identification of mutations in the TBK1 gene (encoding TANK binding kinase 1). One study examined 2869 ALS patients and 6405 controls and found nearly 50 variants throughout TBK1 in ALS patients, including splice-site, loss-of-function (LoF) and missense mutations [1]. Another study used whole exome sequencing and linkage analysis to interrogate 252 fALS cases that were negative for SOD1 and C9orf72 mutation [2]. In this study, eight predicted LoF mutations in TBK1 were found in 13 families (a mixture of truncating and splice-site mutations). The mean age of onset of fALS was 60 years of age, 15% with bulbar onset and with 50% of cases demonstrating cognitive impairment, often frank FTD. LoF mutations were not a contributor to sALS, but nine missense variants were found in both fALS and sALS.

The TBK1 LoF mutations would be predicted to truncate TBK1, but seven of the eight variants identified by Freischmidt et al. produced little or no detectable transcripts or protein products, suggesting that haploinsufficiency of TBK1 is responsible for neurodegeneration. The most 3’ truncation mutation [c.2138+2T>C (p.690–713del)] generated a protein product lacking 22 amino acids from the C-terminal coiled-coil domain 2, which appeared to abolish its ability to bind optineurin (OPTN) in vitro. Several of the missense mutations also abrogated the ability of TBK1 to bind OPTN and/or phosphorylate IRF3. It remains unclear how loss of TBK1 function causes neurodegeneration, but aberrant cellular clearance is a possibility given that both OPTN and p62, another ALS-linked protein that TBK1 interacts with, have roles in autophagy [3,4].

TBK1 interacts with OPTN, which binds to ubiquitin chains on mitochondria. This recruits TBK1 to mitochondria and promotes its kinase activation. TBK1 can then phosphorylate the UBAN domain of OPTN, expanding its ability to bind ubiquitin chains. This positive feedback mechanism promotes recruitment and retention of TBK1/OPTN to damaged mitochondria [5]. Further evidence for a role of TBK1/OPTN in the removal of damaged mitochondria involves the PINK1-PARKIN pathway and mitophagy through the polyubiquitination of defective mitochondria. TBK1 physically associates with autophagy adapters OPTN, NDP52 and p62, strengthening the link between ALS and selective autophagy [6], and TBK1 is able to phosphorylate p62 at S403 in the early period of mitochondrial depolarization [7].

Further TBK1 variants have recently been found in European, Taiwanese and Chinese patients with ALS and/or FTD [8–11]. Strikingly, up to 10.8% of cases of patients presenting with ALS–FTD in a French patient cohort were found to carry LoF mutations in TBK1, suggesting that patients...
Presenting with FTD and ALS who do not have C9orf72 mutations should be screened for TBK1 mutations [12]. In another study of 104 pathologically confirmed FTLD-TDP patients who were negative for C9orf72 and GRN mutations, 4.8% of patients had mutations in TBK1, OPTN or both [13]. These mutations were associated with reduced expression of TBK1 and/or OPTN mRNA and protein in cerebellar tissue. This study implicates the OPTN/TBK1 pathway in FTD with TDP-43 inclusions [13]. Pathological analysis of brain and spinal cord from ALS patients with TBK1 mutations also identified TDP-43 positive inclusions [11,14]. Thus, TBK1-ALS is yet another ‘TDP-43 proteinopathy’.

**CCNF**

Linkage analysis followed by whole-exome sequencing of a large ALS–FTD kindred of British ancestry

<table>
<thead>
<tr>
<th>ALS type</th>
<th>Gene ID</th>
<th>Description</th>
<th>Location</th>
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</thead>
<tbody>
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<td>SOD1</td>
<td>Superoxide dismutase 1, soluble</td>
<td>21q22.11</td>
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<tr>
<td>ALS 2</td>
<td>ALS2</td>
<td>ALS2, asin Rho guanine nucleotide exchange factor</td>
<td>2q33.2</td>
</tr>
<tr>
<td>ALS 3</td>
<td>ALS3</td>
<td>Amyotrophic lateral sclerosis 3 (autosomal dominant)</td>
<td>18q21</td>
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<tr>
<td>ALS 4</td>
<td>SETX</td>
<td>Senataxin</td>
<td>9q34.13</td>
</tr>
<tr>
<td>ALS 5</td>
<td>SPG11</td>
<td>Spastic paraplegia 11 (autosomal recessive)</td>
<td>1q14</td>
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<tr>
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<td>FUS</td>
<td>FUS RNA binding protein</td>
<td>16p11.2</td>
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<td>ALS7</td>
<td>Amyotrophic lateral sclerosis 7</td>
<td>20p13</td>
</tr>
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<td>VAPB</td>
<td>VAMP (vesicle-associated membrane protein) associated protein B and C</td>
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<td>ALS 9</td>
<td>ANG</td>
<td>Angiogenin</td>
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<td>FIG4</td>
<td>FIG4 phosphinositide 5-phosphatase</td>
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<td>OPTN</td>
<td>Optineurin</td>
<td>10p13</td>
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<td>ATXN2</td>
<td>Ataxin 2</td>
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<td>VCP</td>
<td>Valosin containing protein</td>
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<td>D-amino-acid oxidase</td>
<td>12q24</td>
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<td>DCTN1</td>
<td>Dynactin subunit 1</td>
<td>2p13</td>
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<tr>
<td>ALS</td>
<td>NEFH</td>
<td>Neurofilament, heavy polypeptide</td>
<td>22q12.2</td>
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<td>Peripherin</td>
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<td>Sequestosome 1</td>
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<td>17q12</td>
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<td>Spastin</td>
<td>2p24-p21</td>
</tr>
<tr>
<td>ALS</td>
<td>ELP3</td>
<td>Elongator acetyltransferase complex subunit 3</td>
<td>8p21.1</td>
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<tr>
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<td>LMNB1</td>
<td>Lamin B1</td>
<td>5q23.2</td>
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<tr>
<td>ALS</td>
<td>GLE1</td>
<td>GLE1 RNA export mediator</td>
<td>9q34.11</td>
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<tr>
<td>ALS</td>
<td>TBK1</td>
<td>TANK binding kinase 1</td>
<td>12q14.1</td>
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<tr>
<td>ALS</td>
<td>TUBA4A</td>
<td>Tubulin alpha 4a</td>
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<tr>
<td>ALS</td>
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<td>Cyclin F</td>
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<tr>
<td>ALS</td>
<td>NEK1</td>
<td>NIMA related kinase 1</td>
<td>4q33</td>
</tr>
</tbody>
</table>

**Table 2.** Genes implicated in amyotrophic lateral sclerosis

ALS, amyotrophic lateral sclerosis; FTD, frontotemporal dementia.
Gene encodes two isoforms, Gle1A and Gle1B. GLE1 gene causing a F115C/C15/C24 occur throughout mutants also failed to rescue motor Volume 29 500) and FTD (nearly 200) cases from Europe, are important questions requiring www.co-neurology.com variants were identified in vivo encoding cyclin F, a ubiquitously expressed GLE1 600), sALS [15] identified a missense mutation in the Nerve, neuro-muscular junction and motor neuron diseases characterized by increased expression of cyclins. In a cell cycle can lead to apoptosis and neuronal loss [16]. Cyclin F is not a highly abundant protein and appears unstable. Unlike other cyclins, its degradation is independent of the ubiquitin proteasome system but requires the C-terminal PEST region of the protein [17]. Cyclin F is a substrate binding subunit of SCF (Skp1-Cul1-F-box protein) ubiquitin ligase complexes and interacts with the ribonucleotide reductase family member 2. Ribonucleotide reductase plays a critical role in the conversion of ribonucleotides to deoxyribonucleotides necessary for replicative and repair DNA synthesis.

Attempts by postmitotic neurons to re-enter the cell cycle can lead to apoptosis and neuronal loss characterized by increased expression of cyclins. In a neurotrophin withdrawal model of primary motor neuron apoptotic cell death, nuclear condensation and DNA fragmentation can be observed along with activation of caspase-3 and caspase-9. The cyclin-dependent kinase inhibitors olomoucine, roscovitine and flavopiridol have been demonstrated to suppress the death of motoneurons. Taken together with the observation that cyclin D1 and cyclin E expression rises following removal of neurotrophic support, it can be envisaged that postmitotic neuronal loss occurs as neurons attempt to re-enter the cell cycle inappropriately [18]. Treatment with nerve growth factor decreases cyclin F levels in PC12EY cells suggesting that cyclin F is involved in NGF-mediated cell cycle events during the differentiation of these cells [19].

**GLE1**

Recessive mutations in GLE1, encoding the RNA-processing protein Gle1, cause the lethal congenital contracture syndrome 1 and lethal arthrogryposis with anterior horn cell disease [20,21]. Candidate screening of GLE1 in 173 fALS and 760 sALS patients recently identified novel nonsense and missense mutations in two sALS cases and a splice-site mutation in a fALS case [22**]. The nonsense transcript produces no functional protein, whereas the splice-site mutation alters the C-terminus, preventing the mutant isoform from localizing at the nuclear pore where it is critical for mRNA export. These GLE1 mutants also failed to rescue motor neuron pathology in zebrafish lacking GLE1. Thus, the mutations seem to cause a critical loss of function.

**GLE1** encodes two isoforms, Gle1A and Gle1B. Gle1B localizes to the nuclear pore complex and shuttles between the nucleus and cytoplasm [23] and self-associates via its coiled-coil domain forming oligomers necessary for its function in mRNA export [24]. The unique C-terminal 43 amino acid region of Gle1B mediates binding to the C-terminal non-FG region of the nucleoporin CG1/NPL1 [23], whereas nuclear rim localization of Gle1B is dependent on 29 N-terminal residues that interact with Nup155 [25]. These observations are interesting given that nuclear transport has recently been implicated in motor neuron degeneration [26,27].

Gle1 also has important roles in translation termination [28,29] and initiation [30]. Interestingly, the Gle1A isoform specifically has been found to associate in stress granules, which is an area of intense research in ALS [31]. Furthermore, overexpression of Gle1A or a disease-associated isoform led to the formation of cytoplasmic protein aggregates [32]. This suggests that, rather than loss of function, cellular stress may occur through increased cytoplasmic Gle1 activity.

**MATR3**

Exome sequencing of a family of European ancestry with dominantly inherited ALS and dementia identified a mutation in the MATR3 gene causing a F115C missense change [33**]. Analysis of a further 108 fALS cases identified a T622A mutation in a 66-year-old Sardinian and a first cousin who presented with
rapidly progressive ALS at 64 years of age. Analysis of 96 British ALS cases identified a P154S variant in a sALS case. Further large screens of European, Canadian and Australian ALS cohorts identified three variants in MATR3 including a V394M missense mutation and two splice-site mutations [34–36], whereas a Taiwanese study of ~200 cases found just one mutation (A72T) in a patient with bulbar-onset ALS [37]. Overall, MATR3 mutations may account for less than 1% of ALS. Interestingly, there is clinical heterogeneity in the phenotype associated with MATR3 mutations, with some cases having very slow progression longer than 15 years, or clinical and electromyographic findings more in keeping with distal myopathy with vocal cord paresis [38–40].

Matrin 3 is a 125-kDa nuclear matrix protein that binds DNA and RNA. It remains unclear how ALS-linked mutations might cause disease, with initial pathological and cellular studies showing little or no mislocalization of matrin 3 [33**]. There is some evidence that TDP-43 and matrin 3 may interact [42] but more work is needed to determine if MATR3-ALS is indeed a TDP-43 proteinopathy.

**TUBA4A**

Identification of TUBA4A (encoding the Tubulin, Alpha 4A protein) as a candidate gene associated with fALS is intriguing as it further implicates cytoskeletal structural dynamics in ALS pathogenesis [43**]. Exome sequencing and rare variant analysis of a discovery cohort identified five nonsynonymous TUBA4A changes within exon 4. These changes included four missense variants – R320C/H, R215C, A383T and a nonsense mutation W407X. Four of these mutations were predicted to be deleterious, whereas three nonsynonymous changes found in 4300 controls were predicted to be benign. A replication study revealed an excess of TUBA4A mutations among patients and identified a T145P variant that segregated with disease. With the exception of A383T and G43V, no ALS-associated TUBA4A mutations were seen in 13 023 controls. Clinically, patients with TUBA4A mutations display spinal-onset ALS with both upper and lower motor neuron features with a low prevalence of FTD. Time will tell how common a cause of ALS TUBA4A mutations are.

In-vitro studies suggested that TUBA4A mutations confer a dominant-negative property on the protein. Mutant TUBA4A is inefficient at incorporating into microtubules in vitro. Variants also inhibit the general assembly of the cellular microtubule network and thus reduce structural stability. TUBA4A<sup>W407X</sup> can form aggregates and therefore may sequester other tubulin binding proteins or impair cellular proteostasis.

**CHCHD10**

Bannwarth et al. [44**] described a large kindred with slowly progressive ALS, FTD, cerebellar ataxia, mitochondrial myopathy and mitochondrial DNA deletions. Failing to identify mutations in genes normally associated with mitochondrial disease, they undertook whole exome sequencing and identified a missense mutation in CHCHD10 (c176C>T; p.S59L), which encodes the coiled-coil helix domain-containing protein 10. This same mutation was also identified in another kindred with classical ALS–FTD, with or without Parkinsonism. CHCHD10 is a mitochondrial protein located in the intermembrane space and enriched at cristae junctions. Both endogenous and overexpressed CHCHD10<sup>S59L</sup> altered mitochondrial cristae ultrastructure and caused fragmentation of the mitochondrial network. Respiratory chain deficiency was also observed, suggesting that CHCHD10 is critical for maintaining ATP production and oxygen consumption [44**]. Mitochondria from CHCHD10 mutant fibroblasts also showed poor genome repair after oxidative stress [45].

Subsequent studies have identified CHCHD10 mutations in European populations in association with a variety of phenotypes, mostly ALS and FTD, but also Charcot–Marie–Tooth type 2 and spinal muscular atrophy [46–55]. In these populations, CHCHD10 mutation accounts for less than 1% of ALS and FTD. Interestingly, studies from China suggest that although CHCHD10 mutations are also rare in ALS, they may be a more significant cause of FTD, perhaps accounting for nearly 10% of cases in this population [56–58].

**NEK1**

Another minor hit from the exome sequencing study of Cirulli et al. [1*] was dominant LoF mutations in NEK1. More recently, this gene was implicated in a separate whole exome sequencing study of 265 fALS index cases and 827 control individuals [59]. More screens will help determine how common a cause of ALS and/or FTD NEK1 mutations are, but it is notable that initial studies suggest that NEK1 interacts with two known ALS proteins, Alsin and VAPB [1*].

Other studies have shown that the C21orf2 protein can interact with NEK1. C21orf2 affects cell proliferation after DNA damage. DNA repair was
shown to be less efficient in C21ORF2-depleted cells and homologous recombination was impaired. This deficit could be rescued by the overexpression of NEK1 [60]. NEK1 is a member of the NIMA (never in mitosis A) related kinase family of serine/threonine kinases involved in the early cellular response to genotoxic stress and plays an important role in preventing cell death induced by DNA damage [61,62]. Foci of damaged DNA in NEK1 null cells remain after removal of a toxic insult, and NEK1 null cells develop unstable chromosomes at a rate much higher than identically cultured wild-type cells [63]. NEK1 also plays a role in mitochondrial function regulating a pathway of mitochondrial cell death through phosphorylation of voltage-dependent anion channel 1 (VDAC1) on serine 193 [64].

**LATEST DEVELOPMENTS IN C9orf72**

The C9orf72 gene remains the most lucrative area of research in ALS right now. The astounding progress in our understanding of C9orf72 biology is due to the novelty of the gene mutation, an intronic hexanucleotide repeat expansion (HRE) and myriad questions regarding its mode of toxicity; the frequency of the mutation, which accounts for 22–61% of fALS, 6–19% of sALS and around 25% of familial FTD in European populations; the fact that C9orf72 mutation is associated with TDP-43 pathology, the hallmark protein of ALS and half of FTD cases and the fact that C9orf72 links both ALS and FTD, which are on a clinicopathological spectrum. The history and progress of 'C9-ALS–FTD' have recently been well reviewed elsewhere [65–67], but a few points are worthy of note.

1. Although the debate over protein (di)peptide repeats, DPRs, generated by RAN translation) and RNA toxicity (RNA foci, G-quadruplexes, sequestration of RNA-binding proteins) in C9-ALS–FTD continues, mouse knockout studies have shown that loss of C9orf72, while not obviously toxic to the nervous system, can cause severe immune dysregulation and lymphoma [68–70]. One study suggested a role for C9orf72 in autophagy [71]. It has therefore been suggested that human C9orf72 knockdown therapies should specifically target the HRE-containing allele to avoid the potential effects of complete loss of C9orf72.

2. The recent development of novel antibodies directed against the short and long isoforms of the C9orf72 protein is welcome [72**]. These tools demonstrated that the long isoform is present in the cytoplasm in vivo. The short isoform localizes to the nuclear membrane, but in ALS patients it seems to relocate to the plasma membrane, and in these cells TDP-43 mislocalizes as well [72**]. These results are intriguing given recent interest in nucleocytoplasmic transport disruption in models of C9orf72 mutation (reviewed in [27]). More experimental tools are needed to elucidate the normal functions of C9orf72 protein, whose biology remains poorly understood.

3. Novel transgenic mouse models have recently been developed, which variably recapitulate features of ALS [73–76,77**]. One BAC-transgenic model in particular develops physical and pathological phenotypes relevant to human disease, including, importantly, TDP-43 proteinopathy [77**]. Intriguingly, this model found that antisense transcripts were better correlated with disease than sense transcripts. These models may prove valuable in developing biomarkers and therapies for ALS–FTD.

4. Oligogenicity in ALS is a topic of growing interest and appears to be more common in fALS than sALS [78]. One particularly interesting example is the coexistence of intermediate expansions in ATXN2 with the C9orf72 HRE [78–80]. It has been suggested that ATXN2 expansion may push C9orf72 HRE carriers toward an ALS or ALS–FTD phenotype rather than one of the many other phenotypes associated with the mutation. Further genotype–phenotype studies are warranted, as determining how oligogenicity in ALS modifies phenotypes will no doubt lead to important insights into disease pathogenesis and yield credible therapeutic targets.

**CONCLUDING REMARKS**

Significant genetic advances continue to be made in ALS as we have summarized above. These clues implicate almost every area of cell biology, in particular proteostasis, RNA processing and the neuronal cytoskeleton. Having focussed our attention on these new genes, and advances in C9orf72, we have not had an opportunity to describe a number of other intriguing advances made in our understanding of established ALS genes. These include a potential role for TDP-43 in cryptic exon splicing [81], hydrogel studies of hnRNPs, DPRs and FUS, which show that ALS mutations can impair proteostasis and axonal local translation [82–85], the characterization of the first SOD1 ENU mutant knock-in mouse that develops a motor phenotype [86] and a large Turkish study identifying a number of intriguing new rare variants in ALS cases [87]. Thanks to project MinE (www.projectmine.com)
ACKNOWLEDGEMENTS

None.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as

1. Cirulli ET, Lasseigne BN, Petrovski S, et al. Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. Science 2015;347:1436. This publication utilized exome sequencing to identify novel amyotrophic lateral sclerosis (ALS) risk genes and pathways including autophagy and neuroinflammation through mutation of TBKI and OPTN. The study also suggests a possible association between mutation of NEK1 and ALS implicating pathways including endosomal trafficking and endoplasmic reticulum function.


4. Fecto F, Yan J, Vemula SP, et al. SQSTM1 mutations in familial and sporadic amyotrophic lateral sclerosis. Arch Neurol 2011;68:1440–1446. This work proved to be of interest as it not only first implicated MATR3 as a protein of outstanding interest. The study suggests a mechanism of haploinsufficiency and builds on literature implicating defects in RNA metabolism in ALS.


6. Heo JM, Ordouxe A, Paula JA, et al. The PINK1-PARKIN mitochondrial ubiquitylation pathway drives a program of OPTN/NDPS2 recruitment and TBKI activation to promote mitophagy. Molecular Cell 2015;60:7. This work demonstrates the importance of TBKI in the degradation of damaged mitochondria.

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CHCHD10 is not a frequent causative gene in

Neurodegeneration. C9ORF72

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