Genetics of motor neuron disorders: new insights into pathogenic mechanisms

Patrick A. Dion, Hussein Daoud and Guy A. Rouleau

Abstract | The past few years have seen the identification of dozens of genes with causal roles in motor neuron diseases (MNDs), particularly for amyotrophic lateral sclerosis and hereditary spastic paraplegia. Although many additional MND genes remain to be identified, the accumulated genetic evidence has already provided new insights into MND pathogenesis, which adds to the well-established involvement of superoxide dismutase 1 (*SOD1*) mutations. The pathways that have been recently implicated include those that affect RNA processing, axonal transport and mitochondrial function. The functional classes of MND genes identified so far are likely to aid the selection of high-priority candidate genes for future investigation, including those for so-called sporadic cases.

Linkage study
A method of searching for the chromosomal location of a gene by looking for co-segregation of the disease with genetic markers of known chromosomal location within families.

Epigenetics
Changes in gene expression that are stable through cell division but do not involve changes in the underlying DNA sequence. The best-studied example is cellular differentiation, but environmental factors, such as maternal nutrition, can influence epigenetic programming.

Centre Hospitalier Universitaire Sainte-Justine Research Center, Centre of Excellence in Neuromics, Université de Montréal, Quebec H2L 2W5, Canada. Correspondence to G.A.R. e-mail: guy.rouleau@umontreal.ca
doi:10.1038/nrg2680

Motor neuron diseases (MNDs) are an etiologically heterogeneous group of disorders that are characterized by muscle weakness and/or spastic paralysis, which results from the selective degeneration of lower motor neurons (LMNs) and/or upper motor neurons (UMNs), respectively (TABLE 1). Over the past 16 years, more than 30 Mendelian MND genes have been identified and characterized. This is a substantial achievement given the clinical and genetic heterogeneity that was originally suggested by both the large number of sporadic cases observed in some MNDs and the presumed involvement of environmental factors.

Until recently, attention was focused mainly on the pathogenic processes that result from mutations in superoxide dismutase 1 (*SOD1*) and that underlie a fraction of familial amyotrophic lateral sclerosis (FALS) cases. However, exciting recent progress has been made in identifying mutations in other genes that underlie a range of MNDs, particularly ALS and hereditary spastic paraplegia (HSP). Studies of these genes in cell-based systems and animal models have implicated several additional cellular processes in MND pathogenesis, including RNA processing, membrane trafficking and axonal transport, and mitochondrial function. Furthermore, in the case of ALS, some of these genes are mutated in sporadic forms of the disease. Although additional predisposing genes remain to be identified, the insights gained should accelerate further gene discovery and, ultimately, are hoped to provide routes for the development of much-needed new therapies.

Amyotrophic lateral sclerosis

ALS is the most common adult-onset MND, is usually fatal within five years of onset and is characterized by the degeneration of UMNs and LMNs. Approximately 5–10% of patients with ALS have a family history, and these patients most frequently inherit the disease in an autosomal dominant manner. Family-based linkage studies have led to the identification of twelve loci and eight genes for FALS, as well as three loci for ALS with frontotemporal dementia (FTD) (TABLE 2). Although these findings have provided valuable insights, they only explain a small fraction of all ALS cases. The majority of ALS cases have no obvious family history and are referred to as sporadic ALS (SALS). Initial hypotheses about the causes of SALS mainly considered environmental factors; more recently, suggestions have been made about the involvement of epigenetics (BOX 1). However, increasing evidence suggests that genetic factors also contribute to SALS. In the following sections we review recent progress made in understanding the genetic basis of both FALS and SALS.

Mendelian ALS involving *SOD1*

Our understanding of the molecular and genetic basis of ALS began with the identification of mutations in

Table 1 | **Classification and clinical characteristics of motor neuron diseases**

Disease	Age of onset	Prevalence	Motor neuron involvement	Clinical features
ALS	Between 45 and 60 years old	4–6/100,000	UMNs and LMNs	Progressive muscle weakness, atrophy and spasticity
HSP	From early childhood to 70 years old	3–10/100,000	UMNs	Progressive spasticity in the lower limbs
PLS	Between 35 and 66 years	1/10,000,000	UMNs	Spinal and bulbar spasticity
SMA	Between 6 and 18 months old for type I, II and III; between 15 and 50 years old for type IV	1/6,000–10,000	LMNs	Symmetrical muscle weakness and atrophy
SBMA	Between 30 and 50 years old	1–9/100,000	LMNs	Slowly progressive limb and bulbar muscle weakness with fasciculations, muscle atrophy and gynecomastia
LCCS	Fetal	1/25,000	UMNs and LMNs	Early fetal hydrops and akinesia, degeneration of anterior horn neurons and extreme skeletal muscle atrophy

ALS, amyotrophic lateral sclerosis; HSP, hereditary spastic paraplegia; LCCS, lethal congenital contracture syndrome; LMN, lower motor neuron; PLS, primary lateral sclerosis; SBMA, spinal bulbar muscular atrophy; SMA, spinal muscular atrophy; UMN, upper motor neuron.

Reactive oxygen species
Ions or small molecules that include oxygen ions, free radicals and peroxides, both inorganic and organic.

Hu-antigen R
An RNA-stabilizing protein that is a member of the embryonic lethal abnormal visual (ELAV) family. These proteins recognize the 3′ UTR sequences of mRNAs, in particular the adenine/uridine-rich elements, the widespread occurrence of which suggests that they are involved in the regulation of many biological processes.

Astrocyte
One of the three main cell types in the brain, the others being neurons and oligodendrocytes. Astrocytes act as a scaffold that maintains brain structure and they can alter the extracellular milieu and ionic concentration through the expression of various transporters and channel proteins. They support the functions of neurons and oligodendrocytes.

Cre recombinase
A type I topoisomerase from the P1 bacteriophage that catalyses the site-specific recombination of DNA between loxP sites. It binds to the loxP sites to allow DNA that is cloned between the sites to be removed.

Microglia
Small neuroglial cells of the central nervous system. They have long processes and ameboid and phagocytic activity at sites of neural damage or inflammation.

SOD1 (REF. 1), which account for 15–20% of autosomal dominant FALS cases and 1–2% of all ALS cases. As there are many descriptions of the discovery and role of *SOD1* mutations in the existing literature, we keep our discussion brief, describing the pathogenic mechanisms underlying SOD1-mediated toxicity in ALS and discussing the most recent and exciting advances in this area.

Mechanisms of SOD1-mediated pathogenesis. SOD1 is an abundant, ubiquitously expressed, cytosolic enzyme. It functions as a homodimer to convert harmful superoxide radicals to molecular oxygen and hydrogen peroxide, therefore preventing the further generation of reactive oxygen species (ROS). The protein comprises 153 amino acids, and over 125 distinct amino acid changes have been reported to cause ALS[2], some of which affect the active site and others the structure. The pathological effects of *SOD1* mutations are not thought to result from loss of dismutase activity but rather from gain-of-function effects through which the protein acquires one or more toxic properties. This theory is supported by several lines of evidence, including the absence of motor neuron degeneration in *Sod1*-null mice[3] and its occurrence in transgenic mice overexpressing mutant forms of *SOD1* (REF. 4), irrespective of residual dismutase activity.

Several mechanisms have been proposed to explain the toxicity of mutant SOD1 (FIG. 1). In motor neurons, toxicity might result from various effects, including oxidative stress, accumulation of intracellular SOD1-positive aggregates, mitochondrial dysfunction and defects in axonal transport (for an in-depth discussion, see REF. 5). However, it is noteworthy that G93A-SOD1 was recently reported to compete with Hu-antigen R (HuR, also known as embryonic lethal abnormal visual-like protein 1 (ELAVL1)) for binding to adenine/uridine-rich elements in the 3′ UTR of vascular endothelial growth factor (*VEGF*)[6], a gene that has been previously implicated as a modifier of ALS in mice and humans[7]. Although an effect of SOD1 on mRNA stability remains to be shown, this finding suggests that additional mechanisms of toxicity mediated by *SOD1* mutations remain to be identified.

The role of other cell types in neuronal degeneration, which is referred to as non-cell-autonomous toxicity, has been the subject of substantial work in ALS — more so than for any other neurological disorder. Initial evidence came from the analysis of transgenic mice expressing mutant SOD1 exclusively in either neurons[8] or astrocytes[9]; no motor neuron degeneration was observed in either case. Reports describing chimeric mice with mixtures of normal and mutant SOD1-expressing cells showed that toxicity in motor neurons requires their proximity to non-neuronal cells expressing mutant SOD1, in particular astrocytes[10]. This was further investigated by several groups using mice carrying a mutant *SOD1* transgene that can be excised by expressing Cre recombinase; toxicity was examined using Cre-specific expression in motor neurons, astrocytes, microglia, muscle cells and Schwann cells. Although several hypotheses have been proposed for how neighbouring cells confer toxicity to motor neurons, including glutamate excitotoxicity and microglia activation, the specific mechanisms remain unclear (see REF. 11 for a discussion).

Variable penetrance among* SOD1 *mutations. Most *SOD1* mutations are autosomal dominant, but a few show lower penetrance and are recessively inherited[12]. In addition, some *SOD1* mutations vary in penetrance in different populations, although the reasons for this variation are poorly understood. The best-studied example is the D90A-SOD1 mutation, as it can be transmitted in either a dominant or a recessive manner in different populations. Scandinavian patients with ALS who are homozygous for the D90A mutation present with a slow progressing disease, and the high frequency of unaffected Scandinavians who are heterozygous for D90A-SOD1 led to it being considered as a benign polymorphism in this population at first[13]. By contrast, D90A-SOD1 heterozygous cases in other populations develop classical rapidly progressing ALS[14]. Compound heterozygotes have also been reported for D90A and D96N mutations[15].

Another low penetrance *SOD1* mutation was described in a family in which the proband carried a homozygous deletion in *SOD1* (ΔG27/P28), and eight

Table 2 | **Genes and loci that predispose to amyotrophic lateral sclerosis**

ALS disease type	Chromosome	Gene (gene symbol)	Inheritance	Onset	Class	Refs
Mendelian genes						
ALS1	21q22.1	Superoxide dismutase 1 *(SOD1)*	AD	Adult	Detoxification enzyme	1
ALS2	2q33	Amyotrophic lateral sclerosis 2 (*ALS2*)	AR	Juvenile	GEF signalling	41
ALS4	9q34	Senataxin *(SETX)*	AD	Juvenile	DNA and RNA metabolism	47
ALS6	16q12	*FUS*	AD and AR	Adult	RNA binding, exon splicing and DNA repair	34–37
ALS8	20q13.33	Vesicle-associated membrane protein-associated protein B *(VAPB)*	AD	Adult	Vesicular trafficking	49
ALS9	14q11	Angiogenin *(ANG)*	AD	Adult	Neovascularization	50
ALS10	1p36.22	TAR DNA-binding protein *(TARDBP)*	AD	Adult	RNA binding and exon skipping	26,27
ALS	2p13	Dynactin 1 *(DCTN1)*	AD	Adult	Axonal transport	57
ALS–FTDP	17q21.1	Microtubule-associated protein tau *(MAPT)*	AD	Adult	Microtubule assembly and stability	69
Mendelian loci						
ALS3	18q21	Unknown	AD	Adult	Unknown	59
ALS5	15q15.1–21.1	Unknown	AR	Juvenile	Unknown	48
ALS7	20p13	Unknown	AD	Adult	Unknown	38
ALS-X	Xcen	Unknown	XD	Adult	Unknown	60
ALS–FTD1	9q21–22	Unknown	AD	Adult	Unknown	63
ALS–FTD2	9p13.3–21.3	Unknown	AD	Adult	Unknown	64–68

AD, autosomal dominant; ALS, amyotrophic lateral sclerosis; ALS–FTDP, ALS–FTD with Parkinsonian features; AR, autosomal recessive; FTD, frontotemporal dementia; GEF, guanine nucleotide exchange factor; XD, X-linked dominant.

Schwann cell
A type of non-neuronal brain cell that lacks axons and dendrites and forms axons in the peripheral nervous system.

Microglia activation
Microglia can be activated by several factors, including glutamate receptor agonists, pro-inflammatory cytokines, cell necrosis factors and lipopolysaccharide. Once activated, the cells undergo key morphological changes, including the secretion of cytotoxic factors, recruitment molecules and pro-inflammatory molecules. In addition, activated microglia undergo proliferation to increase their numbers.

Penetrance
The proportion of individuals with a specific genotype who manifest the genotype at the phenotypic level. If the penetrance of a disease allele is 100%, all individuals who carry that allele will express the associated disorder and the genotype is said to be 'completely penetrant'.

unaffected relatives (48–85 years of age) were heterozygous for this deletion[16]. The low penetrance of this mutation is explained by the fact that it enhances the naturally occurring alternative splicing of exon 2 of *SOD1*, which leads to reduced transcription of the mutant allele. *SOD1* could be vulnerable to alternative splicing defects, as its first intron contains a GC-motif, which can act as an atypical donor splice site[17]. Abnormal splicing events that affect *SOD1* might therefore have a modifying effect that could explain more generally the phenotypic heterogeneity reported in other ALS families.

SOD1-mediated toxicity in sporadic ALS. A recent hypothesis about SOD1 toxicity focuses on the fact that oxidation can induce the misfolding of wild-type SOD1, which yields a modified SOD1 protein with binding and toxic properties that are similar to those of mutant SOD1 (REF. 18). Misfolded wild-type SOD1 may therefore underlie a subset of SALS cases without known ALS causative mutations[19]. This theory could now be examined, as several antibodies that specifically recognize the mutant or misfolded forms of SOD1 have been developed[20–22]. However, the amount of misfolded SOD1 that is necessary to trigger ALS remains to be clarified.

Non-SOD1-linked Mendelian ALS

In addition to SOD1-linked FALS, seven Mendelian loci have been linked to FALS and several causative genes have been identified (TABLE 2).

The* ALS10 *locus: TAR DNA-binding protein. Following the initial identification of TAR DNA-binding protein 43 (TDP43) as a major constituent of ubiquitylated protein aggregates in patients with ALS and a subset of patients with frontotemporal lobar degeneration[23,24], the presence of such aggregates has been observed in most ALS cases that have been examined. The exception is SOD1-linked FALS cases[25]. Therefore *TARDBP*, which encodes TDP43, was an excellent candidate gene for resequencing in ALS cases, a strategy that our group and others followed in cohorts of SALS and FALS cases. These efforts led to the identification of several missense mutations, mostly in the glycine-rich carboxy-terminal region of the protein[26,27], and a truncating mutation (Y374X) was subsequently identified[28]. In total, over 30 dominantly inherited mutations have been reported that so far account for ~1–3% of all ALS cases[29]. In addition, *TARDBP* mutations have recently been reported in patients with ALS and cognitive deficits[30,31]. To date, no clear genotype–phenotype correlation has emerged, and it remains unclear whether ALS-predisposing *TARDBP* mutations are due to a gain or loss of function. This is likely to be clarified by the generation of model organisms that express TDP43 mutants.

TDP43 is an evolutionarily well-conserved nuclear protein that contains two RNA recognition motifs in addition to the C-terminal domain, which interacts with heterogeneous nuclear ribonucleoproteins (hnRNPs)[32] and is probably involved in other protein–protein

Polymorphism
The contemporary definition is any site in the DNA sequence that is present in the population in more than one state. By contrast, the traditional definition is an allele with a population frequency of between >1% and <99%.

Compound heterozygote
A situation in which an individual is heterozygous for two different mutations at the same locus.

Proband
In a family study, the individual who is first identified in the family as having the disease under study.

interactions. TDP43 shuttles between the nucleus and the cytoplasm and has a role in RNA processing and the regulation of alternative splicing. Its partial mislocalization in nuclear and cytoplasmic aggregates in ALS suggests that its functions relating to the processing of nuclear pre-mRNAs may be compromised, providing one course of investigation for future functional studies.

Notably, new connections between SALS and FALS cases have been made through the identification of *TARDBP* mutations in addition to the TDP43-positive aggregates that are observed in both FALS and SALS cases. *TARDBP* mutations have been reported almost in equal proportions in SALS and FALS cases[33]; by contrast, *SOD1* mutations have been reported in only a few cases of SALS. Functional studies and animal models of TDP43 mutants will be needed to establish whether some of these *TARDBP* mutations, particularly in SALS, are causative or benign rare polymorphisms.

Box 1 | **Environmental and epigenetic factors in ALS**

Several environmental factors that are suspected to contribute to the neuronal degeneration observed in amyotrophic lateral sclerosis (ALS) have been examined. The first evidence of an environmental contribution to ALS came from the high incidence of ALS with Parkinsonism and progressive dementia that was reported in the Chamorro population of Guam. The etiological agents underlying this regional incidence are believed to be neurotoxins, such as sterol glucosides and β-methylamino-L-alanine (BMAA), which are found in the cycad flour that is consumed by the local population[163]. Exposure to neurotoxins has also been suspected to underlie the increased post-war risk of ALS in veterans who participated in the first Gulf war[164]. Similarly, an increased ALS risk has been associated with exposure to electromagnetic fields[165]. Occupational exposure to agricultural chemicals, such as pesticides and insecticides, has also been associated with an increased risk of ALS[166]. This was recently supported by the observation of an ALS-like motor neuron disorder that was induced by chronic inhalation of pyrethroid insecticides[167]. Moreover, in a large prospective study, exposure to formaldehyde was associated with an increased risk of ALS, although this remains to be verified independently[168]. The observation of an increased ALS risk in Italian professional soccer players suggested that excessive physical activity could also be a risk factor[169,170], although this is not a resolved issue[171]. Recent evidence also suggests that repeated head injuries might increase ALS risk[172]. Among other factors, cigarette smoking has been associated with increased ALS risk, although this result is significant only in women[173].

More recently, several lines of evidence have pointed towards the involvement of epigenetic factors in an increasing number of human pathologies, including neurodegenerative diseases[174]. Epigenetic modifications provide a plausible link between the environment and alterations in gene expression that might lead to disease phenotypes. Indeed, environmental factors can perturb long-term gene regulation, starting at early stages of development; however, these perturbations do not have pathological results until much later in life. Such a phenomenon has been described for Alzheimer's disease[175]. Regarding ALS, studies of such epigenetic factors are still in early stages. It has been proposed that the epigenetic silencing of genes that are vital for motor neuron function could underlie ALS; however, analyses of the promoter methylation profiles of superoxide dismutase 1 (*SOD1*), vascular endothelial growth factor (*VEGF*) and genes that are involved in the primary detoxification mechanism for heavy metals (metallothionein 1A (*MT1A*) and *MT2A*) did not reveal inappropriate methylation levels[176,177].

In summary, there is compelling evidence that environmental factors can have a role in ALS susceptibility. The role of epigenetic factors in ALS needs to be better clarified. Additionally, more results from combined epidemiological and genetic studies are needed to understand the contribution of genetic, epigenetic and environmental factors to this highly heterogeneous disease.

***The* ALS6 *locus:* FUS.** The recent identification of ALS causal mutations in the *FUS* gene, which also encodes another DNA/RNA-binding protein, is another promising advance[34,35]. In 2003, three groups defined the *ALS6* locus[36–38]. Following the reports of *TARDBP* mutations in ALS, Vance and colleagues prioritized the sequencing of six *ALS6* positional candidate genes encoding DNA/RNA-binding proteins in a large British family and identified a dominant missense mutation (R521C) in *FUS*[35]. Their screening of 197 additional FALS cases led to the identification of the same mutation in four families and two other missense mutations. Kwiatkowski and colleagues concurrently conducted a homozygosity mapping study of a Cape Verdean family with ALS that had a recessive pattern of inheritance and identified a homozygosity cluster in the *ALS6* locus[34]. Genomic resequencing of the 56 candidate genes in the cluster revealed a homozygous missense *FUS* mutation (H517Q) in all affected individuals, whereas none of the individuals who were heterozygous for H517Q *FUS* had developed ALS. Their subsequent examination of 292 FALS cases revealed 12 dominant mutations in 16 families, including one mutation (R521G) that was reported to have incomplete penetrance. In the same study, no *FUS* mutations were found in 293 SALS cases. Our own resequencing of *FUS* in French and French-Canadian ALS cases identified two previously reported missense mutations and a 3-bp deletion (S57del)[39]; these changes were observed in SALS cases and are therefore the first indication that *FUS* also causes sporadic cases.

FUS is essentially a nuclear protein with some cytoplasmic localization. However, immunoanalysis of brain and spinal cord from cases of ALS with *FUS* mutations suggest cytoplasmic retention and the recruitment of FUS to neuronal aggregates[34,35], which is reminiscent of the aggregates of SOD1 and TDP43 that are observed in other ALS cases. Initial neuropathological examinations suggest that FUS aggregates are absent in healthy individuals, in ALS cases with SOD1 mutations and in SALS cases with TDP43 aggregates. Similarly, TDP43-positive aggregates seem to be absent in cases of ALS with *FUS* mutations, which suggests that FUS pathogenesis is independent of TDP43 aggregation[29]. However, neuropathological examinations have only been carried out in a limited number of cases of ALS, so caution is warranted in interpreting these preliminary results.

There are striking similarities between FUS and TDP43: both proteins directly bind RNA and single- or double-stranded DNA; both are mostly nuclear and shuttle to the cytoplasm; in both the majority of mutations are in the highly conserved C-terminal domains and nearly all mutations result in mislocalization and the formation of aggregates; and, finally, both are multifunctional proteins that are involved in several mRNA-processing steps, such as expression regulation, splicing and localization[40]. These similarities make other RNA/DNA-binding proteins high-priority candidates in future investigations of novel ALS causative genes.

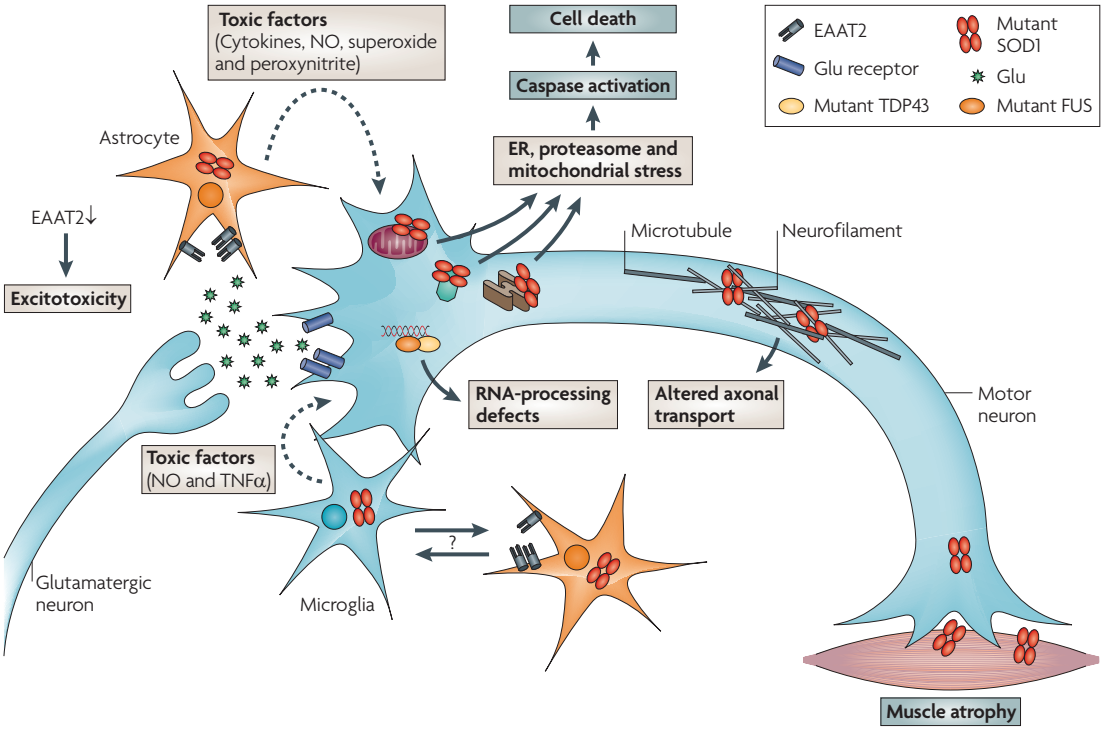

Figure 1 | **Physiopathological mechanisms underlying the specific degeneration of motor neurons in amyotrophic lateral sclerosis.** Mutant superoxide dismutase 1 (*SOD1*) directly affects motor neurons through diverse pathways, such as mitochondrial defects (reduced ATP production and increased free calcium release), dysfunction in the endoplasmic reticulum (ER) and the proteasome, and alterations in axonal transport, all of which lead to the activation of apoptotic cascades. However, *SOD1*-mediated toxicity in motor neurons is insufficient to mediate disease progression; non-neuronal neighbours, such as astrocytes and microglia, contribute to motor neuron damage in what is known as the non-cell-autonomous process. Astrocytes and microglia that express mutant *SOD1* secrete several potentially toxic factors into the cellular environment, which amplify the initial damage and drive the progression of the disease. In addition, the astroglial reuptake of synaptic glutamate (Glu) is reduced through the inactivation of excitatory amino acid transporter 2 (EAAT2), which leads to the excitotoxic death of motor neurons. The exact pathological mechanism by which mutant TAR DNA-binding protein (*TARDBP*, which encodes TDP43) and *FUS* are involved in amyotrophic lateral sclerosis is still unknown, but the implication of these two genes provides persuasive evidence that defects in RNA processing are involved in the selective death of motor neurons. NO, nitric oxide; TNFα, tumour necrosis factor-α.

Other loci implicated in FALS. Homozygous loss-of-function mutations in *ALS2* have been found to underlie a juvenile-onset form of ALS[41,42]. *Als2*-null mice only develop a mild MND, but a recent study showed that knockdown of the *zALS2* homologue in *Danio rerio* (zebrafish) produces severe motor neuron perturbations[43]. The mild phenotype in mice has been proposed to be due to the expression of previously unsuspected alternatively spliced *ALS2* transcripts that escape the effect of the mutation. Interestingly, mutations in *ALS2* have also been observed in juvenile primary lateral sclerosis (JPLS) and infantile-onset ascending spastic paralysis (IAHSP)[44,45]. Alsin (the protein product of *ALS2*) contains three putative guanine nucleotide exchange factor (GEF) domains that activate members of the RAS superfamily of GTPases, and it functions as an exchange factor for the small GTPase RAB5A, which regulates endosomal trafficking and RAC1 activity[46].

Autosomal dominant mutations of senataxin (*SETX*) have been observed in another atypical form of FALS, a juvenile-onset, slow progressing form that is characterized by the absence of bulbar and respiratory symptoms[47]. *SETX* encodes a DNA/RNA helicase domain with homology to regulator of nonsense transcripts 1 (*RENT1*, also known as *UPF1*) and immunoglobulin mu-binding protein 2 (*IGHMBP2*), which encode proteins that are involved in RNA processing[47]. Another autosomal recessive juvenile-onset ALS locus (*ALS5*) was mapped to 15q15.1–q21.1 in North African and European families, but the causative gene remains unknown[48].

The remaining FALS causative genes all predispose to adult-onset ALS. A point mutation (P56S) in the vesicle-associated membrane protein-associated protein B (*VAPB*) gene was identified in one Brazilian family with classical autosomal dominant ALS and in six families with spinal muscular atrophy (SMA)[49]. No other mutations were subsequently reported, and *VAPB* is likely to be a rare cause of ALS.

Missense mutations in angiogenin (*ANG*) were originally reported in 15 FALS and 11 apparent SALS cases

Heterogeneous nuclear ribonucleoprotein
A complex of RNA and protein that is present in the nucleus during transcription and post-transcriptional modification of pre-mRNA. Such complexes serve as a signal that the pre-mRNA is not yet fully processed and ready for export to the cytoplasm.

Homozygosity mapping
An approach for detecting rare disease-promoting variants. This method detects extensive homozygous haplotypes that are hundreds of kilobases or more in length and that are unique to, or enriched in, affected individuals.

from Ireland and Scotland[50], and additional mutations were identified in patients from Italy, North America, France and Germany[51–54]. It is noteworthy that a missense mutation was also found in a large Dutch pedigree with ALS and FTD[55]. ALS-associated angiogenin mutants were shown to have reduced neuroprotective activity against hypoxic injury, which suggests that these mutants result in a loss of function and not a loss of expression[56].

Mutations in the p150 subunit of dynactin 1 (DCTN1), a component of the dynein complex that comprises the major axonal retrograde motor, were identified in a family with a slow progressing autosomal dominant MND[57]. These mutations are predicted to alter the binding of the dynactin–dynein motor to microtubules. Additional *DCTN1* mutations have subsequently been reported in SALS, FALS and a family with ALS and co-occurrence of FTD[58].

Despite extensive gene resequencing, the causative genes for ALS at the remaining loci remain to be found. These include the *ALS3*, *ALS7* and *ALSX* loci on chromosomes 18 (REF. 59) and 20 (REF. 38) and the X chromosome[60], respectively.

ALS and frontotemporal dementia. FTD is the second most common form of dementia and is characterized by the degeneration of neurons in the frontal lobe and anterior temporal lobe. Patients with FTD have personality changes and show impairment of executive functions and language[61]. Growing evidence indicates that in many cases FTD and ALS are two phenotypic manifestations of a common underlying genetic cause[62]. Indeed, parallel examinations of extended pedigrees in which both ALS and FTD segregate have led to the mapping of three loci. The *ALS–FTD1* locus (9q21–q22) was the first to be defined[63], and a second locus, *ALS–FTD2* (9p21.3–13.3), was later identified in families with ALS and FTD from the Netherlands, Scandinavia, Canada, France and Australia[64–68]. The growing number of families with ALS with linkage to 9p makes this a robust locus for the identification of an ALS–FTD-predisposing gene. Mutations in microtubule-associated protein tau (*MAPT*) at chromosome 17q21 have also been reported in patients with ALS or FTD[69,70] and in patients with other neurodegenerative diseases[62]. This locus is referred to as *ALS–FTDP* because of the presence of Parkinsonian features in some affected individuals. Haploinsufficiency of the granulin (*GRN*) gene causes FTD in 17q-linked families with no mutations in *MAPT* and ubiquitin-only pathology[71]. Finally, mutations in three genes — valosin-containing protein (*VCP*)[72], chromatin-modifying protein 2B (*CHMP2B*)[73] and *GRN*[74] — have been reported in patients with FTD. Mutations in *CHMP2B* and *GRN* are also associated with ALS[73,74]. The co-occurrence of ALS and FTD in the same families, and indeed in the same individuals, further highlights the high level of genetic heterogeneity of these diseases and implies a role for other factors in determining the phenotypic outcome of the causal mutation; such factors could include modifier genes and environmental factors.

Complex genetic models for sporadic ALS

Despite the increasing success in identifying genes that underlie FALS, which results from penetrant monogenic mutations, the aetiology of most cases of ALS (that is, cases of SALS) remains unknown, and more complex genetic models may need to be considered. Such models could, for example, take into account mutations in several genes — possibly with variable penetrance — with additive effects in the presence or absence of environmental factors. In contrast to family-based linkage studies, population-based association studies are more suitable for identifying such common variants with small effects and are therefore useful for investigating complex diseases, such as SALS.

Candidate-gene association studies. Several candidate gene-based association studies have been carried out to identify genetic susceptibility factors for SALS, and previously investigated candidates include *VEGF*, neurofilament, heavy polypeptide (*NEFH*), *DCTN1*, leukaemia inhibitory factor (*LIF*), *APEX*, apolipoprotein E (*APOE*), survival of motor neuron 1 (*SMN1*), the paraoxonase cluster (*PON1–PON3*) and haemochromatosis (*HFE*) (see REF. 75 for a review of these studies). These genes were selected based on their functions or their implications in other MNDs. However, the associations found for these genes in one population have rarely been validated in a second population. Among these candidate genes, the *PON1–PON3* cluster has recently been extensively studied. Six case–control association studies reported an association of polymorphisms located in the coding, intronic or promoter regions of *PON1–PON3* with ALS. However, a meta-analysis recently excluded the *PON* genes as susceptibility factors for ALS[76], therefore indicating that caution is necessary when interpreting positive associations derived from small studies, as population stratification seems to greatly influence the small candidate-gene studies, resulting in false-positive associations. We will not discuss these genes further as, in our opinion, the evidence for their role as ALS-predisposing genes is tentative at best.

Genome-wide association studies. Genome-wide association studies (GWA studies) have rapidly become a powerful tool in genetics and have been successfully used to identify susceptibility genes for complex diseases, such as type 2 diabetes and multiple sclerosis[77]. The main advantage of GWA studies is the ability to efficiently genotype hundreds of thousands of SNPs without the need to select candidate genes based on their function or their possible role in disease. Several GWA studies have been conducted for SALS. The first, which included 276 cases and 271 controls, was reported in 2007 and failed to identify significant hits but formed the basis for additional studies[78]. The SNP data was made freely available and served as a replication set in a follow-up GWA study of 766 cases and 750 controls that identified FGGY carbohydrate kinase domain-containing (*FGGY*) as a candidate gene[79]. A third study of 461 cases and 450 controls from the Netherlands reported a significant association for the inositol 1,4,5-triphosphate receptor 2

Retrograde motor
Motor proteins bind and transport several different cargoes in nerve cells, including organelles, polymers and vesicles containing neurotransmitters. Retrograde transport runs towards the minus end of the axons.

Frontal lobe
An area of the brain located at the front of each cerebral hemisphere that is involved in higher mental functions. The executive functions of the frontal lobe include the ability to recognize future consequences, override and suppress unacceptable social responses, and determine similarities and differences between things or events.

Anterior temporal lobe
The temporal lobes are regions of the cerebral cortex that are located beneath the Sylvian fissure on both the left and right hemispheres of the brain. The anterior part of the lobes is involved in visual processing and object perception and recognition.

Haploinsufficiency
A condition in a diploid organism in which a single functional copy of a gene results in a phenotype, such as a disease.

Association studies
A gene-discovery strategy that compares allele frequencies in cases and controls to assess the contribution of genetic variants to phenotypes in specific populations.

Meta-analysis
An approach that combines the results of several studies that address a set of related research hypotheses to overcome the problem of reduced statistical power in studies with small sample sizes.

Population stratification
A population that contains several subpopulations that differ in their genetic characteristics.

(*ITPR2*) gene[80]. However, when this group expanded their analysis by including data from the first GWA study[78], *ITPR2* did not present a significant association, but a distinct association for the dipeptidyl-peptidase 6 (*DPP6*) gene was found[81] and was concurrently replicated in an Irish study[82]. However, the Irish study also included the populations used previously by the Dutch group, which explained the *DPP6* replication. The *DPP6* association was subsequently replicated in an Italian population[83], but not in a set of samples from Poland[84].

A recent and extensive follow-up study using 2,160 tested patients and 3,008 controls failed to confirm any significant association for the genes identified in these GWA studies[85]. In a recent microsatellite-based GWA study in which 1,884 microsatellite markers were genotyped using a pooled set of patients with ALS and controls (from the United Kingdom, United States and Belgium), a set of alleles at the D8S1820 marker (which is located in the intron of elongation protein 3 (*ELP3*), which encodes a component of RNA polymerase II) were found to be associated with ALS[86]. Finally, a large-scale GWA study recently identified two susceptibility loci for SALS; one on chromosome 19p13.3 that maps to a haplotype block in unc-13 homologue A (*UNC13A*) and one on chromosome 9p21.2 (REF. 87). However, none of the previously associated SNPs from the GWA and candidate gene-based studies mentioned above was associated with SALS in this study.

The limitations of GWA studies in ALS are clear. The availability of several thousand well-phenotyped cases is a key element of any GWA study aimed at finding genetic factors with modest effects. Furthermore, the high degree of genetic (allelic and non-allelic) heterogeneity of SALS cases might be a limiting factor for the identification of causative alleles, as different disease-causing alleles may exist in the same gene. If this is the case, the strength of association signals for that gene will be diluted. Lastly, given that many rare, highly penetrant mutations could cause ALS, as recently observed for *TARDBP* and *FUS* mutations in SALS, the identification of such variations by GWA studies will inherently be difficult given that these studies can only detect common, low-penetrance variants. By contrast, such rare variants may be more easily identified with the whole-genome sequencing methods that will probably supersede the current SNP-based technologies in the near future as high-throughout sequencing methods become affordable and allow the resequencing of human genomes (which is already being done by initiatives such as the 1000 Genomes Project).

Chromosomal rearrangements and copy number variants. Chromosomal abnormalities that affect the structure and/or number of chromosomes and copy number variants (CNVs) may cause a small number of ALS cases. Two apparently balanced translocations were described in a patient with ALS and another with ALS–FTD[88,89]. These observations suggest that these two phenotypes could be caused by deletion, disruption or deregulation through a positional effect of a gene or genes near the breakpoint areas, although no causative genes have been identified. The high-density SNP arrays that have enabled the high-throughput genotyping reported in GWA studies can also be used to detect CNVs. Any CNV that affects expression rather than protein structure could be a low-penetrance variant. Two CNV analyses have been conducted with SALS cases from the Netherlands and Ireland[90,91]. In the Dutch study, no CNV locus was significantly associated with ALS; however, a larger number of deletions were found to be exclusive to patients than those that were exclusive to controls[90]. The Irish study found no significant association between CNVs and ALS[91].

Hereditary spastic paraplegia

The various manifestations of HSP comprise the second most important group of MNDs in terms of the number of mutations identified and the resulting insights into the pathogenesis of MND. The various spastic paraplegia (SPG) loci are associated with different forms of HSP (which are listed in TABLE 3 and Supplementary information S1 (table)). Currently, 45 SPG loci and 20 causative genes have been identified, 7 and 5 of which, respectively, have been reported since 2007. The identification of these genes has led to insights into potential pathological mechanisms (FIG. 2).

Axonal transport and membrane trafficking in HSP. Eight HSP genes — spastin (*SPAST*), atlastin GTPase 1 (*ATL1*), kinesin family member 5A (*KIF5A*), non-imprinted in Prader–Willi/Angelman syndrome 1 (*NIPA1*), zinc finger, FYVE domain-containing 26 (*ZFYVE26*), *SPG20*, *SPG21* and *SPG11* — support dysfunction of axonal transport and membrane trafficking as causes of HSP. *SPAST*-associated cases account for ~40% of autosomal dominant HSP cases, making mutations in this gene the most common cause of HSP[92]. Over 150 mutations were described in *SPAST*, which encodes spastin, a member of the ATPase-associated (AAA) family of proteins, which are components of the dynein motor and are involved in axonal retrograde cargo transport[93,94]. Different *SPAST* mutations lead to either haploinsufficiency or gain of function[95,96], and the interaction of spastin with CHM1B indicates that spastin is also involved in the endocytic pathway and the formation of the midbody during cytokinesis[97,98]. Mutations in another gene, *ATL1*, cause ~10% of autosomal dominant HSP cases with an early-onset pure phenotype (SPG3A)[99,100]. The primary role of atlastin 1 is in endoplasmic reticulum and Golgi morphogenesis, but it also has a role in neurite outgrowth[101,102]. Interestingly, atlastin and spastin directly interact, which suggests a common pathway of HSP pathogenesis[103].

HSP cases associated with *KIF5A* (SPG10) were initially only reported in early-onset pure HSP. However, more recently mutations were reported in 10% of cases of complicated HSP in patients of European descent[104,105]. KIF5A is a subunit of kinesin 1, and *in vitro* studies indicated that expression of its mutant forms led to reduced cargo flux along microtubules. Mutations in *NIPA1* usually cause a slow progressing pure HSP[106,107], and the NIPA1 protein is known to

Genome-wide association studies
The examination of DNA variation (typically SNPs) across the whole genome in a large number of individuals who have been matched for population ancestry and assessed for a disease or trait of interest. Correlations between variants and the trait are used to locate genetic risk factors.

Microsatellite
A class of repetitive DNA that is made up of repeats that are 2–8 nucleotides in length. Microsatellites can be highly polymorphic and are frequently used as molecular markers in population genetics studies.

1000 Genomes Project
An international research effort, launched in 2008, to establish by far the most detailed catalogue of human genetic variation. Plans are to sequence the genomes of at least 1,000 anonymous participants of different ethnic groups over the next 3 years using newly developed technologies.

Copy number variant
A DNA sequence variant (including deletions and duplications) in which the result is a departure from the expected diploid representation of the DNA sequence.

Balanced translocation
A translocation between non-homologous chromosomes in which the exchange occurs with no gain or loss of genetic material.

Midbody
A transient organelle-like structure that is formed during mammalian cell division and persists until just before the complete separation of the dividing cells.

Table 3 | **Genetic causes of other motor neuron diseases**

Disease	Chromosome	Gene	Protein	Inheritance	Associated clinical features	Refs
SPG1	Xq28	*L1CAM*	L1 cell adhesion molecule	X-linked	MASA syndrome and X-linked hydrocephalus	124
SPG2	Xq22	*PLP1*	Myelin proteolipid protein	X-linked	Pure and complicated (mental retardation and seizures) HSP	125
SPG3A	14q12–q21	*ATL1*	Atlastin	AD	Pure and complicated HSP with early onset and slow progression	99
SPG4	2p22	*SPAST*	Spastin	AD	Mainly pure HSP with variable onset	92
SPG5A	8q21.3	*CYP7B1*	Cytochrome P450, family 7, subfamily B, polypeptide 1	AR	Pure HSP with variable onset	128
SPG6	15q11.2–q12	*NIPA1*	Non-imprinted in Prader–Willi/Angelman syndrome region protein 1	AD	Pure HSP with adult onset	106
SPG7	16q24.3	*SPG7*	Paraplegin	AR	Pure and complicated HSP (optic, cortical and cerebellar atrophies)	118
SPG8	8q24	*KIAA0196*	Strumpellin	AD	Pure HSP with adult onset (marked spasticity)	133
SPG10	12q13	*KIF5A*	Kinesin family member 5A	AD	Pure and complicated HSP with early onset	104
SPG11	15q21.1	*SPG11*	Spatacsin	AR	Pure HSP with variable onset and thin corpus callosum with early onset	117
SPG13	2q24–q34	*HSPD1*	Heat shock protein 60	AD	Pure HSP with adult onset	122
SPG15	14q24.1	*ZFYVE26*	Spastizin	AR	Complicated HSP (Kjellin syndrome) with adolescent onset	111
SPG17	11q12–q14	*BSCL2*	Seipin	AD	Complicated HSP (Silver syndrome) with variable onset	134
SPG20	13q12.3	*SPG20*	Spartin	AR	Complicated HSP (Troyer syndrome) with childhood onset	112
SPG21	15q21–q22	*SPG21*	Maspardin	AR	Complicated HSP (Mast syndrome) with early adult onset	113
SPG22	Xq13.2	*SLC16A2*	Solute carrier family 16, member 2	X-linked	Allan–Herndon–Dudley syndrome (AHDS)	135
SPG31	2p12	*REEP1*	Receptor expression-enhancing protein 1	AD	Pure HSP with variable onset	136
SPG39	19p13	*PNPLA6*	Neuropathy target esterase	AR	Very rare HSP with marked distal wasting in all four limbs	138
SPG42	3q25.31	*SLC33A1*	Acetyl-CoA transporter	AD	Pure HSP with variable onset	129
SPG44	1q42.13	*GJC2*	Connexin 47	AR	Complicated HSP (ataxia, dysarthria and seizures) with late onset	132
JPLS	2q33.1	*ALS2*	Alsin	AR	Juvenile ALS2 and infantile-onset ascending spastic paralysis	41
PLSA1	4ptel–p16.1	*Unknown*	Unknown	AD	Progressive asymmetric spastic paraparesis and weakness of the lower limbs followed by upper limb involvement	140
SMA	5q12.2–q13.3	*SMN1*	Survival of motor neuron protein 1, telomeric	AR	SMA type I, II, III and IV	148
SBMA	Xq11–q12	*AR*	Androgen receptor	X-linked	Kennedy disease (X-linked recessive form of SMA)	150
LCCS1	9q34	*GLE1*	GLE1	AR	Lethal arthrogryposis with anterior horn cell disease	153
LCCS2	12q13	*ERBB3*	ERBB3	AR	Early fetal hydrops and akinesia with anterior horn disease with neurogenic bladder defects	155
LCCS3	19p13.3	*PIP5K1C*	Phosphatidylinositol-4-phosphate 5-kinase, type I, gamma	AR	Similar to LCCS2 but without neurogenic bladder defects	154

AD, autosomal dominant; ALS, amyotrophic lateral sclerosis; AR, autosomal recessive; *BSCL2*, Berardinelli–Seip congenital lipodystrophy 2; *GLC2*, gap junction protein, gamma 2; HSP, hereditary spastic paraplegia; JPLS, juvenile primary lateral sclerosis; LCCS, lethal congenital contracture syndrome; MASA, mental retardation, aphasia, shuffling gait and adducted thumbs; *PLP1*, proteolipid protein 1; PLSA1, adult primary lateral sclerosis; *PNPLA6*, phospholipase domain-containing 6; SBMA, spinal bulbar muscular atrophy; SMA, spinal muscular atrophy; SPG, spastic paraplegia; *ZFYVE26*, zinc finger, FYVE domain-containing 26.

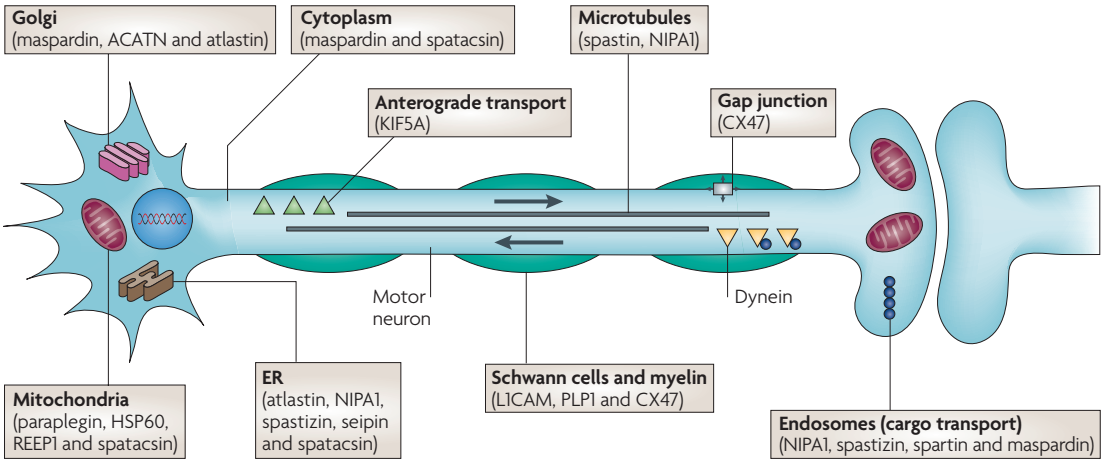

Figure 2 | **Potential pathogenic mechanisms in hereditary spastic paraplegia.** The genes known to predispose to hereditary spastic paraplegia (HSP) support the view that defects at various cellular sites can be detrimental to the long axons of upper motor neurons. This representation of a motor neuron shows where these HSP-predisposing proteins have been suggested to reside or function. Motor neurons have a regulated, dynamic membrane-trafficking system that involves HSP-predisposing proteins that function in the endoplasmic reticulum (ER) and Golgi. Endosomes are transported along the microtubule cytoskeleton to various subcellular locations, a process that involves HSP-predisposing proteins that are located on endosomes and microtubules. Motor neurons rely on mitochondria to drive the efficient transport of signals, molecules and organelles to and from nerve terminals, and this process also involves HSP-predisposing proteins that function in mitochondria. For other HSP-predisposing proteins (for example, strumpellin, connexin 47 (CX47) and cytochrome P450, family 7, subfamily B, polypeptide 1 (CYP7B1); not shown), their localization is unclear and/or their contribution to motor neuron diseases is not yet understood. ACATN, acetyl-CoA transporter; HSP60, heat shock protein 60; KIF5A, kinesin family member 5A; L1CAM, L1 cell adhesion molecule; NIPA1, non-imprinted in Prader–Willi/Angelman syndrome region protein 1; PLP1, proteolipid protein 1; REEP1, receptor expression-enhancing protein 1.

act as an Mg^{2+} transporter and to interact with early endosomes and the cell surface[108]. The introduction of human *NIPA1* mutations into the *Caenorhabditis elegans* orthologue *nipa-1* causes neurodegeneration[109]. Depletion of the NIPA1 *Drosophila melanogaster* orthologue, spichtyin, suggests that it also has a role in axonal transport[110].

SPG15 cases are associated with truncating mutations in *ZFYVE26*, which encodes a zinc finger protein named spastizin, and have a characteristic autosomal recessive complicated phenotype[111]. Initial cellular examination of spastizin indicated that it colocalizes with endosomal and endoplasmic reticulum markers, and so it is thought to be involved in membrane trafficking at these sites[111]. SPG20- and SPG21-associated HSP, which are respectively due to mutations in *SPG20* (spartin) and *SPG21* (maspardin), are two autosomal recessive forms[112,113]. In patients with SPG20 or SPG21, both *SPG20* and *SPG21*, respectively, are affected by protein-truncating mutations, which suggests that these forms result from a loss of function[112,114]. Spartin has been shown to associate with the surface of lipid droplets and to regulate their size and number. The deregulation of lipid droplets is predicted to provoke axonal damage[115]. Little is known about the function of maspardin, but a report indicated that it localizes prominently to the cytoplasm as well as to membranes, possibly at *trans*-Golgi network/late endosomal compartments. Cases of SPG11 are due to mutations in *SPG11*, which encodes spatacsin. The function of this protein is unknown, but the accumulation of membrane material was observed in the nerve biopsy of a patient with SPG11 (REF. 116). The types of *SPG11* mutations that have been identified suggest that they cause loss of function[117].

Mitochondrial dysfunction in HSP. Three HSP causative genes (*SPG7*, heat shock 60-kDa protein 1 (*HSPD1*) and receptor expression-enhancing protein 1 (*REEP1*)) support a role for mitochondrial dysfunction. Mutations in *SPG7*, which encodes paraplegin, account for ~5% of autosomal recessive HSPs. These mutations produce both pure and complicated HSP forms[118,119]. Paraplegin is part of the metalloprotease AAA complex, which is an ATP-dependent proteolytic complex that is located on the inner mitochondrial membranes and that controls protein quality and ribosomal assembly[120]. Paraplegin-null mice develop axonal swellings owing to an accumulation of mitochondria and neurofilaments[121]. This event precedes axonal degeneration but correlates with the onset of motor impairment, therefore suggesting that both axonal transport and mitochondrial dysfunction may be implicated and that the disease is due to the loss of paraplegin function. SPG13 cases are associated with *HSPD1*, which participates in the folding of mitochondrial proteins, and are usually late-onset, pure forms[122]. A missense mutation in *HSPD1* was suggested to be associated with early onset in patients who also have spastin mutations[123]. This HSP is thought to result from a reduced protein-folding activity in mitochondrial protein quality control.

Schwann cell-related HSP. Two HSP causative genes (L1 cell adhesion molecule (*L1CAM*) and proteolipid protein 1 (*PLP1*)), which underlie two X-linked forms of HSP, were reported in early studies of this MND[124,125]. The *L1CAM*-associated HSP (SPG1) is the most common form of complicated HSP. L1CAM is a transmembrane glycoprotein protein that is expressed in neurons and Schwann cells and is believed to have a role in the development of the central nervous system (CNS)[126]. Mutations in *PLP1* (which is associated with SPG2) have been found in families with complicated HSP and also cause Pelizaeus–Merzbacher disease (PMD)[127]. The difference in phenotype between PMD and SPG2 is thought to arise from the differential effect that *PLP1* mutations can have on the two isoforms of the protein that it encodes: PLP1 and DM20. Both are integral membrane proteins that account for ~50% of the protein content of adult CNS myelin[127]. Mutations in exon 3B of *PLP1* might predominantly result in SPG2 rather than PMD because this exon is excluded from DM20, which is thought to therefore be left intact. PMD is thought to arise only when DM20 is also affected by mutation.

Other or unknown cellular dysfunctions in HSP. Mutations in cytochrome P450, family 7, subfamily B, polypeptide 1 (*CYP7B1*) are found in families with autosomal recessive HSP (SPG5A)[128]. SPG5A was originally viewed as a pure HSP form with variable age of onset and slow progression, but it is now believed to have both pure and complex forms[105]. CYP7B1 mutants are proposed to affect cholesterol homeostasis because the normal protein is involved in myelin formation. A mutation in solute carrier family 33, member 1 (*SLC33A1*), which encodes the acetyl-CoA transporter (ACATN), was recently reported to cause a novel HSP (SPG42)[129]. ACATN transports acetyl-CoA into the lumen of the Golgi apparatus and is believed to have a role in the outgrowth and maintenance of motor neuron axons[130,131]. Knockdown of *slc33a1* in *D. rerio* was shown to cause defective axonal outgrowth from the spinal cord[129]. A missense mutation was reported in a family with a late-onset, slow progressing complicated form of HSP (SPG44); the mutation was found in gap junction protein, gamma 2 (*GJC2*), which encodes the gap junction protein connexin 47 (CX47)[132]. CX47–CX43 channels are involved in the normal maintenance of myelin, and the expression of mutant CX47 in a cell culture model indicated that it could still form gap junction plaques with CX43 (REF. 132), although they had a severely altered voltage-dependent gating that was predicted not to function under physiological conditions; therefore, SPG44 may result from a loss of function[132].

The dysfunctions underlying mutations of *CYP7B1*, *SLC33A1* and *GJC2* suggest that several pathways can lead to HSP, but the mechanisms involved in some other forms of HSP remain largely unknown. SPG8 cases are associated with mutations in *KIAA0196*, which encodes strumpellin, and are characterized by severe spasticity and reduced vibration sense[133]. The function of strumpellin and the mode of action of its mutants remain unknown, but in *D. rerio* both the knockdown of its homologue and the expression of the mutated human protein were shown to produce shorter motor axons and abnormal branching. The causative genes of other HSP forms, SPG17, SPG22, SPG31 and SPG39, were respectively reported to be Berardinelli–Seip congenital lipodystrophy 2 (*BSCL2*)[134], *SLC16A2* (REF. 135), *REEP1* (REFS 136,137) and patatin-like phospholipase domain-containing 6 (*PNPLA6*)[138], but little is known about how these proteins contribute to HSP pathogenesis.

Primary lateral sclerosis

Adult primary lateral sclerosis (PLSA1) is an autosomal disorder that only affects UMNs in the corticospinal and corticobulbar tracts. The distinction between PLSA1 and adult ALS is based on the absence of LMN involvement in PLS[139]. The two disorders are sometimes regarded as clinically distinct progressive neurodegenerative disorders, but although this is likely to be true for many PLS cases, there are patients who present pure UMN signs for years but later progress to typical ALS, so there is an overlap with ALS. Following a genome-wide analysis with a French-Canadian family with PLSA1, our group mapped a locus to 4ptel–4p16.1 (REF. 140). It is noteworthy that along with JPLS and IAHSP, PLS is part of a range of diseases that can result from *ALS2* mutations[141–143]. Furthermore, some overlaps exist between PLS and HSP, as both disorders are characterized by pure UMN signs and symptoms.

Spinal muscular atrophy

SMA is an autosomal recessive MND and is one of the most common genetic diseases that cause infant mortality[144]. In humans, *SMN2* is a nearly identical copy of *SMN1*, and the protein products of these genes have functions in the spliceosome. *SMN2* differs from *SMN1* by a few nucleotides, and the crucial difference in their coding region is the C-to-T transition at position +6 of exon 7 (REF. 145). This difference is translationally silent but leads to the skipping of exon 7 in SMN2. SMN proteins without exon 7 are less stable, self-oligomerize less efficiently and have less efficient splicing functions[146,147]. SMA type I, II and III are all caused by loss-of-function mutations or deletions of *SMN1* (REF. 148). Several groups have also examined *SMN* genes in ALS; however, a meta-analysis of these studies found no evidence for either homozygous deletions or CNVs of *SMN1* and/or *SMN2* as ALS risk factors[149].

Spinal bulbar muscular atrophy

Spinal bulbar muscular atrophy (SBMA) was the first triplet repeat disease to be identified and is due to the expansion of a trinucleotide CAG repeat that encodes the polyglutamine (polyQ) tract in the first exon of the androgen receptor (*AR*) gene[150]. SBMA is an X-linked recessive polyglutamine expansion disease and, like other polyglutamine expansion diseases, it is characterized by the formation of aggregates. A mouse model expressing *AR* with an expanded polyglutamine stretch has been used to examine treatment strategies[151]. Leuprorelin rescued the motor deficits of

Myelin
An electrically insulating material that usually forms a layer around the axon of a neuron. It is essential for the proper functioning of the nervous system. Schwann cells supply the myelin for peripheral neurons, whereas oligodendrocytes supply it to neurons of the central nervous system.

these animals, which suggests that ligand binding is important for pathogenesis.

Lethal congenital contracture syndrome

Although this pathology is not a classical MND, some types of lethal congenital contracture syndrome (LCCS1 and LCCS3) could be considered as severe *in utero* forms of MND. In 1998, an autosomal recessive condition that was characterized by fetal akinesia was reported in a Finnish population, and a locus (*LCCS1*) was mapped[152]. LCCS1 is primarily characterized by the highly focused degeneration of motor neurons in the spinal cord, although other defects occur concurrently. The mutations causing LCCS1 were identified in *GLE1*, which encodes a protein that is required for the export of mRNAs from the nucleus to the cytoplasm[153]. In this population, 51 out of 52 LCCS1 cases were homozygous for a single substitution in intron 3 (c.432-10A>G). This substitution creates a cryptic splice acceptor site that results in nine extra nucleotides and consequentially inserts three amino acids into the predicted coiled-coil domain of the protein. GLE1 is expressed ubiquitously but the pathological changes are only observed in the anterior horn motor neurons, and so it has been suggested that the three-amino-acid insertion could prevent interactions with a motor neuron-specific protein (or another ligand)[153].

Two other genes were reported to cause similar conditions (LCCS2 and LCCS3) but only the causative gene of LCCS3, phosphatidylinositol-4-phosphate 5-kinase, type I, gamma (*PIP5K1C*), seems to produce a similar atrophy of the spinal cord anterior horn[154,155]. The pathological changes associated with the LCCS2 causative gene, *ERBB3*, are not as restricted to motor neurons, but they nonetheless cause neuronal apoptosis[155]. Both *PIP5K1C* and *ERBB3* encode modulators of the phosphatidylinositol (PtdIns) pathway and, interestingly, mutations in *FIG4* — a phosphoinositide 5-phosphatase that regulates the cellular abundance of PtdIns(3,5)P_2, which is associated with endosomal vesicle traffic to the *trans*-Golgi network — were recently identified in ALS[156].

Emerging mechanistic themes in MND

The variety of genes reported to be mutated in MND indicates that mutant proteins acting at several intracellular sites can lead to the axonal degeneration that causes these diseases — namely, degeneration of the terminal portion of corticospinal tracts and dorsal column motor neurons. The selective involvement of these axons could result from the fact that they are the longest in the CNS. The characterization of mutant proteins involved in MND indicates that some MNDs are essentially the consequence of defects in one specific pathway (for example, splicing in SMA), whereas others may involve several pathways (for example, excitotoxicity and misfolding in ALS).

One of the themes observed in several MNDs is RNA processing. The recent discovery of the involvement of *TARDBP* and *FUS* in ALS provides direct links to defects in RNA processing as a broad pathway that contributes to motor neuron degeneration. Other MND genes (for example, *SETX*, *ANG*, *SMN1* and *GLE1*) also encode RNA-processing proteins, and even SOD1 is now suspected to act as an mRNA stabilizer. Indirect evidence also comes from the alternative splicing of genes such as *ALS2*, *SMN1*, *SMN2*, *PLP1* and *SPAST*. Although preliminary, the *ELP3* association with ALS described above also supports an RNA-processing theme[86]. However, it is still too early to know whether RNA-processing defects genuinely have a substantial role in ALS pathogenesis. Nonetheless, mutations in several genes with known roles in the various stages of RNA processing now underlie a broad range of MNDs with variable onsets, ranging from *in utero* to late in life. As the evidence for the susceptibility of motor neurons to RNA-processing defects grows, the identification of the precise mechanisms behind these defects has become a new goal for MND researchers, in particular for researchers interested in sporadic MNDs, such as SALS. Motor neurons from the CNS may be more susceptible to RNA-processing defects because the CNS expresses more alternative splicing transcripts than other tissues[157] and hence has a lower tolerance for the disturbance of its mRNA.

Conversely, RNA-processing defects are not an emerging theme for any of the forms of HSP in which other processes (including axonal transport and membrane trafficking, mitochondrial dysfunction and various other pathogenetic pathways) have been suggested to be crucial for axonal homeostasis. For further details on the mechanisms that are believed to underlie HSPs, see REF. 158. HSP causative proteins are involved in membrane-trafficking processes (including budding, transport, tethering and fusions of membrane vesicles), which suggests that these processes are key to the survival of motor neurons (FIG. 2). The reports of mutations in *FIG4*, *PIP5K1C* and *ERBB3* in LCCS and ALS also support the susceptibility of motor neurons to membrane-trafficking dysfunction.

Mitochondrial dysfunction, which has been reported in the pathogenesis of ALS[159] and various non-MND neurodegenerative disorders[160], also affects axonal transport, which is an ATP-dependent process. However, there is more than one way in which mitochondrial dysfunction might lead to motor neuron damage. In ALS, mitochondrial abnormalities, such as vacuolated and dilated mitochondria with disorganized cristae and inner mitochondrial membrane defects, are observed in both sporadic and familial cases[161,162]. Moreover, mutant SOD1 is present in fractions enriched for mitochondria derived from affected tissues but not for mitochondria from unaffected tissues. Although the mechanism by which mutant SOD1 affects mitochondrial function is not yet entirely clear, it may be linked to the fact that mitochondria are the gatekeepers of apoptosis; they contain several pro-apoptotic molecules (for example, B cell leukaemia/lymphoma 2 (BCL-2)-like proteins) that activate cytosolic proteins to execute apoptosis, block anti-apoptotic proteins in the cytosol and directly cleave nuclear DNA.

Anterior horn
The ventral column of grey matter in the spinal cord that contains the cell bodies of motor (efferent) neurons.

Cristae
Internal compartments that are formed by the inner membranes of mitochondria. They contain several key proteins for aerobic respiration, including ATP synthase and various cytochromes.

Conclusions

The studies we have described above, which have identified many mutations in MND-causing genes, show the continued power of working on large pedigrees that segregate causative mutations in a classical Mendelian fashion. However, such transmission explains only a small fraction of MNDs. GWA studies have attempted to identify causal alleles for SALS, in which several genes with small effects are likely to be involved, but these studies have yet to reach their full potential for the identification of novel ALS-predisposing genes. The development of novel promising strategies, such as whole-genome or -exome sequencing, would be helpful for identifying rare variants underlying ALS. The newly emerged insights into the molecular basis of disease processes in familial forms of MND, such as FALS and HSP, suggest candidate genes for prioritization — for example, those associated with RNA processing, axonal transport and membrane trafficking. How these genes potentially interact to cause a motor neuron-specific phenotype remains unclear, and intensive efforts will be needed to examine whether some of these genes connect in a unifying pathological process — for example, a burning question in ALS is whether a connection exists among *SOD1*, *TARDBP* and *FUS* that might underlie pathogenesis in sporadic cases.

The rapidly increasing knowledge of MND pathogenesis is exciting, and although these discoveries do not translate into immediate benefits for patients, they nonetheless offer avenues of investigation for future treatments. Because the deregulation of several cellular processes underlies the various MNDs, cocktails of neuroprotective agents that target different pathways may one day offer the hope of treating some MNDs. From a clinical trials perspective, genetically diagnosed individuals might be more likely to benefit from early therapeutic intervention.

1. Rosen, D. R. *et al.* Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* **362**, 59–62 (1993).
This report describes the original discovery of *SOD1* mutations in FALS cases. *SOD1* was the first ALS causative gene to be identified.
2. Wroe, R., Wai-Ling Butler, A., Andersen, P. M., Powell, J. F. & Al-Chalabi, A. ALSOD: the Amyotrophic Lateral Sclerosis Online Database. *Amyotroph. Lateral Scler.* **9**, 249–250 (2008).
3. Reaume, A. G. *et al.* Motor neurons in Cu/Zn superoxide dismutase-deficient mice develop normally but exhibit enhanced cell death after axonal injury. *Nature Genet.* **13**, 43–47 (1996).
4. Gurney, M. E. *et al.* Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. *Science* **264**, 1772–1775 (1994).
This paper reports the first generation and description of an ALS transgenic mouse model.
5. Rothstein, J. D. Current hypotheses for the underlying biology of amyotrophic lateral sclerosis. *Ann. Neurol.* **65**, S3–S9 (2009).
6. Li, X. *et al.* Mutant copper-zinc superoxide dismutase associated with amyotrophic lateral sclerosis binds to adenine/uridine-rich stability elements in the vascular endothelial growth factor 3′-untranslated region. *J. Neurochem.* **108**, 1032–1044 (2009).
7. Lambrechts, D. *et al.* VEGF is a modifier of amyotrophic lateral sclerosis in mice and humans and protects motoneurons against ischemic death. *Nature Genet.* **34**, 383–394 (2003).
8. Pramatarova, A., Laganiere, J., Roussel, J., Brisebois, K. & Rouleau, G. A. Neuron-specific expression of mutant superoxide dismutase 1 in transgenic mice does not lead to motor impairment. *J. Neurosci.* **21**, 3369–3374 (2001).
This paper offers evidence that SOD1 toxicity is not cell autonomous.
9. Gong, Y. H., Parsadanian, A. S., Andreeva, A., Snider, W. D. & Elliott, J. L. Restricted expression of G86R Cu/Zn superoxide dismutase in astrocytes results in astrocytosis but does not cause motoneuron degeneration. *J. Neurosci.* **20**, 660–665 (2000).
This paper offers evidence surrounding the crucial contribution of astrocytes during the pathogenesis of ALS.
10. Yamanaka, K. *et al.* Astrocytes as determinants of disease progression in inherited amyotrophic lateral sclerosis. *Nature Neurosci.* **11**, 251–253 (2008).
11. Lobsiger, C. S. & Cleveland, D. W. Glial cells as intrinsic components of non-cell-autonomous neurodegenerative disease. *Nature Neurosci.* **10**, 1355–1360 (2007).
12. Andersen, P. M. Amyotrophic lateral sclerosis associated with mutations in the CuZn superoxide dismutase gene. *Curr. Neurol. Neurosci. Rep.* **6**, 37–46 (2006).
13. Andersen, P. M. *et al.* Autosomal recessive adult-onset amyotrophic lateral sclerosis associated with homozygosity for Asp90Ala CuZn-superoxide dismutase mutation. A clinical and genealogical study of 36 patients. *Brain* **119**, 1153–1172 (1996).
14. Andersen, P. M. *et al.* Amyotrophic lateral sclerosis associated with homozygosity for an Asp90Ala mutation in CuZn-superoxide dismutase. *Nature Genet.* **10**, 61–66 (1995).
15. Hand, C. K. *et al.* Compound heterozygous D90A and D96N SOD1 mutations in a recessive amyotrophic lateral sclerosis family. *Ann. Neurol.* **49**, 267–271 (2001).
16. Zinman, L. *et al.* A mechanism for low penetrance in an ALS family with a novel *SOD1* deletion. *Neurology* **72**, 1153–1159 (2009).
17. Mount, S. M. A catalogue of splice junction sequences. *Nucleic Acids Res.* **10**, 459–472 (1982).
18. Ezzi, S. A., Urushitani, M. & Julien, J. P. Wild-type superoxide dismutase acquires binding and toxic properties of ALS-linked mutant forms through oxidation. *J. Neurochem.* **102**, 170–178 (2007).
19. Kabashi, E., Valdmanis, P. N., Dion, P. & Rouleau, G. A. Oxidized/misfolded superoxide dismutase-1: the cause of all amyotrophic lateral sclerosis? *Ann. Neurol.* **62**, 553–559 (2007).
20. Rakhit, R. *et al.* An immunological epitope selective for pathological monomer-misfolded SOD1 in ALS. *Nature Med.* **13**, 754–759 (2007).
21. Jonsson, P. A. *et al.* Minute quantities of misfolded mutant superoxide dismutase-1 cause amyotrophic lateral sclerosis. *Brain* **127**, 73–88 (2004).
22. Urushitani, M., Ezzi, S. A. & Julien, J. P. Therapeutic effects of immunization with mutant superoxide dismutase in mice models of amyotrophic lateral sclerosis. *Proc. Natl Acad. Sci. USA* **104**, 2495–2500 (2007).
23. Arai, T. *et al.* TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem. Biophys. Res. Commun.* **351**, 602–611 (2006).
24. Neumann, M. *et al.* Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* **314**, 130–133 (2006).
The two reports above showed that protein aggregates in ALS contain TDP43 and therefore prompted ongoing and extensive investigations into the role of this protein in ALS.
25. Mackenzie, I. R. *et al.* Pathological TDP-43 distinguishes sporadic amyotrophic lateral sclerosis from amyotrophic lateral sclerosis with *SOD1* mutations. *Ann. Neurol.* **61**, 427–434 (2007).
26. Kabashi, E. *et al.* *TARDBP* mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. *Nature Genet.* **40**, 572–574 (2008).
This paper describes eight missense mutations in *TARDBP* in patients with SALS and FALS and shows that the mutations correspond with the accumulation of a lower-molecular-weight TDP43 fragment.
27. Sreedharan, J. *et al.* TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science* **319**, 1668–1672 (2008).
The authors identify three missense mutations in *TARDBP* in patients with ALS (including one large family) and provide proof of the effects of these mutations by injecting chick embryos.
28. Daoud, H. *et al.* Contribution of *TARDBP* mutations to sporadic amyotrophic lateral sclerosis. *J. Med. Genet.* **46**, 112–114 (2009).
29. Lagier-Tourenne, C. & Cleveland, D. W. Rethinking ALS: the FUS about TDP-43. *Cell* **136**, 1001–1004 (2009).
30. Corrado, L. *et al.* High frequency of *TARDBP* gene mutations in Italian patients with amyotrophic lateral sclerosis. *Hum. Mutat.* **30**, 688–694 (2009).
31. Benajiba, L. *et al.* *TARDBP* mutations in motoneuron disease with frontotemporal lobar degeneration. *Ann. Neurol.* **65**, 470–473 (2009).
32. Buratti, E. *et al.* TDP-43 binds heterogeneous nuclear ribonucleoprotein A/B through its C-terminal tail: an important region for the inhibition of cystic fibrosis transmembrane conductance regulator exon 9 splicing. *J. Biol. Chem.* **280**, 37572–37584 (2005).
33. Valdmanis, P. N., Daoud, H., Dion, P. A. & Rouleau, G. A. Recent advances in the genetics of amyotrophic lateral sclerosis. *Curr. Neurol. Neurosci. Rep.* **9**, 198–205 (2009).
34. Kwiatkowski, T. J. Jr *et al.* Mutations in the *FUS/TLS* gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science* **323**, 1205–1208 (2009).
35. Vance, C. *et al.* Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science* **323**, 1208–1211 (2009).
The two papers above describe *FUS* mutations in the *ALS6* locus in cases of FALS.
36. Abalkhail, H., Mitchell, J., Habgood, J., Orrell, R. & de Belleroche, J. A new familial amyotrophic lateral sclerosis locus on chromosome 16q12.1–16q12.2. *Am. J. Hum. Genet.* **73**, 383–389 (2003).
37. Ruddy, D. M. *et al.* Two families with familial amyotrophic lateral sclerosis are linked to a novel locus on chromosome 16q. *Am. J. Hum. Genet.* **73**, 390–396 (2003).
38. Sapp, P. C. *et al.* Identification of two novel loci for dominantly inherited familial amyotrophic lateral sclerosis. *Am. J. Hum. Genet.* **73**, 397–403 (2003).
39. Belzil, V. V. *et al.* Mutations in *FUS* cause FALS and SALS in French and French Canadian populations. *Neurology* 9 Sep 2009 (doi:10.1212/WNL.0b013e3181bbfeef).
40. Buratti, E. & Baralle, F. E. Multiple roles of TDP-43 in gene expression, splicing regulation, and human disease. *Front. Biosci.* **13**, 867–878 (2008).
41. Hadano, S. *et al.* A gene encoding a putative GTPase regulator is mutated in familial amyotrophic lateral sclerosis 2. *Nature Genet.* **29**, 166–173 (2001).
42. Hentati, A. *et al.* Linkage of recessive familial amyotrophic lateral sclerosis to chromosome 2q33–q35. *Nature Genet.* **7**, 425–428 (1994).

43. Gros-Louis, F. *et al. Als2* mRNA splicing variants detected in KO mice rescue severe motor dysfunction phenotype in *Als2* knock-down zebrafish. *Hum. Mol. Genet.* **17**, 2691–2702 (2008).
44. Eymard-Pierre, E. *et al.* Novel missense mutation in *ALS2* gene results in infantile ascending hereditary spastic paralysis. *Ann. Neurol.* **59**, 976–980 (2006).
45. Gros-Louis, F. *et al.* An *ALS2* gene mutation causes hereditary spastic paraplegia in a Pakistani kindred. *Ann. Neurol.* **53**, 144–145 (2003).
46. Otomo, A. *et al.* ALS2, a novel guanine nucleotide exchange factor for the small GTPase Rab5, is implicated in endosomal dynamics. *Hum. Mol. Genet.* **12**, 1671–1687 (2003).
47. Chen, Y. Z. *et al.* DNA/RNA helicase gene mutations in a form of juvenile amyotrophic lateral sclerosis (ALS4). *Am. J. Hum. Genet.* **74**, 1128–1135 (2004).
48. Hentati, A. *et al.* Linkage of a commoner form of recessive amyotrophic lateral sclerosis to chromosome 15q15–q22 markers. *Neurogenetics* **2**, 55–60 (1998).
49. Nishimura, A. L. *et al.* A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. *Am. J. Hum. Genet.* **75**, 822–831 (2004).
50. Greenway, M. J. *et al. ANG* mutations segregate with familial and 'sporadic' amyotrophic lateral sclerosis. *Nature Genet.* **38**, 411–413 (2006).
51. Fernandez-Santiago, R. *et al.* Identification of novel angiogenin (*ANG*) gene missense variants in German patients with amyotrophic lateral sclerosis. *J. Neurol.* **256**, 1432–1459 (2009).
52. Gellera, C. *et al.* Identification of new *ANG* gene mutations in a large cohort of Italian patients with amyotrophic lateral sclerosis. *Neurogenetics* **9**, 33–40 (2008).
53. Paubel, A. *et al.* Mutations of the *ANG* gene in French patients with sporadic amyotrophic lateral sclerosis. *Arch. Neurol.* **65**, 1333–1336 (2008).
54. Wu, D. *et al.* Angiogenin loss-of-function mutations in amyotrophic lateral sclerosis. *Ann. Neurol.* **62**, 609–617 (2007).
55. van Es, M. A. *et al.* A case of ALS-FTD in a large FALS pedigree with a K17I *ANG* mutation. *Neurology* **72**, 287–288 (2009).
56. Sebastia, J. *et al.* Angiogenin protects motoneurons against hypoxic injury. *Cell Death Differ.* (2009).
57. Puls, I. *et al.* Mutant dynactin in motor neuron disease. *Nature Genet.* **33**, 455–456 (2003).
58. Munch, C. *et al.* Heterozygous R1101K mutation of the *DCTN1* gene in a family with ALS and FTD. *Ann. Neurol.* **58**, 777–780 (2005).
59. Hand, C. K. *et al.* A novel locus for familial amyotrophic lateral sclerosis, on chromosome 18q. *Am. J. Hum. Genet.* **70**, 251–256 (2002).
60. Siddique, T. *et al.* X-linked dominant locus for late-onset familial amyotrophic lateral sclerosis. *Am. J. Hum. Genet.* **63** (Suppl.), A308 (1998).
61. Neary, D., Snowden, J. & Mann, D. Frontotemporal dementia. *Lancet Neurol.* **4**, 771–780 (2005).
62. Talbot, K. & Ansorge, O. Recent advances in the genetics of amyotrophic lateral sclerosis and frontotemporal dementia: common pathways in neurodegenerative disease. *Hum. Mol. Genet.* **15**, R182–R187 (2006).
63. Hosler, B. A. *et al.* Linkage of familial amyotrophic lateral sclerosis with frontotemporal dementia to chromosome 9q21–q22. *JAMA* **284**, 1664–1669 (2000).
64. Morita, M. *et al.* A locus on chromosome 9p confers susceptibility to ALS and frontotemporal dementia. *Neurology* **66**, 839–844 (2006).
65. Valdmanis, P. N. *et al.* Three families with amyotrophic lateral sclerosis and frontotemporal dementia with evidence of linkage to chromosome 9p. *Arch. Neurol.* **64**, 240–245 (2007).
66. Vance, C. *et al.* Familial amyotrophic lateral sclerosis with frontotemporal dementia is linked to a locus on chromosome 9p13.2–213. *Brain* **129**, 868–876 (2006).
67. Le Ber, I. *et al.* Chromosome 9p-linked families with frontotemporal dementia associated with motor neuron disease. *Neurology* **72**, 1669–1676 (2009).
68. Luty, A. A. *et al.* Pedigree with frontotemporal lobar degeneration — motor neuron disease and Tar DNA binding protein-43 positive neuropathology: genetic linkage to chromosome 9. *BMC Neurol.* **8**, 32 (2008).
The six references above describe families with ALS and FTD that were mapped to the chromosome 9p locus.
69. Hutton, M. *et al.* Association of missense and 5′-splice-site mutations in *tau* with the inherited dementia FTDP-17. *Nature* **393**, 702–705 (1998).
70. Zarranz, J. J. *et al.* A novel mutation (K317M) in the *MAPT* gene causes FTDP and motor neuron disease. *Neurology* **64**, 1578–1585 (2005).
71. Cruts, M. *et al.* Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. *Nature* **442**, 920–924 (2006).
72. Watts, G. D. *et al.* Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. *Nature Genet.* **36**, 377–381 (2004).
73. Parkinson, N. *et al.* ALS phenotypes with mutations in *CHMP2B* (charged multivesicular body protein 2B). *Neurology* **67**, 1074–1077 (2006).
74. Spina, S. *et al.* Clinicopathologic features of frontotemporal dementia with *Progranulin* sequence variation. *Neurology* **68**, 820–827 (2007).
75. Valdmanis, P. N. & Rouleau, G. A. Genetics of familial amyotrophic lateral sclerosis. *Neurology* **70**, 144–152 (2008).
76. Wills, A. M. *et al.* A large-scale international meta-analysis of paraoxonase gene polymorphisms in sporadic ALS. *Neurology* **73**, 16–24 (2009).
77. McCarthy, M. I. *et al.* Genome-wide association studies for complex traits: consensus, uncertainty and challenges. *Nature Rev. Genet.* **9**, 356–369 (2008).
78. Schymick, J. C. *et al.* Genome-wide genotyping in amyotrophic lateral sclerosis and neurologically normal controls: first stage analysis and public release of data. *Lancet Neurol.* **6**, 322–328 (2007).
79. Dunckley, T. *et al.* Whole-genome analysis of sporadic amyotrophic lateral sclerosis. *N. Engl. J. Med.* **357**, 775–788 (2007).
80. van Es, M. A. *et al. ITPR2* as a susceptibility gene in sporadic amyotrophic lateral sclerosis: a genome-wide association study. *Lancet Neurol.* **6**, 869–877 (2007).
81. van Es, M. A. *et al.* Genetic variation in *DPP6* is associated with susceptibility to amyotrophic lateral sclerosis. *Nature Genet.* **40**, 29–31 (2008).
82. Cronin, S. *et al.* A genome-wide association study of sporadic ALS in a homogenous Irish population. *Hum. Mol. Genet.* **17**, 768–774 (2008).
83. Del Bo, R. *et al. DPP6* gene variability confers increased risk of developing sporadic amyotrophic lateral sclerosis in Italian patients. *J. Neurol. Neurosurg. Psychiatry* **79**, 1085 (2008).
84. Cronin, S., Tomik, B., Bradley, D. G., Slowik, A. & Hardiman, O. Screening for replication of genome-wide SNP associations in sporadic ALS. *Eur. J. Hum. Genet.* **17**, 213–218 (2009).
85. Chiò, A. *et al.* A two-stage genome-wide association study of sporadic amyotrophic lateral sclerosis. *Hum. Mol. Genet.* **18**, 1524–1532 (2009).
86. Simpson, C. L. *et al.* Variants of the elongator protein 3 (*ELP3*) gene are associated with motor neuron degeneration. *Hum. Mol. Genet.* **18**, 472–481 (2009).
87. van Es, M. A. *et al.* Genome-wide association study identifies 19p13.3 (*UNC13A*) and 9p21.2 as susceptibility loci for sporadic amyotrophic lateral sclerosis. *Nature Genet.* 6 Sep 2009 (doi:10.1038/ng.442).
88. Kaneko, K., Saito, F., Sunohara, N. & Ikeuchi, T. Cytogenetic analysis of 23 Japanese patients with amyotrophic lateral sclerosis. *Clin. Genet.* **47**, 158–160 (1995).
89. Meyer, T. *et al.* High rate of constitutional chromosomal rearrangements in apparently sporadic ALS. *Neurology* **60**, 1348–1350 (2003).
90. Blauw, H. M. *et al.* Copy-number variation in sporadic amyotrophic lateral sclerosis: a genome-wide screen. *Lancet Neurol.* **7**, 319–326 (2008).
This paper presents the first examination of potential CNVs in ALS.
91. Cronin, S. *et al.* Analysis of genome-wide copy number variation in Irish and Dutch ALS populations. *Hum. Mol. Genet.* **17**, 3392–3398 (2008).
92. Hazan, J. *et al.* Spastin, a new AAA protein, is altered in the most frequent form of autosomal dominant spastic paraplegia. *Nature Genet.* **23**, 296–303 (1999).
This study shows that mutations in *SPAST* are the most common cause of autosomal dominant HSP.
93. Hollenbeck, P. J. & Saxton, W. M. The axonal transport of mitochondria. *J. Cell Sci.* **118**, 5411–5419 (2005).
94. van Niekerk, E. A. *et al.* Sumoylation in axons triggers retrograde transport of the RNA-binding protein La. *Proc. Natl Acad. Sci. USA* **104**, 12913–12918 (2007).
95. Svenson, I. K., Ashley-Koch, A. E., Pericak-Vance, M. A. & Marchuk, D. A. A second leaky splice-site mutation in the spastin gene. *Am. J. Hum. Genet.* **69**, 1407–1409 (2001).
96. Solowska, J. M. *et al.* Quantitative and functional analyses of spastin in the nervous system: implications for hereditary spastic paraplegia. *J. Neurosci.* **28**, 2147–2157 (2008).
97. Reid, E. *et al.* The hereditary spastic paraplegia protein spastin interacts with the ESCRT-III complex-associated endosomal protein CHMP1B. *Hum. Mol. Genet.* **14**, 19–38 (2005).
98. Yang, D. *et al.* Structural basis for midbody targeting of spastin by the ESCRT-III protein CHMP1B. *Nature Struct. Mol. Biol.* **15**, 1278–1286 (2008).
99. Zhao, X. *et al.* Mutations in a newly identified GTPase gene cause autosomal dominant hereditary spastic paraplegia. *Nature Genet.* **29**, 326–331 (2001).
100. Namekawa, M. *et al.* SPG3A is the most frequent cause of hereditary spastic paraplegia with onset before age 10 years. *Neurology* **66**, 112–114 (2006).
This paper reports that mutations in *ATL1* account for ~10% of cases of autosomal dominant HSP.
101. Namekawa, M. *et al.* Mutations in the *SPG3A* gene encoding the GTPase atlastin interfere with vesicle trafficking in the ER/Golgi interface and Golgi morphogenesis. *Mol. Cell Neurosci.* **35**, 1–13 (2007).
102. Zhu, P. P., Soderblom, C., Tao-Cheng, J. H., Stadler, J. & Blackstone, C. SPG3A protein atlastin-1 is enriched in growth cones and promotes axon elongation during neuronal development. *Hum. Mol. Genet.* **15**, 1343–1353 (2006).
103. Evans, K. *et al.* Interaction of two hereditary spastic paraplegia gene products, spastin and atlastin, suggests a common pathway for axonal maintenance. *Proc. Natl Acad. Sci. USA* **103**, 10666–10671 (2006).
104. Reid, E. *et al.* A kinesin heavy chain (*KIF5A*) mutation in hereditary spastic paraplegia (SPG10). *Am. J. Hum. Genet.* **71**, 1189–1194 (2002).
105. Goizet, C. *et al.* Complicated forms of autosomal dominant hereditary spastic paraplegia are frequent in SPG10. *Hum. Mutat.* **30**, E376–E385 (2009).
106. Rainier, S., Chai, J. H., Tokarz, D., Nicholls, R. D. & Fink, J. K. *NIPA1* gene mutations cause autosomal dominant hereditary spastic paraplegia (SPG6). *Am. J. Hum. Genet.* **73**, 967–971 (2003).
107. Reed, J. A. *et al.* A novel *NIPA1* mutation associated with a pure form of autosomal dominant hereditary spastic paraplegia. *Neurogenetics* **6**, 79–84 (2005).
108. Goytain, A., Hines, R. M., El-Husseini, A. & Quamme, G. A. *NIPA1*(*SPG6*), the basis for autosomal dominant form of hereditary spastic paraplegia, encodes a functional Mg^{2+} transporter. *J. Biol. Chem.* **282**, 8060–8068 (2007).
109. Zhao, J. *et al.* Hereditary spastic paraplegia-associated mutations in the *NIPA1* gene and its *Caenorhabditis elegans* homolog trigger neural degeneration *in vitro* and *in vivo* through a gain-of-function mechanism. *J. Neurosci.* **28**, 13938–13951 (2008).
110. Wang, X., Shaw, W. R., Tsang, H. T., Reid, E. & O'Kane, C. J. *Drosophila* spichthyin inhibits BMP signaling and regulates synaptic growth and axonal microtubules. *Nature Neurosci.* **10**, 177–185 (2007).
111. Hanein, S. *et al.* Identification of the *SPG15* gene, encoding spastizin, as a frequent cause of complicated autosomal-recessive spastic paraplegia, including Kjellin syndrome. *Am. J. Hum. Genet.* **82**, 992–1002 (2008).
112. Patel, H. *et al. SPG20* is mutated in Troyer syndrome, an hereditary spastic paraplegia. *Nature Genet.* **31**, 347–348 (2002).
113. Simpson, M. A. *et al.* Maspardin is mutated in mast syndrome, a complicated form of hereditary spastic paraplegia associated with dementia. *Am. J. Hum. Genet.* **73**, 1147–1156 (2003).
114. Bakowska, J. C., Jupille, H., Fatheddin, P., Puertollano, R. & Blackstone, C. Troyer syndrome protein spartin is mono-ubiquitinated and functions in EGF receptor trafficking. *Mol. Biol. Cell* **18**, 1683–1692 (2007).
115. Hanna, M. C. & Blackstone, C. Interaction of the *SPG21* protein ACP33/maspardin with the aldehyde dehydrogenase ALDH16A1. *Neurogenetics* **10**, 217–228 (2009).
116. Hehr, U. *et al.* Long-term course and mutational spectrum of *spatacsin*-linked spastic paraplegia. *Ann. Neurol.* **62**, 656–665 (2007).

117. Stevanin, G. *et al.* Mutations in *SPG11*, encoding spatacsin, are a major cause of spastic paraplegia with thin corpus callosum. *Nature Genet.* **39**, 366–372 (2007).
118. Casari, G. *et al.* Spastic paraplegia and OXPHOS impairment caused by mutations in paraplegin, a nuclear-encoded mitochondrial metalloprotease. *Cell* **93**, 973–983 (1998).
119. Wilkinson, P. A. *et al.* A clinical, genetic and biochemical study of *SPG7* mutations in hereditary spastic paraplegia. *Brain* **127**, 973–980 (2004).
120. Koppen, M., Metodiev, M. D., Casari, G., Rugarli, E. I. & Langer, T. Variable and tissue-specific subunit composition of mitochondrial m-AAA protease complexes linked to hereditary spastic paraplegia. *Mol. Cell Biol.* **27**, 758–767 (2007).
121. Ferreirinha, F. *et al.* Axonal degeneration in paraplegin-deficient mice is associated with abnormal mitochondria and impairment of axonal transport. *J. Clin. Invest.* **113**, 231–242 (2004).
122. Hansen, J. J. *et al.* Hereditary spastic paraplegia SPG13 is associated with a mutation in the gene encoding the mitochondrial chaperonin Hsp60. *Am. J. Hum. Genet.* **70**, 1328–1332 (2002).
123. Hewamadduma, C. A. *et al. HSP60* is a rare cause of hereditary spastic paraparesis, but may act as a genetic modifier. *Neurology* **70**, 1717–1718 (2008).
124. Jouet, M. *et al.* X-linked spastic paraplegia (SPG1), MASA syndrome and X-linked hydrocephalus result from mutations in the *L1* gene. *Nature Genet.* **7**, 402–407 (1994).
125. Saugier-Veber, P. *et al.* X-linked spastic paraplegia and Pelizaeus–Merzbacher disease are allelic disorders at the proteolipid protein locus. *Nature Genet.* **6**, 257–262 (1994).
126. Hortsch, M. Structural and functional evolution of the L1 family: are four adhesion molecules better than one? *Mol. Cell Neurosci.* **15**, 1–10 (2000).
127. Inoue, K. *PLP1*-related inherited dysmyelinating disorders: Pelizaeus–Merzbacher disease and spastic paraplegia type 2. *Neurogenetics* **6**, 1–16 (2005).
128. Tsaousidou, M. K. *et al.* Sequence alterations within CYP7B1 implicate defective cholesterol homeostasis in motor-neuron degeneration. *Am. J. Hum. Genet.* **82**, 510–515 (2008).
129. Lin, P. *et al.* A missense mutation in *SLC33A1*, which encodes the acetyl-CoA transporter, causes autosomal-dominant spastic paraplegia (SPG42). *Am. J. Hum. Genet.* **83**, 752–759 (2008).
130. Hirabayashi, Y., Kanamori, A., Nomura, K. H. & Nomura, K. The acetyl-CoA transporter family SLC33. *Pflugers Arch.* **447**, 760–762 (2004).
131. Kanamori, A. *et al.* Expression cloning and characterization of a cDNA encoding a novel membrane protein required for the formation of O-acetylated ganglioside: a putative acetyl-CoA transporter. *Proc. Natl Acad. Sci. USA* **94**, 2897–2902 (1997).
132. Orthmann-Murphy, J. L. *et al.* Hereditary spastic paraplegia is a novel phenotype for *GJA12/GJC2* mutations. *Brain* **132**, 426–438 (2009).
133. Valdmanis, P. N. *et al.* Mutations in the *KIAA0196* gene at the *SPG8* locus cause hereditary spastic paraplegia. *Am. J. Hum. Genet.* **80**, 152–161 (2007).
134. Windpassinger, C. *et al.* Heterozygous missense mutations in *BSCL2* are associated with distal hereditary motor neuropathy and Silver syndrome. *Nature Genet.* **36**, 271–276 (2004).
135. Bohan, T. P. & Azizi, P. Allan–Herndon–Dudley syndrome: should the locus for this hereditary spastic paraplegia be designated SPG 22? *Arch. Neurol.* **61**, 1470–1471 (2004).
136. Beetz, C. *et al. REEP1* mutation spectrum and genotype/phenotype correlation in hereditary spastic paraplegia type 31. *Brain* **131**, 1078–1086 (2008).
137. Zuchner, S. *et al.* A new locus for dominant hereditary spastic paraplegia maps to chromosome 2p12. *Neurogenetics* **7**, 127–129 (2006).
138. Rainier, S. *et al.* Neuropathy target esterase gene mutations cause motor neuron disease. *Am. J. Hum. Genet.* **82**, 780–785 (2008).
139. Gordon, P. H., Cheng, B., Katz, I. B., Mitsumoto, H. & Rowland, L. P. Clinical features that distinguish PLS, upper motor neuron-dominant ALS, and typical ALS. *Neurology* **72**, 1948–1952 (2009).
140. Valdmanis, P. N., Dupre, N. & Rouleau, G. A. A locus for primary lateral sclerosis on chromosome 4ptel–4p16.1. *Arch. Neurol.* **65**, 383–386 (2008). **The authors describe a French-Canadian family with PLSA1 that maps to chromosome 4p.**
141. Mintchev, N., Zamba-Papanicolaou, E., Kleopa, K. A. & Christodoulou, K. A novel *ALS2* splice-site mutation in a Cypriot juvenile-onset primary lateral sclerosis family. *Neurology* **72**, 28–32 (2009).
142. Panzeri, C. *et al.* The first *ALS2* missense mutation associated with JPLS reveals new aspects of alsin biological function. *Brain* **129**, 1710–1719 (2006).
143. Yang, Y. *et al.* The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. *Nature Genet.* **29**, 160–165 (2001).
144. Lorson, C. L. *et al.* SMN oligomerization defect correlates with spinal muscular atrophy severity. *Nature Genet.* **19**, 63–66 (1998).
145. Monani, U. R. *et al.* A single nucleotide difference that alters splicing patterns distinguishes the SMA gene *SMN1* from the copy gene *SMN2*. *Hum. Mol. Genet.* **8**, 1177–1183 (1999).
146. Lorson, C. L. & Androphy, E. J. An exonic enhancer is required for inclusion of an essential exon in the SMA-determining gene *SMN*. *Hum. Mol. Genet.* **9**, 259–265 (2000).
147. Pellizzoni, L., Kataoka, N., Charroux, B. & Dreyfuss, G. A novel function for SMN, the spinal muscular atrophy disease gene product, in pre-mRNA splicing. *Cell* **95**, 615–624 (1998).
148. Lefebvre, S. *et al.* Identification and characterization of a spinal muscular atrophy-determining gene. *Cell* **80**, 155–165 (1995). **This paper shows that loss-of-function mutations or deletions of the *SMN1* gene cause SMA.**
149. Corcia, P. *et al.* The importance of the *SMN* genes in the genetics of sporadic ALS. *Amyotroph. Lateral Scler.* 6 Mar 2009 (doi:10.1080/17482960902759162).
150. La Spada, A. R., Wilson, E. M., Lubahn, D. B., Harding, A. E. & Fischbeck, K. H. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature* **352**, 77–79 (1991). **The authors identified a trinucleotide CAG repeat expansion in the first exon of the *AR* gene as being responsible for SBMA.**
151. Katsuno, M. *et al.* Leuprorelin rescues polyglutamine-dependent phenotypes in a transgenic mouse model of spinal and bulbar muscular atrophy. *Nature Med.* **9**, 768–773 (2003).
152. Makela-Bengs, P. *et al.* Assignment of the disease locus for lethal congenital contracture syndrome to a restricted region of chromosome 9q34, by genome scan using five affected individuals. *Am. J. Hum. Genet.* **63**, 506–516 (1998).
153. Nousiainen, H. O. *et al.* Mutations in mRNA export mediator GLE1 result in a fetal motoneuron disease. *Nature Genet.* **40**, 155–157 (2008).
154. Narkis, G. *et al.* Lethal contractural syndrome type 3 (LCCS3) is caused by a mutation in *PIP5K1C*, which encodes PIPKI gamma of the phophatidylinsitol pathway. *Am. J. Hum. Genet.* **81**, 530–539 (2007).
155. Narkis, G. *et al.* Lethal congenital contractural syndrome type 2 (LCCS2) is caused by a mutation in *ERBB3* (*Her3*), a modulator of the phosphatidylinositol-3-kinase/Akt pathway. *Am. J. Hum. Genet.* **81**, 589–595 (2007).
156. Chow, C. Y. *et al.* Deleterious variants of *FIG4*, a phosphoinositide phosphatase, in patients with ALS. *Am. J. Hum. Genet.* **84**, 85–88 (2009).
157. Yeo, G., Holste, D., Kreiman, G. & Burge, C. B. Variation in alternative splicing across human tissues. *Genome Biol.* **5**, R74 (2004).
158. Salinas, S., Proukakis, C., Crosby, A. & Warner, T. T. Hereditary spastic paraplegia: clinical features and pathogenetic mechanisms. *Lancet Neurol.* **7**, 1127–1138 (2008).
159. Boillee, S., Vande Velde, C. & Cleveland, D. W. ALS: a disease of motor neurons and their nonneuronal neighbors. *Neuron* **52**, 39–59 (2006).
160. Lin, M. T. & Beal, M. F. Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. *Nature* **443**, 787–795 (2006).
161. Hirano, A., Donnenfeld, H., Sasaki, S. & Nakano, I. Fine structural observations of neurofilamentous changes in amyotrophic lateral sclerosis. *J. Neuropathol. Exp. Neurol.* **43**, 461–470 (1984).
162. Hirano, A. *et al.* Fine structural study of neurofibrillary changes in a family with amyotrophic lateral sclerosis. *J. Neuropathol. Exp. Neurol.* **43**, 471–480 (1984).
163. Khabazian, I. *et al.* Isolation of various forms of sterol β-D-glucoside from the seed of *Cycas circinalis*: neurotoxicity and implications for ALS–parkinsonism dementia complex. *J. Neurochem.* **82**, 516–528 (2002).
164. Horner, R. D. *et al.* Occurrence of amyotrophic lateral sclerosis among Gulf War veterans. *Neurology* **61**, 742–749 (2003).
165. Li, C. Y. & Sung, F. C. Association between occupational exposure to power frequency electromagnetic fields and amyotrophic lateral sclerosis: a review. *Am. J. Ind. Med.* **43**, 212–220 (2003).
166. Qureshi, M. M. *et al.* Analysis of factors that modify susceptibility and rate of progression in amyotrophic lateral sclerosis (ALS). *Amyotroph. Lateral Scler.* **7**, 173–182 (2006).
167. Doi, H. *et al.* Motor neuron disorder simulating ALS induced by chronic inhalation of pyrethroid insecticides. *Neurology* **67**, 1894–1895 (2006).
168. Weisskopf, M. G. *et al.* Prospective study of chemical exposures and amyotrophic lateral sclerosis. *J. Neurol. Neurosurg. Psychiatry* **80**, 558–561 (2009).
169. Chio, A., Benzi, G., Dossena, M., Mutani, R. & Mora, G. Severely increased risk of amyotrophic lateral sclerosis among Italian professional football players. *Brain* **128**, 472–476 (2005).
170. Chio, A. *et al.* ALS in Italian professional soccer players: the risk is still present and could be soccer-specific. *Amyotroph. Lateral Scler.* **10**, 205–209 (2009).
171. Valenti, M. *et al.* Amyotrophic lateral sclerosis and sports: a case–control study. *Eur. J. Neurol.* **12**, 223–225 (2005).
172. Chen, H., Richard, M., Sandler, D. P., Umbach, D. M. & Kamel, F. Head injury and amyotrophic lateral sclerosis. *Am. J. Epidemiol.* **166**, 810–816 (2007).
173. Weisskopf, M. G. *et al.* Prospective study of cigarette smoking and amyotrophic lateral sclerosis. *Am. J. Epidemiol.* **160**, 26–33 (2004).
174. Santos-Rebouças, C. B. & Pimentel, M. M. Implication of abnormal epigenetic patterns for human diseases. *Eur. J. Hum. Genet.* **15**, 10–17 (2007).
175. Migliore, L. & Coppedè, F. Genetics, environmental factors and the emerging role of epigenetics in neurodegenerative diseases. *Mutat. Res.* **667**, 82–97 (2008).
176. Oates, N. & Pamphlett, R. An epigenetic analysis of SOD1 and VEGF in ALS. *Amyotroph. Lateral Scler.* **8**, 83–86 (2007).
177. Morahan, J. M., Yu, B., Trent, R. J. & Pamphlett, R. Are metallothionein genes silenced in ALS? *Toxicol. Lett.* **168**, 83–87 (2007).

Acknowledgements

The authors wish to thank C. Vande Velde and I. A. Meijer for their careful and insightful reading of the manuscript; their ideas and suggestions were welcomed and appreciated. G.A.R. has received MND-related funding from the Canadian Institutes of Health, the Muscular Dystrophy Association ALS Division, the ALS Association and the ALS Society of Canada.

DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene

ALS2 | *ANG* | *CHMP2B* | *FUS* | *GRN* | *MAPT* | *SETX* | *SOD1* | *TARDBP* | *VAPB* | *VCP*

FURTHER INFORMATION

1000 Genomes Project: http://www.1000genomes.org

SUPPLEMENTARY INFORMATION

See online article: S1 (table)

ALL LINKS ARE ACTIVE IN THE ONLINE PDF