Genetics of Amyotrophic Lateral Sclerosis

Mehdi Ghasemi and Robert H. Brown, Jr.

Department of Neurology, University of Massachusetts Medical School, Worcester, Massachusetts 01655

Correspondence: robert.brown@umassmed.edu

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Amyotrophic lateral sclerosis (ALS) is a devastating, uniformly lethal degenerative disorder of motor neurons that overlaps clinically with frontotemporal dementia (FTD). Investigations of the 10% of ALS cases that are transmitted as dominant traits have revealed numerous gene mutations and variants that either cause these disorders or influence their clinical phenotype. The evolving understanding of the genetic architecture of ALS has illuminated broad themes in the molecular pathophysiology of both familial and sporadic ALS and FTD. These central themes encompass disturbances of protein homeostasis, alterations in the biology of RNA binding proteins, and defects in cytoskeletal dynamics, as well as numerous downstream pathophysiological events. Together, these findings from ALS genetics provide new insight into therapies that target genetically distinct subsets of ALS and FTD.

Amyotrophic lateral sclerosis (ALS or Lou Gehrig's disease) is a devastating neurodegenerative disorder that affects lower motor neurons (LMNs) in the brainstem and spinal cord and upper motor neurons (UMNs) in the motor cortex, which are predominantly large, layer V neurons that comprise the corticospinal tract. The core clinical phenotype is characterized by (1) focal onset of weakness that progresses to involve all of the limbs and bulbar muscles, leading ultimately to total paralysis and death from respiratory failure, and (2) hyperreflexia. At autopsy, degeneration of corticospinal ("upper") and spinal and bulbar ("lower") motor neurons is often associated with activation of neuroimmune cells within the central nervous system (CNS) (microglia, astrocytes, and oligodendroglia) (Kang et al. 2013; Philips and Rothstein 2014). In ~15% of ALS patients, neurons in the prefrontal and temporal cortex are also variably affected (Ringholz et al. 2005), leading to frontal executive dysfunction, speech production disorders, and concurrent frontotemporal dementia (FTD). ALS and FTD can be viewed as divergent ends of the spectrum of a single heterogeneous disease. Moreover, although a combination of UMN and LMN findings characterizes classic ALS, the continuum of disease ranges from exclusively UMN (primary lateral sclerosis or PLS) to exclusively LMN involvement (progressive muscular atrophy or PMA). As discussed below, some cases of ALS with FTD occur with other multisystem manifestations, including pathology in muscle and bone. Rarely, "ALS-plus" syndromes are encountered in which ALS patients also develop autonomic nervous system involvement, extrapyramidal signs, cerebellar findings, and sensory abnormalities in addition to classic ALS signs.

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ALS has a worldwide incidence of about two cases per 100,000 per year and a prevalence of about five cases per 100,000. In the United States, ALS affects about 25,000 individuals and thus formally is classified as an "orphan" disease (fewer than 200,000 affected cases) (Rowland and Shneider 2001). However, it is remarkable that ALS accounts for about one in 500 adult deaths, a statistic that predicts that more than 500,000 individuals now in the United States will succumb to this disease. Onset typically occurs between 55 and 60 years of age, with wide-ranging severity. Median survival is 27.5 mo from symptom onset (Robberecht and Philips 2013); the 4-year survival rate is ~40%. The major cause of death is respiratory failure due to respiratory muscle weakness.

Although most cases of ALS are sporadic (sALS), ~10% are familial (fALS), with autosomal dominant transmission. Rarely, fALS may be transmitted as either X-linked or recessive traits (Andersen and Al-Chalabi 2011; Deng et al. 2011). fALS and sALS share similar clinical presentations; either can develop concurrently with FTD.

Efforts to identify Mendelian genes whose mutations cause ALS have identified more than 100 gene mutations that increase susceptibility to ALS or modify its phenotype (Fig. 1A,B) (Andersen and Al-Chalabi 2011; Peters et al. 2015b). Multiple genetic variants may interact simultaneously to increase ALS susceptibility; such oligogenic cases of ALS may not appear to be familial in a conventional Mendelian manner yet may underlie the apparently sporadic form of the disease (Luigetti et al. 2011; Chio et al. 2012; van Blitterswijk et al. 2012; Pottier et al. 2015). Analysis of pathways implicated by mutant ALS genes has provided new insights into the pathogenesis of both fALS and sALS, although, regrettably, this has not yet yielded definitive treatments.

As summarized in Figure 2, three themes have emerged from the investigations of the molecular biology of diverse mutant ALS genes in fALS and sALS.

1. Analyses of one set of genes encoding a range of proteins (e.g., cytosolic superoxide dismutase or adaptor proteins that mediate protein quality control such as ubiquilin-2) indicate that motor neuron death in ALS involves misfolding, aggregation, and deposition of proteins that are often ubiquitinated and predominantly cytoplasmic. A growing literature suggests that the mutant proteins may induce misfolding of their wild-type (WT) counterparts and thus have prion-like properties.
2. Another set of genes (e.g., TAR DNA-binding protein [TDP-43], fused in sarcoma/translocated in liposarcoma [FUS/TLS]) encodes proteins that are implicated in interacting with RNA and DNA and illustrates the principle that perturbations in multiple aspects of RNA function and homeostasis are toxic to motor neurons. Particularly exciting in this gene set is the demonstration of nonnuclear localization and function of protein-bound RNA, with roles in axonal transport and local protein translation in dendrites and neuromuscular junctions.
3. In most cases, there are disturbances of cytoskeletal architecture and dynamic function of distal axons and dendrites.

Three additional points regarding this formulation deserve mention. First, elegant studies document that the non-cell-autonomous, neuroinflammatory aspect of ALS can accelerate the disease course. Yet, to date, every ALS gene is expressed in motor neurons; none of the known ALS genes are predominantly expressed in nonneural cells. This suggests that expression of a mutant ALS gene within the motor neuron is the sine qua non of motor neuron degeneration in fALS. Second, these themes in pathogenesis are not mutually exclusive; mutations in RNA-binding proteins from category 2 predispose the proteins to self-assembly and to propagating aggregation, as described in category 1. Third, regardless of how the primary gene defects are categorized, they all are associated with common downstream pathologic processes, including induction of endoplasmic reticulum (ER) stress, accelerated autophagy, mitochondrial dysfunction, altered axonal transport, disturbed

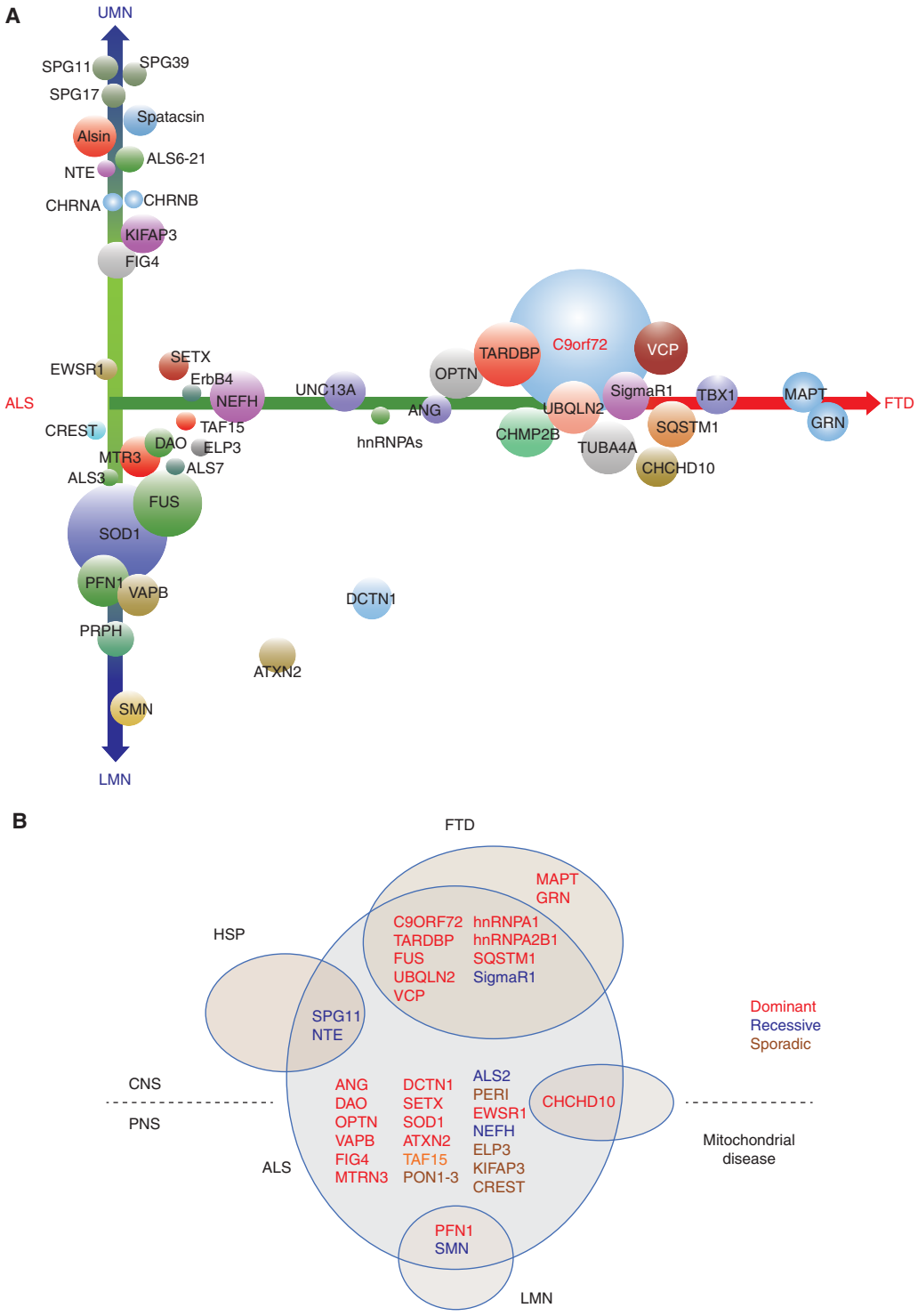

Figure 1. The phenotypic spectrum of amyotrophic lateral sclerosis (ALS) genetics. (*A*) Forty-six ALS-related genes are arrayed along axes that depict two major phenotypic aspects: the extent to which corticospinal versus lower motor neurons are involved (*y*-axis) and the overlap with frontotemporal dementia (FTD) (*x*-axis). The diameters of each gene approximate their relative frequencies. (*B*) The phenotypic overlap of ALS genes with hereditary spastic paraplegia (HSP), frontotemporal dementia (FTD), mitochondrial disease, and lower motor neuropathies (LMN) is shown.

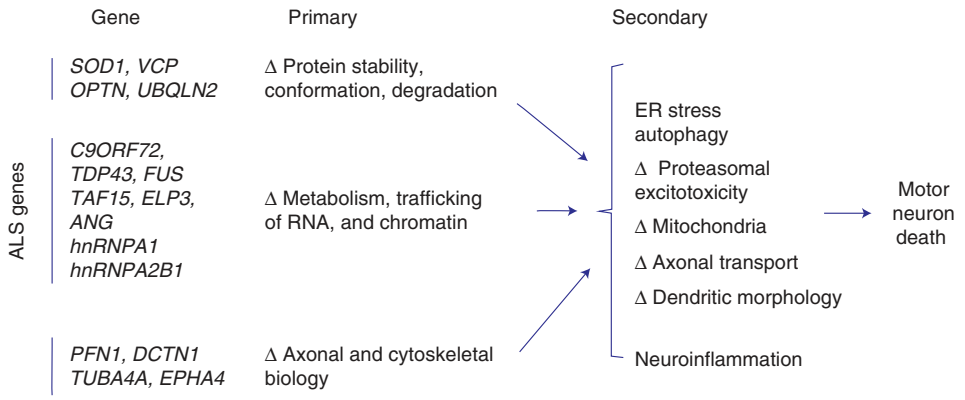

Figure 2. The common ALS genes define three primary themes in pathophysiology: conformational instability and aggregation of proteins, impaired trafficking of RNA, and altered cytoskeletal dynamics. These converge on multiple secondary, downstream pathologic processes that include activation of endoplasmic reticulum (ER) stress and autophagy, proteasomal dysfunction, altered mitochondrial function, disturbed axonal transport, altered dendritic morphology, and excitotoxicity.

cytoskeletal dynamics in dendrites and growth cones, and altered membrane excitability (Vucic et al. 2014). A detailed discussion of these diverse but important secondary phenomena, which are also evident in sALS, is beyond the scope of this review. Table 1 provides a detailed summary of the major ALS genes; a full listing can be found at alsod.iop.kcl.ac.uk.

OVERVIEW OF ALS GENETICS

Adult-Onset ALS Genes Transmitted as Mendelian Traits

Genes Implicated in Disturbances of Protein Homeostasis

Superoxide Dismutase. The first ALS gene, cytosolic copper-zinc superoxide dismutase (SOD1), was identified in 1993 (Fig. 3) (Rosen et al. 1993). In most families harboring *SOD1* gene mutations, disease penetrance is >90% by age 70 (Cudkowicz et al. 1997). More than 170 mutations have now been detected in the *SOD1* gene in fALS (Abel et al. 2012); together these account for ~20% of fALS cases. Almost all of these mutations are missense changes, scattered across the coding sequence without focal mutation hotspots. A few mutations truncate the terminal segment of the SOD1 protein; none of the ALS-related mutations in SOD1 are predicted to eliminate production of the protein. This strongly suggests that the mutant protein must be present to initiate motor neuron death.

The clinical phenotype associated with SOD1 mutations tends to be only ALS without FTD or other neurological features. In North America, the most common mutation is a substitution of valine for alanine in codon 4 (A4V). This is associated with an extremely aggressive course, with survival in many cases only 11–12 mo; both clinically and pathologically, these individuals usually show relatively little evidence of corticospinal tract involvement (Cudkowicz et al. 1997, 1998).

Although it is distinctly uncommon, there are some instances in which SOD1-related ALS is transmitted as a recessive trait. In northern Scandinavia, the variant D90A is present in ~1% of the population; in that setting, the mutation only triggers ALS when present homozygously (Andersen et al. 1995). This suggests that there is a dose effect; more of the mutant gene product is required to initiate motor neuron degeneration in this population. The clinical phenotype of ALS associated with the D90A/D90A genotype in northern Sweden is also distinctive, with very slow progression, long survival (12–14 yr), and a predominance of lower limb spasticity at onset. It is intriguing that the D90A mutation can cause ALS as a

Table 1. Gene mutations that cause ALS

Gene	Fraction fALS (%)	Locus	Encoded protein	Functionality	Clinical phenotype	Neuropathology	Reference(s)
C9ORF72	40–50	9p21.3	C9ORF72	Transcription and pre-mRNA splicing regulation; membrane traffic via Rab GTPase family	ALS; ALS+ FTLD; FTLD	NCI; DN; GCI; intranuclear RNA foci (sense, antisense); cytoplasmic RNA peptide aggregates	DeJesus-Hernandez et al. 2011; Renton et al. 2011; Cooper-Knock et al. 2012; Diekstra et al. 2014
SOD1	20–25	21q22	SOD1	Major cytosolic antioxidant	ALS; PMA	NCI; NII; DN; GCI; aggregates—p62, C9ORF72, ubiquilin 2, others; impaired axonal transport, mitochondrial function; disturbed dendritic arborization of neurons; oxidative stress-related neuronal toxicity	Rosen et al. 1993; Watanabe et al. 2001; Forsberg et al. 2011
TARDBP	4–5	1p36.2	TDP-43	Transcription and pre-mRNA splicing regulation; micRNA biogenesis; RNA transport and stabilization; translational regulation of *ApoE-II* and *CFTR*	ALS; ALS+ FTLD; FTLD	NCI; NII; DN; GCI	Rutherford et al. 2008; Sreedharan et al. 2008; Neumann 2009
FUS	4–5	16p11.2	FUS (or TLS)	Transcription and pre-mRNA splicing regulation; micRNA processing; mRNA transport and stabilization; maintenance of genomic integrity; regulating protein synthesis at synapse	ALS; ALS+ FTLD; FTLD	NCI; DN; GCI	Kwiatkowski et al. 2009; Vance et al. 2009; Huang et al. 2010
OPTN	2–3	10p13	Optineurin	Golgi maintenance; exocytosis; vesicular trafficking; regulator of NF-kB signaling pathway; autophagy process	ALS; ALS+ FTLD	NCI; ↑ TDP-43, FUS, and SOD1 aggregates	Maruyama et al. 2010
PFN1	1–2	17p13	Profilin 1	Regulates ATP-mediated actin polymerization	ALS	NCI; ↓ axonal distension and growth cone elongation; co-aggregation with TDP-43	Wu et al. 2012

Continued

Table 1. *Continued*

Gene	Fraction fALS (%)	Locus	Encoded protein	Functionality	Clinical phenotype	Neuropathology	Reference(s)
VCP	1–2	9p13	VCP or p97	Protein degradation via UPS, autophagy, and the ER; membrane fusion	ALS; ALS+ FTLD; FTLD	NCI; NII; DN; ↑ TDP-43 aggregates; ↓ stress-granule clearance	Forman et al. 2006; Kimonis et al. 2008; Johnson et al. 2010
ANG	1–2	14q11.2	Angiogenin	RNA processing and tRNA modification; vascularization; RNAase activity and assembly of stress granules; neurite outgrowth and pathfinding	ALS; ALS+ FTLD	↓ Stress-granule formation in motor neurons	Greenway et al. 2006; Thiyagarajan et al. 2012
TUBA4A	1	2q35	Tubulin α4A	Major component of microtubules; neuronal cell skeleton	ALS; ALS+ FTLD	NCI; destabilized microtubule network; ↓ microtubules repolymerization capability	Smith et al. 2014
UBQLN2	<1	Xp11	Ubiquilin 2	Protein degradation via UPS	ALS; ALS+ FTLD; FTLD (Rare)	NCI; ↑ TDP-43, p62, FUS, and OPTN inclusions	Deng et al. 2011; Ugwu et al. 2014
TAF15	<1	17q11	TAF15	Transcription initiation; RNA polymerase II gene component	ALS	NCI	Couthouis et al. 2011
EWSR1	<1	22q12.2	EWSR1	Transcriptional repressor	ALS	NCI; DN	Couthouis et al. 2012
hnRNPA1	<1	12q13	hnRNPA1	Packing and transport of mRNA; micRNA biogenesis	Rare ALS; ALS+ FTLD[a]; FTLD (rare)	NCI	Bekenstein and Soreq 2013; Kim et al. 2013; Le Ber et al. 2014
hnRNPA2B1	<1	7p15	hnRNPA2/B1	Packing and transport of mRNA; micRNA biogenesis	ALS+ FTLD (rare)[a]; FTLD (rare)	NCI	Kim et al. 2013; Le Ber et al. 2014
SETX	<1	9q34.13	Senataxin	DNA/RNA helicase activity; DNA/RNA metabolism	ALS[b]	↓ neuronal differentiation; ↓ neurite growth	Chen et al. 2004

Continued

Table 1. *Continued*

Gene	Fraction fALS (%)	Locus	Encoded protein	Functionality	Clinical phenotype	Neuropathology	Reference(s)
CREST	<1	20q13.3	SS18L1	Ca^{2+}-dependent transcriptional activator	ALS	DN; ↓ dendrite outgrowth; ↑ interaction with FUS	Chesi et al. 2013; Teyssou et al. 2014
MATR3	<1	5q31.2	Matrin 3	RNA processing; stabilizing mRNAs; gene silencing; chromatin organization	ALS; ALS+ FTLD (Rare)	NCI; NII; ↑ interaction with TDP-43	Johnson et al. 2014b; Millecamps et al. 2014
ATXN2	1-2	12q24	Ataxin 2	RNA processing; regulation of receptor tyrosine kinase endocytosis	ALS; ALS+ FTLD; PMA	NCI; ↑ interaction with TDP-43	Elden et al. 2010; Baumer et al. 2014
ELP3	<1	8p21.1	ELP3	RNA processing; transcript elongation; histone acetylation; modification of tRNA wobble nucleosides	ALS	NCI; abnormal branching in motor axons; co-localization with TDP-43 and FUS aggregates	Simpson et al. 2009; Fujita et al. 2014
SQSTM1	<1	5q35	p62 or SQSTM1	Autophagy and UPS degradation; regulator of NF-kB signaling pathway; immune response	ALS; ALS+ FTLD; FTLD	NCI; NII; GCI; ↓ mutSOD1 autophagic degradation	Gal et al. 2009; Fecto et al. 2011; Rubino et al. 2012
CHMP2B	<1	3p11	CHMP2B	MVBs formation; protein trafficking between plasma membrane, trans-Golgi network, and lysosome	ALS[c]; PMA[d]; FTLD	NCI; DN; GCI; disrupted endosomal structure; aggregates of autophagosomes and multilamellar structures; ↑ TDP-43, p62, and ubiquitin inclusions	Cannon et al. 2006; Parkinson et al. 2006
ALS2	<1	2q33.1	Alsin	Activation of the small GTPase Rac1 macropinocytosis-associated endosome fusion and trafficking; neurite outgrowth	ALS[e]; PLS	↓ axonal growth; ↓ lysosome-dependent clearance of p62 and LC3-II	Hadano et al. 2001; Yang et al. 2001; Otomo et al. 2008
VAPB	<1	20q13	VAPB	Regulation of ER–Golgi transport and secretion	ALS; PLS; PMA	NCI; ↑ TDP-43 toxicity and inclusions; aberrant synaptic microtubule cytoskeleton; nuclei mispositioning and aberrant architecture	Nishimura et al. 2004; Sanhueza et al. 2014

Continued

Table 1. *Continued*

Gene	Fraction fALS (%)	Locus	Encoded protein	Functionality	Clinical phenotype	Neuropathology	Reference(s)
SIGMAR1	<1	9p13.3	SIGMAR1	Lipid transport through ER; BDNF and EGF signaling	ALS; ALS+ FTLD; FTLD	NCI; ↑ apoptosis induced by ER stress; ↑ interaction with VAPB	Al-Saif et al. 2011; Belzil et al. 2013; Prause et al. 2013
DCTN1	<1	2p13	Dynactin	ER–Golgi transport; centripetal movement of lysosomes and endosomes; spindle formation, chromosome movement; nuclear positioning; axonogenesis	ALS	NCI; $p150^{glued}$ aggregation; ↑ SOD1 aggregates	Munch et al., 2004
FIG4	<1	6q21	$PI_{3,5}P_2$	Phosphoinositide phosphatase activity; endosomal vesicle trafficking to the trans-Golgi network; regulation of autophagy	ALS; PLS	NCI; ↑ swollen intracellular vacuoles; ↑ LC3-II, p62, and LAMP-2 aggregates in neurons and astrocytes	Zhang et al. 2007; Chow et al. 2009
SPG11	1	15q21.1	Spatascin	Neuronal cell skeleton; axonal transport; involved in synaptic vesicles	ALS; HSP	NCI; DN; ↓ acetylated stabilized tubulin; ↓ synaptic vesicles in neurites; disrupted anterograde axonal transport	Stevanin et al. 2007; Orlacchio et al. 2010; Manole et al. 2016
NEFH	<1	22q12.2	NEFH	Maintaining axon diameter	ALS	NCI; ↑ neurofilament aggregates	Figlewicz et al. 1994
PRPH	<1	12q13	Peripherin	Regulating neurite elongation during development and axonal regeneration after injury	ALS	NCI; DN; ↓ ability of the neurofilament network to assemble; ↑ ubiquitinated inclusions; coaggregation with mutSOD1	Beaulieu et al. 1999; Julien and Beaulieu 2000; Gros-Louis et al. 2004
NTE	<1	19p13	Neuropathy target esterase	Regulating the neuronal membrane composition	ALS; HSP	Disruption of ER; ↑ reticular aggregates; ↑ vacuolization of nerve cell bodies	Akassoglou et al. 2004; Rainier et al. 2008; Song et al. 2013
PON1-3	<1	7q21	Paraoxonase 1-3	Enzymatic breakdown of nerve toxins	ALS	Oxidative stress-related neuronal toxicity	Saeed et al. 2006; Slowik et al. 2006; Wills et al. 2009

Continued

Table 1. *Continued*

Gene	Fraction fALS (%)	Locus	Encoded protein	Functionality	Clinical phenotype	Neuropathology	Reference(s)
DAO	<1	12q22	DAO	Regulating levels of D-serine, NMDAR function	ALS	NCI; NII; ↑ D-serine levels in motor neurons and glia; ↑ ubiquitinated inclusions	Mitchell et al. 2010
CHRNA3, *CHRNA4*, *CHRNB4*	<1	15q24, 20q13, 15q24	nAChR	Cholinergic neurotransmission	ALS[§]	Cationic overloading, Ca^{+2} toxicity in MNs	Sabatelli et al. 2009, 2012; Moriconi et al. 2011)
ERBB4	<1	2q34	Receptor tyrosine-protein kinase ErbB-4	Neuronal cell mitogenesis and differentiation	ALS		Takahashi et al. 2013
CHCHD10	<1	22q11	Mitochondrial protein	Mitochondrial genome stability; cristae integrity and mitochondrial fusion	ALS+ FTLD	Mitochondrial fragmentation and DNA instability; mitochondrial crystalloid inclusions	Bannwarth et al. 2014; Chaussenot et al. 2014; Johnson et al. 2014a
C19orf12	<1	9q12	Mitochondrial protein	Unknown	ALS		Deschauer et al. 2012
ALS3	<1	18q21	Disulfide redox protein	Unknown	ALS		Hand et al. 2002
ALS7	<1	20p13	Unknown	Unknown	ALS		Hand et al. 2002
ALS6-21	<1	6p25, 21q22	Unknown	Unknown	ALS[§]		Butterfield et al. 2009
ALS-FTD	<1	16p12	Unknown	Unknown	ALS+ FTLD	NCI; DN; Type B TDP pathology; phosphorylated tau pathology	Dobson-Stone et al. 2013
Gene variants that influence ALS phenotype							
UNC13A		19p13	Unc-13 homolog A	Regulating neurite outgrowth and synaptic neurotransmission	ALS; ALS+ FTLD	↓ synaptogenesis at neuromuscular junction; possible glutamate excitotoxicity	Varoqueaux et al. 2005; van Es et al. 2009; Diekstra et al. 2014

Continued

Table 1. *Continued*

Gene	Fraction fALS (%)	Locus	Encoded protein	Functionality	Clinical phenotype	Neuropathology	Reference(s)
EPHA4		2q36.1	Ephrin receptor A4	Receptor tyrosine kinase activity Modulation of cell morphology and integrin-dependent cell adhesion; regulation of synaptic plasticity and CNS development	ALS	NII; neurite outgrowth deficits in mutant TDP-43 expressed neurons	Van Hoecke et al. 2012; Uyan et al. 2013
CHGB		20p12.3	CHGB	Involved in the ER–Golgi system	ALS	NCI; ↓ density of synaptophysin-like immunoreactivity; ↑ interaction with mutSOD1	Gros-Louis et al. 2009; Schrott-Fischer et al. 2009
KIFAP3		1q24.2	Kinesin-associated protein 3	Tethering chromosomes to spindle pole; chromosome movement; axonal transport of choline acetyl-transferase	ALS[§]	NCI; KIFAP3-SOD1 coaggregation in Lewy-body-like hyaline inclusions	Landers et al. 2009; Tateno et al. 2009; Orsetti et al. 2011
SMN		5q13	Germin 1	Regulating biogenesis of snRNPs	ALS; LMN	NCI; NII; DN; coaggregation with mutFUS, mutSOD1; axonal defects	Jackson et al. 1996; Moulard et al. 1998; Gertz et al. 2012; Groen et al. 2013

ALS, amyotrophic lateral sclerosis; BDNF, brain derived neurotrophic factor; C9ORF72, chromosome 9 open reading frame 72; CHGB, chromogranin B (secretogranin 1); CHMP2B, charged multivesicular body protein 2B; DAO, D-amino acid oxidase; DN, dystrophic neurites; EGF, epidermal growth factor; ELP3, elongator acetyltransferase complex subunit 3; ER, endoplasmic reticulum; EWSR1, Ewing sarcoma breakpoint region 1; FTLD, frontotemporal lobe dementia; FUS, fused in sarcoma; GCIs, glial cell inclusions; hnRNPA1, heterogeneous nuclear ribonucleoprotein A1; hnRNPA2B1, heterogeneous nuclear ribonucleoproteins A2/B1; HSP, hereditary spastic paraplegia; LAMP-2, lysosomal-associated membrane protein 2; LC3-II, microtubule-associated protein 1A/1B-light chain 3-II; LMN, lower motor neuron disease; micRNA, micro RNA; mRNA, messenger RNA; mutSOD1, mutant superoxide dismutase 1; MVBs, multivesicular bodies; nAChR, nicotinic acetylcholine receptor; NCI, neuronal cytoplasmic inclusions; NEFH, neurofilament heavy chain; NII, neuronal intranuclear inclusions; NMDAR, *N*-methyl-D-aspartate receptor; $PI_{3,5}P_2$, phosphatidylinositol 3,5-bisphosphate 5-phosphatase; PLS, primary lateral sclerosis; PMA, progressive muscular atrophy; SIGMAR1, σ non-opioid intracellular receptor 1; SQSTM1, sequestosome 1; SS18L1, synovial sarcoma translocation gene on chromosome 18-Like 1; TAF15, TATA box binding protein-associated factor 15; TDP-43, TAR DNA-binding protein; TLS, translocated in liposarcoma; UMN, upper motor neuron; UPS, ubiquitin-proteasome system; VAPB, vesicle-associated membrane protein B; VCP, valosin-containing protein.

[a]As part of multisystem proteinopathy.

[b]Phenotype more similar to Silver syndrome than to ALS.

[c]Predominant LMN phenotype.

[d]As part of ALS.

[e]Predominant UMN phenotype.

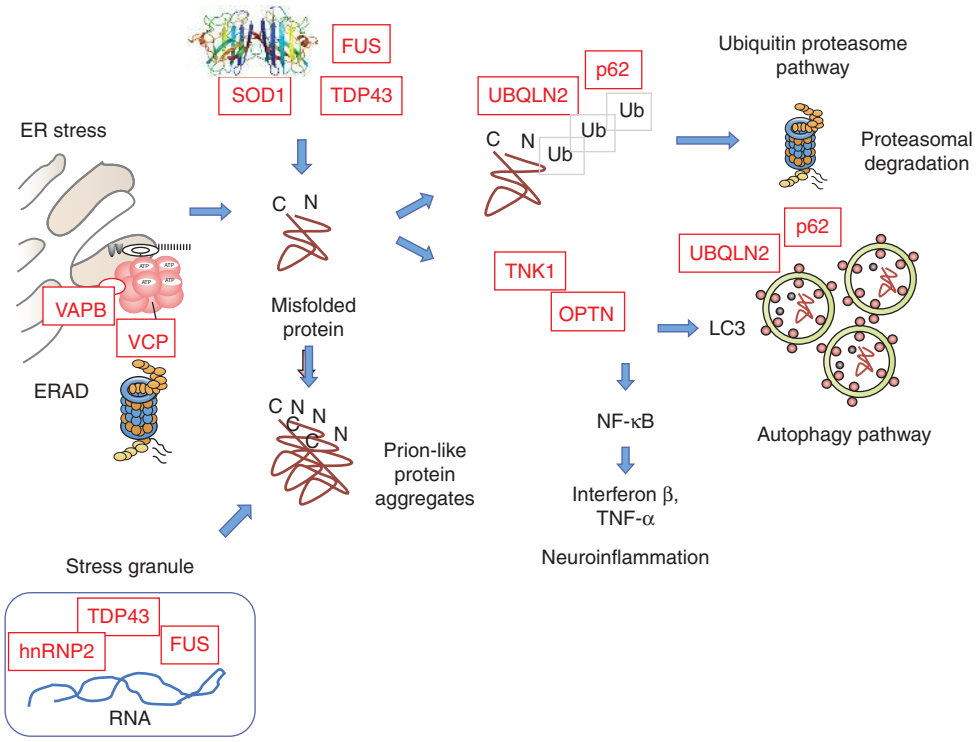

Figure 3. Protein homeostasis is disturbed in ALS. Many ALS genes highlight loss of protein quality control as a central feature of the disease. Misfolding of proteins may reflect many factors including aberrant folding and quality control at the ER (disturbed ER-associated degradation or ERAD), inherent instability due to mutations, aggregation during residence in stress granules, or imperfect clearance via proteasomes or autophagy. Self-assembly of misfolded proteins may lead to propagated instability and prion-like spreading of pathology.

heterozygous variant in other regions of the world. One hypothesis to explain this is that a closely linked variant co-migrating with the D90A substitution in northern Scandinavia confers neuroprotection; this view is consistent with haplotype analyses showing that the D90A allele in Scandinavia is derived from a single founder. However, extensive analyses have failed to identify the linked beneficial variant (Broom et al. 2008). An alternate hypothesis is that there are several variants across the genome in the northern Scandinavian population that are not linked to the SOD1-D90A locus but are nonetheless neuroprotective. There is at least one other recessive SOD1 mutation (ΔG27/P28). By altering exon splicing, this deletion of two codons may reduce expression of the SOD1 transcript (Zinman et al. 2009); it also is predicted to result in a structurally abnormal, potentially unstable, and cytotoxic protein.

Much excitement resulted shortly after the discovery of mutations in the SOD1 gene from the finding that forced expression of high levels of mutant SOD1 protein from human SOD1 transgenes produced an adult-onset, focal, and then spreading motor neuron disease in transgenic mice (Gurney 1994). This was not seen in mice with WT SOD1 transgenes expressing equivalent levels of WT SOD1. Several alleles of SOD1 transgenic mice have been generated, beginning with $SOD1^{G93A}$ and followed by multiple other mutant alleles. Transgenic rats followed; the first were also $SOD1^{G93A}$ transgenics (Nagai et al. 2001). A general principle that has emerged is that the development of motor neu-

ron disease is dose-dependent, requiring a threshold level of mutant protein.

Microscopically as well as clinically, the pathological features of the transgenic SOD1 mice and rats closely resemble those in human ALS. For this reason, these transgenic models have been used extensively to investigate the molecular pathogenesis of SOD1-mediated ALS as well as a range of therapeutic interventions. The utility of these mice in predicting clinical efficacy in humans has been challenged, in part because minor beneficial effects that are of uncertain significance in underpowered mouse trials have led to unsuccessful ALS treatment trials in humans (Scott et al. 2008).

Valosin-Containing Protein. fALS may sometimes arise in association with three other disorders, including not only FTD but also a myopathy with inclusion bodies and Paget's disease. This constellation of four concurrent disorders has been named multisystem proteinopathy or MSP. Most cases of MSP are caused by dominantly inherited mutations in the gene encoding valosin-containing protein (VCP) (Watts et al. 2007). From a clinical perspective, an important additional observation is that there are some instances of MSP in which the predominant phenotype is ALS (Johnson et al. 2010). It is rare that sALS patients have also been found to have mutations in the VCP gene (Abramzon et al. 2012). There is wide phenotypic variation even within the same family. Findings at autopsy include pathological changes including mislocalization of ubiquitinated TDP-43 (see section below) to the cytoplasm, indicating that the degenerative process initiated by mutant VCP shares common elements with other types of ALS and ALS-FTD.

Ubiquilin-2. In ~1% of ALS cases, missense mutations are detected in the proline-rich, PXX domain (residues 491–526) of the ubiquilin-2 gene on the X chromosome (Deng et al. 2011). This is important in that the UBQLN2 protein interacts with TDP-43; aggregates that are UBQLN2-positive are detected in many cases of both sporadic and familial ALS and FTD. Because ubiquilin-2 assists in regulating proteasomal degradation of ubiquitinated proteins, it may regulate many protein levels including TDP-43.

Optineurin, TANK-Binding Kinase 1. In patients with fALS, multiple mutations have been identified in the gene encoding optineurin (OPTN). Although some are transmitted as recessive traits (for example, in the initial families from Japan), it is now evident that others are dominantly inherited. According to one estimate, OPTN may account for ~1% of fALS cases. More recently, a whole-exome mutational screen of 2874 sALS cases and 8475 controls (with a large replication set) concluded that dominant, loss-of-function mutations in the OPTN gene are overrepresented in sALS (Cirulli et al. 2015). OPTN is a protein of ~58 kDa that serves multiple functions; it is implicated in the activation of inflammatory cascades via regulation of interferon and TNF-α. Coordinately with the protein sequestosome-1/p62 (SQSTM1), OPTN is also involved in membrane trafficking and chaperoning of organelles (e.g., mitochondria) and ubiquitinated, misfolded proteins for degradation via autophagic vacuoles and the proteasome.

The function of OPTN is regulated in part by the protein TANK-binding kinase 1 (TBK1). TBK1 has also been implicated as an ALS gene in the above full exome sequencing scan, which disclosed loss-of-function TBK1 variants in sALS (Cirulli et al. 2015). In a parallel study of 252 fALS pedigrees, 13 families were also found to harbor loss-of-function mutations in the TBK1 gene; in the same report, TBK1 mutations were overrepresented in 1010 sALS cases compared with 650 controls (Freischmidt et al. 2015). Some functions of TBK1 are interlocked with OPTN, which is phosphorylated and activated via the coiled-coil domain of TBK1. TBK1 phosphorylation of OPTN accelerates binding of OPTN to LC3, a protein required in the autophagosome. TBK1 itself is directly involved in activating the inflammatory cascade via NF-kB. Functional studies suggested that a critical aspect of some TBK1 variants is loss of function of the C-terminal coiled-coil CCD2 domain of TBK1, which is the CCD2 domain that interacts with multiple other proteins, including OPTN and SQSTM1. The importance of the OPTN-TBK1 axis was further highlighted by a report that full genome sequencing of 104 cases with

pathologically proven FTD (with TDP-43 inclusions) divulged two cases that harbored loss-of-function mutations in both the OPTN and TBK1 genes; three others carried heterozygous missense mutations in the TBK1 gene (Pottier et al. 2015). Combined studies using mRNA and protein expression levels in the cerebellum confirmed marked reductions in OPTN in two cases and reductions in TBK1 levels in two other cases.

CHCHD10 (C22orf2). Within the last three years, several families have been described in which ALS, FTD, ataxia, and ragged red fibers in muscle are co-inherited with missense mutations in the gene CHCHD10 (Bannwarth et al. 2014; Ajroud-Driss et al. 2015). Several investigators have reproduced this finding; CHCHD10 mutations may account for >2% of fALS cases (Johnson et al. 2014a; Ronchi et al. 2015). This nuclear gene is a member of a family of proteins with coiled-coil helix domains (CHCHD); it encodes a mitochondrial protein within the intermembrane space that is thought to be important in maintaining the architecture of cristae and oxidative phosphorylation. This represents the first gene that directly implicates mitochondrial dysfunction as a cause for ALS.

Genes Implicated in Altered Function and Homeostasis of RNA

Transactive Response DNA Binding Protein. A landmark study in 2006 discovered that TDP-43, a 43-kDa DNA binding protein, is the most abundant ubiquitinated protein in pathological cytosolic inclusions in sALS and sporadic FTD (Fig. 4) (Neumann et al. 2006). Subsequently, the importance of this finding was underscored by reports demonstrating that germline TDP-43 mutations can cause familial forms of both ALS and ALS-FTD (Sreedharan et al. 2008). TDP-43, a heterogeneous nuclear ribonucleoprotein or hnRNP, is predominantly nuclear, but shuttles into the cytoplasm and axons during cellular stress. It is multifunctional, with roles in gene transcription, gene translation, the biogenesis of microRNA, gene silencing and RNA binding and transport, and suppression of aberrantly spliced cryptic RNA transcripts (Ling et al. 2015). The TDP-43 protein has several distinctive domains that underlie RNA binding, nuclear export and import, and protein–protein interactions. The latter are glycine-rich and inherently of low complexity. More than 40 mutations have been identified in the TDP-43 gene; almost all are missense changes (Janssens and Van Broeckhoven 2013; Lattante et al. 2013). Mutations in the TDP-43 gene account for ~5% of fALS cases. In both ALS and FTD, TDP-43 accumulates in the cytoplasm, where it is cleaved and hyperphosphorylated; C-terminal fragments accumulate in insoluble cytoplasmic aggregates (Neumann et al. 2006). These aggregates are present in both sALS and fALS. Many forms of fALS, regardless of the offending primary gene mutation, show TDP-43 pathology; exceptions are cases associated with SOD1 and FUS gene mutations (see section below). The same types of TDP-43 pathology have also been reported in other CNS disorders including head trauma, stroke, and Parkinson's disease. This observation implicates the biology of TDP-43 broadly across numerous subcategories of neurologic injury (Neumann et al. 2006).

Several lines of transgenic TDP-43 mice have been generated. Although some mice develop paralysis, the molecular and clinical correlations are difficult to interpret, in contrast with SOD1 mice, because levels of TDP-43 are tightly autoregulated. Elevated or depressed levels of WT TDP-43 are potentially as adverse as overexpressed mutant TDP-43. These mice have been extensively reviewed (Tsao et al. 2012).

Fused in Sarcoma. In 2009, FUS (fused in sarcoma, also known as TLS, translocated in liposarcoma) was identified as another ALS gene (Kwiatkowski et al. 2009; Vance et al. 2009), a point of considerable interest because FUS shares structural and functional features with TDP–43. FUS is an ~75-kDa protein that, like TDP-43, is predominantly nuclear and is also found in ALS cases to be mislocalized to aggregates within the cytoplasm. Like TDP-43, FUS serves multiple functions related to the generation and trafficking of diverse types of RNA. It contributes to local protein translation and dynamic morphological changes in dendrites in response to glutamatergic stimulation,

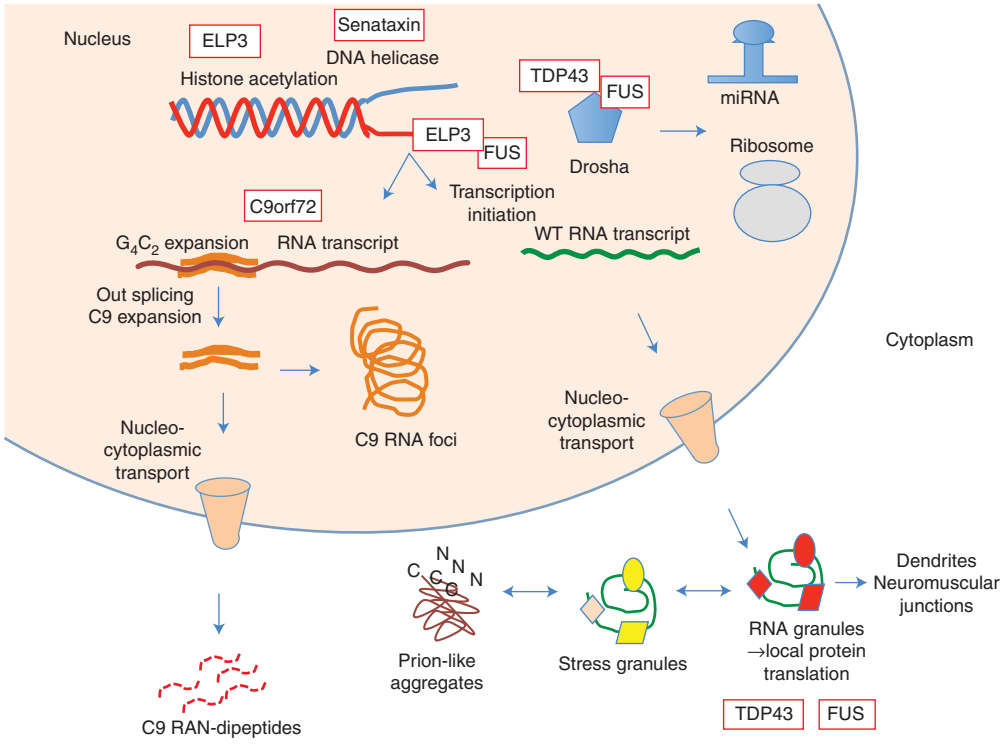

Figure 4. Multiple ALS genes indicate disturbances of RNA-binding proteins and RNA synthesis, trafficking, and function. A diversity of pathologies may arise including altered histone acetylation (elongator acetyltransferase complex subunit 3 [ELP3]), disturbed helicase activity (senataxin), altered splicing and synthesis of microRNA (TAR DNA-binding protein [TDP-43], fused in sarcoma [FUS]), deposition of RNA foci and generation of RNA dipeptides (C9orf72), impaired nucleocytoplasmic transport (C9orf72), and formation of aggregates via stress granules (many of the RNA-binding proteins). Axonal movement of RNA granules may be impaired, with secondary alterations in local protein translation in dendrites and neuromuscular junctions.

a property that bears on synaptic learning. The clinical phenotype of FUS-related ALS is generally not distinctive, except for rare cases in which a missense mutation that alters the nuclear localization signal (P525L) correlates with extremely aggressive, childhood onset ALS. Childhood ALS caused by FUS mutations is associated with basophilic inclusions in the spinal cord (Munoz et al. 2009). Pathologically, it is striking but puzzling that cytoplasmic inclusions with FUS do not also contain TDP-43. This distinction can also be appreciated in FTD cases with frontotemporal lobar degeneration (FTLD), wherein there is yet a third category of inclusions characterized by deposition of tau protein, to the exclusion of TDP43 and FUS. This dichotomy (or trichotomy in the case of FTLD) illustrates that there are patterns of specificity in the molecular events generating these different types of inclusions, although the basis for the specificity remains unclear. Overall, FUS mutations account for about 5% of fALS cases. Although the original FUS mutation was identified as homozygous recessive, most cases are dominantly inherited. FUS shares structural features with two other multifunctional DNA/RNA-binding proteins, Ewing's sarcoma protein (EWS) and TATA-binding associated factor 15 (TAF15) (Morohoshi et al. 1998). This triad has been labeled the TET proteins (translated-in-liposarcoma, Ewing's, and TAF15). Very rare mutations in the EWS and

TAF15 genes have been detected in fALS cases (Ticozzi et al. 2011; Couthouis et al. 2012). Two other hnRNPs with domain structures that are broadly homologous to TDP-43, FUS, and the other TET proteins are hnRNPA2B1 and hnRNPA1; rare mutations in the genes encoding each of these proteins have been implicated in cases of ALS associated with MSP (see section below).

There are now two reports of transgenic FUS models of ALS. In one study, rather fulminant motor axonopathy without motor neuron degeneration but with astrogliosis and ubiquitinated aggregates was triggered by transgenic expression of mutant FUS (R521C) under the control of doxycycline. WT FUS induced some late-onset neuron death in frontal regions of the brain without paralysis (Huang et al. 2011). More recently, slowly progressive motor neuron degeneration with some weakness but no paralysis was detected in mice in which conditional WT and mutant FUS genes were introduced in the tau locus (Sharma et al. 2016). The phenotype required expression of the mutant FUS protein in motor neurons; the mechanism was cell autonomous and did not involve loss of FUS function.

hnRNPA2B1 and hnRNPA1. Rare mutations in genes encoding two other RNA binding proteins, hnRNPA2B1 and hnRNPA1, also cause MSP (see above section) (Kim et al. 2013). The mutations in these genes augment the propensity for the low complexity domains to interact with other proteins and thus for the mutant proteins to induce propagated, prion-like misfolding. Both proteins also interact with TDP-43 (Kim et al. 2013).

C9orf72. In 2011, three landmark studies identified the most common cause of ALS and ALS-FTD: an expansion of a hexanucleotide intronic repeat (GGGGCC) within the first intron of a poorly understood gene, termed *C9orf72* (chromosome 9 open reading frame 72) (Dejesus-Hernandez et al. 2011; Renton et al. 2011; Gijselinck et al. 2012). Clinical diagnosis presently involves repeat primed polymerase chain reaction (PCR) coupled with Southern blotting to define the size of the expansion. Although the most common repeat size is a triplet of the hexanucleotides, controls may have up to 25 or 30 repeats. By contrast, the disease alleles may have hundreds or even thousands of the hexanucleotide repeats. These C9orf72 intronic expansions underlie 45%–50% of fALS and 20%–25% of familial FTD cases in the United States. They also account for 5%–10% of sporadic ALS and FTLD. There is a tendency for bulbar ALS to be more common in the C9-expanded populations.

The relationship between expansion size and phenotype is not completely delineated. The issue is confounded somewhat by somatic variability in expansion size; in the same individual, the number of repeat expansions in the frontal cortex generally exceeds that in the cerebellum or blood, and there are fewer repeats in samples from fibroblasts. In one analysis (van Blitterswijk et al. 2013), expansion size did not vary with the phenotypes (ALS vs. ALS-FTD vs. FTD). Curiously, there was a positive correlation between the expansion length in the frontal cortex and the age of onset; shorter expansions were associated with earlier onset. In the same study, shorter expansions in the cerebellum conferred a survival advantage.

Little is known about the normal function of the *C9orf72* gene. It expresses three transcripts that generate proteins with two isoforms (54 and 24 kDa). These include DENN domains (differentially expressed in normal and neoplastic cells) that are predicted to function as guanine nucleotide exchange factors (GEFs) that interact with Rab GTPases to regulate membrane trafficking events. The scope of this review permits only a brief summary of potential mechanisms of pathogenesis of the hexanucleotide expansion in the *C9orf72* gene. Because at autopsy affected brains show a reduction in levels of the full C9 mRNA transcript, it was originally suggested that one mechanism for the pathology of the C9 expansion might be haploinsufficiency. However, there are several arguments against this hypothesis, including the observation that mice that are hemizygous or fully deleted for the *C9orf72* gene do not develop significant motor neuron loss. Two other models offer alternate explanations for the neurotoxicity of the C9 intronic expansions.

One is based on a major pathological finding in C9orf72 ALS/FTD: the intranuclear deposition of foci of expanded RNA. It is proposed that, as occurs with RNA foci in myotonic dystrophy, the intranuclear RNA deposits may sequester critical intranuclear proteins (like muscleblind proteins in myotonic dystrophy type 1 [DM1]). However, although several candidates for the sequestered protein have been proposed, there is no consensus yet on a single candidate. A second, intriguing model is that the primary pathology is the production, via noncanonical, repeat-associated, non-ATG-dependent translation (RAN-translation), of dipeptide repeat proteins (DPRs) across the expanded G_4C_2 repeat. The production of such DPRs was first discovered in postmortem tissue from patients with spinocerebellar atrophy (SCA8) (Zu et al. 2013); the expanded domains in C9orf72 undergo RAN-translation across both sense and antisense RNA, in all six possible reading frames; this generates five different DPRs (Ash et al. 2013). Studies indicate that some of these DPRs (such as poly-[proline-arginine]) are highly neurotoxic. Recent studies in flies that examine the respective toxicities of the expanded RNA versus the DPR favor the view that the primary offending agent is the DPR (Mizielinska et al. 2014; Tran et al. 2015). Whether a consequence of RNA foci or the DPR, other potentially downstream pathologies are triggered by the G_4C_2 expansions, including substantial disruption of the nuclear pore complex and the process of nuclear-cytoplasmic transport (export of RNA, import of proteins) (Freibaum et al. 2015; Jovicic et al. 2015; Zhang et al. 2015).

Four lines of bacterial artificial chromosome (BAC) transgenic C9orf72 mice have been reported. In one, the BAC expresses the short isoform of the C9orf72 protein, whereas the other expresses the full-length isoform. Both lines show the molecular stigmata of the C9orf72 pathology (abundant intranuclear RNA foci, both soluble and aggregated DPR), but curiously neither showed paralysis, even after two years (O'Rourke et al. 2015; Peters et al. 2015a; Jiang et al. 2016). Still another set of BAC C9orf72 transgenics, in a different mouse strain, show paralysis in a subset of female mice (Liu et al. 2016).

Angiogenin and Ribonuclease 4. Angiogenin (ANG, or ribonuclease 5) is a 123-amino-acid protein with multiple functions including stimulation of angiogenesis, hydrolysis of nucleic acids, and context-dependent activation of proteases. In conditions of cellular stress, ANG induces expression of a class of tRNAs (tiRNAs, or transfer RNA-derived, stress-induced, small RNAs) that suppress protein synthesis, presumably thereby conserving cellular energy reserves. Because it has neuroprotective properties, it is of considerable interest that rare variants in ANG are overrepresented in both ALS (sALS and in some small families) and Parkinson's disease (Greenway et al. 2006; van Es et al. 2011). Ribonuclease 4 is a paralog of ANG that also has angiogenic and neuroprotective properties; one study, as yet unconfirmed, has described a hypofunctional missense mutation in this enzyme that is more abundant in ALS than controls (Li et al. 2013).

Matrin-3. In 2014 it was reported that missense mutations in the gene encoding matrin-3 cause motor neuron disease with prominent myopathic features (a scenario not unlike the multisystem proteinopathy caused by VCP gene mutations) (Johnson et al. 2014b; Leblond et al. 2016). Matrin-3 is predominantly a nuclear protein with multiple functions. It binds DNA and RNA and some RNA binding proteins, including TDP-43. Additionally, it is part of the nuclear pore complex and is likely involved in shuttling hnRNP in and out of the nucleus. It probably is also involved in chaperoning edited RNA. How these mutations disrupt these multiple functions is not yet clear. A recent study indicates that, unlike TDP-43 and FUS, in conditions of cellular stress leading to stress-granule formation, matrin-3 remains largely within the nucleus (Gallego-Iradi et al. 2015).

Genes That Implicate Cytoskeletal and Axonal Dynamics

Dynactin (DCTN1). Genetic analyses have identified several ALS genes that, as a group,

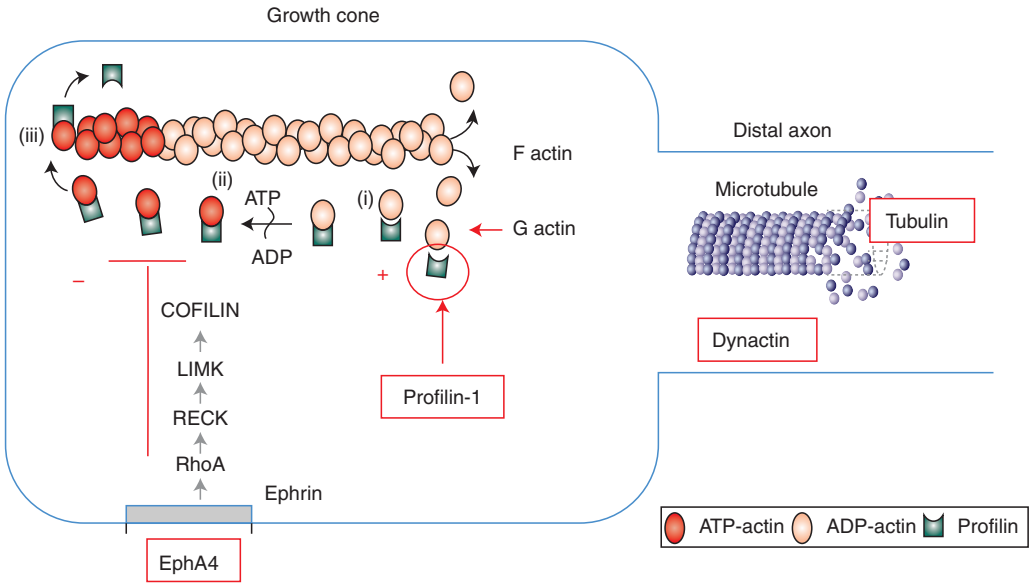

Figure 5. Cytoskeletal dynamics are altered in ALS. Mutations in profilin-1 are likely to impair energy-dependent extension of filamentous actin and elongation of growth cones, a process that is enhanced by reduction in signaling from ephrinA4. Tubulin mutations compromise the structures of microtubules. Mutations in dynactin are predicted to impair retrograde transport along the microtubule backbone.

identify perturbations of cytoskeletal and axonal dynamics as a central element in ALS pathogenesis (Fig. 5). This concept is not new in the field of motor neuron disease. As early as 1987, studies of median motor nerve twigs isolated from ALS patients revealed altered axonal transport ex vivo (Breuer et al. 1987). Disrupted axonal transport has been documented in numerous studies of transgenic ALS mice (Morfini et al. 2009). It was shown that mutant but not WT SOD1 selectively impairs anterograde axonal transport in the isolated axons from squid (Bosco et al. 2010). For these reasons, it was therefore of considerable interest that mutations have now been documented, albeit infrequently, in the dynactin gene (*DCTN1*), which encodes an accessory protein, $p150^{glued}$, that participates in retrograde axonal transport (Puls et al. 2003). At least two clinical phenotypes were reported. In one family, affected individuals developed an early adult onset mainly with lower motor neuropathy with distinctive early laryngeal involvement; in the other, the phenotype was ALS-FTD (Munch et al. 2005).

Profilin-1. PFN1 is a protein that accelerates ATP-initiated polymerization of filamentous actin, a process that is important in many diverse cellular functions including extension of growth cones from the distal motor neuron axon. In 2012, exome sequencing identified mutations in the PFN1 gene in a small set of ALS families, clinically characterized by primarily lower motor neuron disease of adult onset (Wu et al. 2012). Importantly, at least two groups have recently generated transgenic PFN1 mice that, like the transgenic SOD1 mice, develop age- and PFN1-dose-dependent, lethal paralytic disease (Yang et al. 2016; M Kiaei, pers. comm.). This is expected to be an important asset in the field, as comparative studies of the two models should enable definition of the common elements that underlie motor neuron death in both ALS models. The availability of two models will also improve testing of therapeutic compounds.

TubulinA4A. Microtubules, the essential cytoskeletal backbones in axons, are composed of multiple copies of two subunits, α and β tubu-

lin, each a member of a family of tubulin proteins. Four mutations in the gene encoding one form of α tubulin, tubulinA4A (TUBA4A), were identified in an exome scan of 363 cases of fALS and 4300 individuals and confirmed in a replication study of 272 fALS cases and 5510 controls. The same variants were not detected in a large cohort (1355) of sALS cases (Smith et al. 2014). These mutations were predicted to be damaging; all were located within exon 4. In in vitro assays using primary neurons and COS cells, the mutations impaired polymerization of microtubules. Although these mutations were detected in fALS cases, none were tested for co-migration with other affected individuals in the same pedigree, in part because the pedigrees were small. This suggests that penetrance of the TUBA4A variants may be low in contrast with the SOD1 or C9orf72 families.

Juvenile-Onset ALS Genes

Because they are almost always dominantly transmitted, it is thought that most adult-onset neurodegenerative disorders like fALS reflect acquired, adverse properties that are neurotoxic. By contrast, most mutant genes implicated in developmental disorders of juvenile onset often are recessively inherited and reflect the loss of function of genes that are important in development. When the recessive nature of a genetic defect is likely, suggested perhaps by consanguinity, an approach to gene identification is to locate chromosomal regions of preserved homozygosity; fine analysis of the homozygous locus may disclose the mutant disease gene. One example of a recessive, young-onset ALS gene mapped via preserved homozygosity is *ALS2*, which encodes a rho GEF that is abundant in the brain and spinal cord (Hadano et al. 2001; Yang et al. 2001). As described above for the DENN proteins like C9orf72, this GEF is likely involved in membrane trafficking. It activates membrane-associated Rab5 protein (converting a bound GDP to GTP), permitting its interaction with target proteins and vesicles. In neurons, these activities are important in multiple membrane-processing activities, not only in the Golgi and ER but also in neuronal development and remodeling, synaptic function, and neurotransmitter receptor trafficking. Individuals with loss-of-function mutations in alsin develop early-onset corticospinal tract and lower motor degeneration, which, unlike typical adult-onset ALS, is slowly progressive with survival measured in decades (Flor-de-Lima et al. 2014).

Several other gene defects have now been associated with juvenile ALS. Recessive loss-of-function mutations in the spastic paraplegia gene *SPG11* (spatacsin) also commonly cause childhood-onset motor neuron disease; the predominant clinical feature is usually corticospinal tract signs with thinning of the corpus callosum (Del Bo et al. 2007) although a distal axonopathy has also been described (Montecchiani et al. 2016). This protein has an important role in neuronal growth, at least in part because it functions in autophagic pathways (Chang et al. 2014). Several other genes that primarily cause hereditary spastic paraparesis can also include degeneration of spinal motor neurons (Fink 2013). Among these are SPG3 and SPG4, which, respectively, encode atlastin and spastin. Atlastin is a dynamin-like GTPase that mediates ER membrane fusion and interacts with a number of other proteins mutated in hereditary spastic paraplegia (HSP), including spastin, REEP1, and NIPA1 (all of which may be associated with lower motor neuron degeneration, albeit infrequently). Recessive, loss-of-function mutations in the gene-designated neuropathy target esterase also produce early corticospinal tract and more widespread motor neuron degeneration (Rainier et al. 2008). This phospholipase B is localized to ER membranes; it is thought to be neuroprotective following exposure to toxic organophosphorus compounds (Richardson et al. 2013).

Juvenile ALS has also been attributed to recessive mutations in the *SIGMAR1* gene, which encodes a σ non-opioid intracellular receptor (Al-Saif et al. 2011). The phenotype is slowly progressive, predominantly with upper motor neuron involvement. The receptor, Sig-1R, is multifunctional, regulating potassium channels, Ca^{2+} signaling, and chaperone activities in the ER. SigR1 is abundant in motor neurons

at excitatory postsynaptic densities; these are reportedly disturbed in sALS, which may reveal cytoplasmic accumulation of SigR1.

Some genes normally associated with adult-onset ALS also are implicated in juvenile ALS. For example, a very specific mutation in the FUS gene, P525L, is now well documented to cause fulminant juvenile-onset motor neuron disease, associated pathologically with distinctive intranuclear basophilic inclusions (Huang et al. 2010). The fulminant toxicity may reflect the fact that this mutation alters a canonical sequence for nuclear import of proteins. Another dominantly inherited form of juvenile ALS (onset in the late teens and early adulthood) is caused by heterozygous mutations in the senataxin gene (Chen et al. 2004), which encodes a helicase that is important in DNA and RNA coiling and repair. This underscores the importance of the DNA repair pathway in neurons, which are terminally differentiated; this is further highlighted by the fact that numerous recessively transmitted mutations in the senataxin gene are associated with a cerebellar and peripheral nerve neurodegenerative syndrome (ataxia with oculomotor apraxia).

Infantile-onset forms of severe pontobulbar palsy that may be associated with hearing loss, oculomotor palsy, and ataxia are also important; historically, these are designated Brown–Vialetto–Van Laere (BVVL) and Fazio–Londe (FL) disease. In some BVVL–FL families, homozygosity mapping and candidate gene sequencing identified homozygous and compound heterozygous mutations in the gene C20orf54 (SLC52A3), which encodes an intestinal riboflavin transporter (Bosch et al. 2011); subsequently, a second riboflavin transporter has also been implicated in BVVL (Johnson et al. 2012). In another family, without the SLC52A3 defect, a missense mutation of UBQLN1 (encoding ubiquilin1) was reported (Gonzalez-Perez et al. 2012). In southern India, many individuals have been identified with elements of the same phenotype: juvenile onset of deafness accompanied by upper motor neuron signs and denervation, sometimes with optic atrophy and behavioral disturbances. Because some cases recur in families, this entity, originally labeled Madras motor neuron disease, is likely to be recessively inherited; however, an offending gene defect has not been identified (Nalini et al. 2013).

Genome-Wide Association Studies

Conventional gene identification strategies (e.g., genetic linkage followed by candidate gene or exome sequencing) have identified many genes related to ALS or ALS-FTD. However, a limitation in this approach is that linkage studies may not have adequate power to detect disease loci when familial traits have low penetrance. If adequately powered with appropriately large sample sizes, genome-wide association studies (GWAS) analyses can define risk alleles with low odds ratios (Purcell et al. 2003). Although early GWAS studies in ALS were only moderately powered, recent collaborative GWAS studies of increasing sizes have identified several potential ALS loci and genes (Table 2).

Prior to the discovery of the role of C9orf72 in ALS, it was of considerable interest that the strongest GWAS peak was directly on top of the ALS-FTD locus on chromosome 9p21, within which the C9orf72 expansions were subsequently found (van Es et al. 2009; Laaksovirta et al. 2010; Shatunov et al. 2010). Another GWAS locus that has been replicated is proximal to the *UNC13A* gene, which regulates the release of neurotransmitters, including glutamate, and thus is a plausible candidate ALS gene; independent studies have revealed that the minor allele of rs12608932 in *UNC13A* is not only associated with susceptibility but also with shorter survival of ALS patients (van Es et al. 2009; Diekstra et al. 2012b; Chio et al. 2013).

One of the largest GWAS to date in ALS involved 6100 European cases and 7125 controls (Fogh et al. 2014). In addition to consistent results for chromosome 9p association, this study also showed an intriguing hit at chromosome 17q11.2, near the *SARM1* gene. The *SARM1* protein participates in active axonal degeneration following axonal injury; it is quite striking that in mice devoid of *sarm1*, Wallerian degeneration is profoundly attenuated. That said, a focused biological relation of the ALS-

Table 2. Genome-wide association studies in ALS

Single nucleotide polymorphism	Chromosome	Gene	Encoded protein or function	Sample region	Minor allele frequency controls	Patients	Pooled odds ratio (95% confidence interval)	Pooled *P* value	Reference
rs230667	12	*ITPR2*	A Ca^{2+} channel on the ER	Netherlands/ Belgium/ Sweden	0.07	0.11	1.58 (1.30–1.91)	3.28×10^{-6}	van Es et al. 2007
				Ireland	0.11	0.10	–	6.4×10^{-1}	Cronin et al. 2008
				USA/UK/ France/ Netherlands	–	–	–	4×10^{-2}	Landers et al. 2009
				USA/Italy/ Germany	0.11	0.11	1.01	8.7×10^{-1}	Chio et al. 2009
				USA/Ireland/ Sweden/ Belgium/ Netherlands	0.18	0.20	1.21	3.72×10^{-5}	van Es et al. 2009
				UK	0.10	0.10	–	9.8×10^{-1}	Shatunov et al. 2010
				USA	–	–	1.3 (0.9–1.7)	1.4×10^{-1}	Kwee et al. 2012
				China	0.20	0.21	1.02	7.4×10^{-1}	Deng et al. 2013
rs6700125	1	*FLJ10986 (FGGY)*	Unknown; possible role in energy metabolism	USA	0.32	0.41	1.35 (1.13–1.62)	6×10^{-4}	Dunckley et al. 2007
				Ireland	0.33	0.34	–	7.9×10^{-1}	Cronin et al. 2008
				USA/UK/ France/ Netherlands	–	–	–	9.9×10^{-2}	Landers et al. 2009
				USA/Italy/ Germany	–	–	–	8.0×10^{-2}	Chio et al. 2009
				USA/Ireland/ Sweden/ Belgium/ Netherlands	0.34	0.33	1.03	1.1×10^{-1}	van Es et al. 2009

Continued

Table 2. *Continued*

Single nucleotide polymorphism	Chromosome	Gene	Encoded protein or function	Sample region	Minor allele frequency controls	Patients	Pooled odds ratio (95% confidence interval)	Pooled *P* value	Reference
				UK	0.33	0.34	–	7.1×10^{-1}	Shatunov et al. 2010
rs10260404	7	*DPP6*	A transmembrane protein binding A-type neuronal K^+ channel	Ireland	0.34	0.42	1.37 (1.2–1.56)	2.53×10^{-6}	Cronin et al. 2008
				USA/UK/ France/ Netherlands	–	–	–	1.2×10^{-2}	Landers et al. 2009
				USA/Italy/ Germany	–	–	–	6.2×10^{-1}	Chio et al. 2009
				Ireland/Poland/ USA/ Netherland	0.36	0.41	1.23 (1.1–1.38)	2.62×10^{-4}	Cronin et al. 2009
				USA/Ireland/ Sweden/ Belgium/ Netherlands	0.38	0.41	1.16	1.6×10^{-3}	van Es et al. 2009
				UK	0.4	0.4	–	8.9×10^{-1}	Shatunov et al. 2010
				Italy	0.39	0.38	0.97 (0.84–1.09)	6.3×10^{-1}	Fogh et al. 2011
				USA	–	–	1.0 (0.9–1.1)	9.7×10^{-1}	Kwee et al. 2012
				Belgium/ Ireland/USA/ Sweden/ Netherlands	0.53	0.15	3.70	3.6×10^{-3}	Blauw et al. 2010
				China	0.17	0.16	0.94	3.5×10^{-1}	Deng et al. 2013
rs12608932	19	*UNC13A*	A presynaptic protein regulating neurotransmitter release in central and neuromuscular synapses	USA/Ireland/ Sweden/ Belgium/ Netherlands	0.37	0.40	1.2	2.5×10^{-14}	van Es et al. 2009
				UK	0.37	0.35	–	4.2×10^{-1}	Shatunov et al. 2010

Continued

Table 2. *Continued*

Single nucleotide polymorphism	Chromosome	Gene	Encoded protein or function	Sample region	Minor allele frequency controls	Patients	Pooled odds ratio (95% confidence interval)	Pooled *P* value	Reference
				USA	–	–	1.2 (1.0–1.4)	4.6×10^{-2}	Kwee et al. 2012
				Belgium/ Sweden/ Netherlands	0.36	0.40	1.33 (1.10–1.60)	1×10^{-3}	Diekstra et al. 2012a
				China[a]	0.26	0.39	–	–	Deng et al. 2013
rs2814707	9	*MOBKL2B*	May regulate kinase activity	USA/Ireland/ Sweden/ Belgium/ Netherlands	0.23	0.26	1.16	7.45×10^{-9}	van Es et al. 2009
				China	0.03	0.03	1.08	5.2×10^{-1}	Deng et al. 2013
rs16856202	1	*DISC1*	Regulates neurite outgrowth and cortical development	USA/UK/ France/ Netherlands	0.04	0.02	0.499	7.98×10^{-6}	Landers et al. 2009
rs3849942	9	*C9orf72*	Unknown function	USA/Ireland/ Sweden/ Belgium/ Netherlands	0.23	0.26	1.15	1.01×10^{-8}	van Es et al. 2009
				China[a]	0.05	0.05	–	–	Deng et al. 2013
rs2708909	7	*SUNC1* (Intronic)	A nuclear envelope protein Sadl and UNC84 domain containing 1	USA/Italy/ Germany	0.45	0.50	1.17 (1.11–1.23)	6.98×10^{-7}	Chio et al. 2009
rs2708851	7	*SUNC1* (Intergenic)			0.45	0.50	1.17 (1.11–1.23)	1.16×10^{-6}	
rs2275294	20	*ZNF512B*	Regulates TGF-β signaling	Japan	0.41	0.48	1.32 (1.21–1.44)	6.70×10^{-10}	Iida et al. 2011
rs6703183	1	*CAMK1G*	A calmodulin-dependent protein kinase	China	0.34	0.41	1.31	2.92×10^{-8}	Deng et al. 2013
rs8141797	22	*SUSD2*	A novel tumor suppressor in brain, kidney, and lung	China	0.10	0.10	1.52	2.35×10^{-9}	Deng et al. 2013

Continued

Table 2. *Continued*

Single nucleotide polymorphism	Chromosome	Gene	Encoded protein or function	Sample region	Minor allele frequency controls	Patients	Pooled odds ratio (95% confidence interval)	Pooled *P* value	Reference
rs34517613	17	*Unknown*	Unknown	China	0.13	0.11	0.82 (0.76–0.87)	1.11×10^{-8}	Fogh et al. 2014
rs1541160	1	*KIFAP3*	Regulates Rho and Rap1 family members and Ki-Ras by stimulating their GDP/GTP exchange reactions and inhibiting their interactions with membranes	USA/UK/ France/ Netherlands	–	–	–	1.84×10^{-8}	Landers et al. 2009
				USA	–	–	0.95 (0.82–1.2)	4.9×10^{-1}	Kwee et al. 2012
rs3849942	9	9p21 locus *C9orf72*[b]	–	USA/Ireland/ Sweden/ Belgium/ Netherlands	0.23	0.26	1.23	1.58×10^{-6}	van Es et al. 2009
				UK	0.24	0.30	1.39 (1.21–1.59)	2.22×10^{-6}	Shatunov et al. 2010
				Belgium/France/ UK/ Netherlands/ Ireland/ Italy/ Sweden/USA	0.23	0.27	1.22 (1.15–1.30)	4.64×10^{-10}	Shatunov et al. 2010
				Finland	0.16	0.29	2.16 (1.72–2.70)	9.11×10^{-11}	Laaksovirta et al. 2010
				Belgium/ Sweden/USA/ Ireland/ Netherlands	0.22	0.25	1.23 (1.11–1.29)	1.58×10^{-6}	Deng et al. 2013
rs2814707	9	9p21 locus	–	USA/Ireland/ Sweden/ Belgium/ Netherlands	0.23	0.26	1.22	3.33×10^{-6}	van Es et al. 2009
				UK	0.24	0.30	1.38 (1.20–1.58)	3.32×10^{-6}	Shatunov et al. 2010

Continued

Table 2. *Continued*

Single nucleotide polymorphism	Chromosome	Gene	Encoded protein or function	Sample region	Minor allele frequency controls	Patients	Pooled odds ratio (95% confidence interval)	Pooled *P* value	Reference
				Belgium/France/UK/Netherlands/Ireland/Italy/Sweden/USA	0.27	0.23	1.22 (1.15–1.30)	4.47×10^{-10}	Shatunov et al. 2010
				Belgium/Sweden/USA/Ireland/Netherlands	0.23	0.26	1.22 (1.10–1.28)	3.33×10^{-6}	Deng et al. 2013
rs13048019	21	*TIAM1*	–	Finland	0.17	0.30	4.4 (2.32–7.40)	2.58×10^{-8}	Laaksovirta et al. 2010
rs2225389	9	*MOB3B*	May regulate kinase activity	Finland	0.09	0.18	2.26 (1.7–3.0)	2.88×10^{-8}	Laaksovirta et al. 2010
rs75932628	6	*TREM2*	Role in glial cell function and inflammation	North America	0.08	0.54	2.4 (1.29–4.15)	4.1×10^{-3}	Cady et al. 2014

[a]The SNPs failed to be validated in the study for it is significantly deviated from Hardy–Weinberg equilibrium.
[b]Nearby gene.

associated *SARM1* nucleotide polymorphism (SNP) and sarm1 function has not been defined. Another large ALS GWAS has confirmed the SARM1 association and pointed to still other ALS genes (SCFD1, C21orf2, MOBP) (van Rheenen et al. 2016).

Another GWAS-identified gene of interest is *ELP3*, which encodes an RNA polymerase component. A microsatellite GWAS has identified risk alleles in the *ELP3* gene, which were associated with reduced brain expression of *ELP3* in postmortem studies (Simpson et al. 2009). A recent GWAS analysis of 533 Han Chinese with ALS failed to define hits related to C9orf72 but did divulge two ALS-associated loci that are not detected in ALS cohorts in Europe or North America (chromosome 1q32 covering *CAMK1G* [calcium/calmodulin-dependent protein 1] and *SUSD2* [sushi domain containing 2]) (Deng et al. 2013). This illustrates the principle that genetic factors that drive susceptibility may differ in populations with differing genetic architectures.

An innovative study by Diekstra and colleagues correlated SNP variants with levels of expression of the corresponding genes, based on mRNA from blood samples, to define a set of SNPs that are expression quantitative trait loci (eQTLs) (Diekstra et al. 2012a). The resulting set of SNPs at eQTLs was tested for association with ALS. After validation, this identified eight SNP pairs in a single gene, CYP27A1, as associated with ALS. The product of this gene, sterol 27-hydroxylase, is necessary for cholesterol breakdown; loss-of-function mutations in the enzyme cause cerebrotendinous xanthomatosis, which is characterized by deposition of cholesterol products in multiple tissues, and progressive multisystem disease with neurodegeneration. The implication of this eQTL association study—that cholesterol metabolism may be relevant in ALS—is further supported by epidemiological studies relating cholesterol levels to ALS (Diekstra et al. 2012a).

Genomic Structural Variations In ALS

The etiologic role of genomic structural variation (e.g., deletions, duplications, copy number variants [CNVs], insertions, inversions, translocations) in late-onset diseases such as ALS has not yet been completely elucidated. Two studies showed chromosomal translocations or inversions in ALS (Kaneko et al. 1995), but in 4 out of 5 families, these aberrations were also found in several related healthy persons (Meyer et al. 2003).

A well-established CNV within the *SMN1* gene, whose loss-of-function mutations cause spinal muscular atrophy, has been the subject of several ALS studies. It has been reported that deletions of *SMN1* correlate with shortened survival in ALS (Veldink et al. 2005). The most consistent structural variants in the *SMN1* gene are duplications, not deletions. The comprehensive investigation by Blauw (2012a) failed to detect changes in copy numbers of *SMN2* in ALS; moreover, sequencing of the SMN gene disclosed only one missense variant (G26D) of uncertain significance.

Two studies have failed to find changes in the global genomic burden of CNVs in sALS (Blauw et al. 2008; Blauw et al. 2010). An international study involving more than 19,000 individuals (a discovery cohort of 1875 cases and 8732 controls plus a replication cohort of 2559 cases and 5887 controls) screened ~ 300,000 SNPs for structural variations across the genome. The overall burden of CNVs in sALS did not differ from controls (Blauw et al. 2010). However, examination of CNVs within genes suggested an association with duplications within the 5′ end of the DPP6 gene on chromosome 7 and deletions in the NIPA1 gene on chromosome 15. The former locus has been implicated but not uniformly confirmed in ALS by prior GWAS studies (see Table 2); variants in the latter gene have been reported to modulate survival in ALS (see below). These findings are consistent with the view that, at least as presently analyzed, common CNVs are not associated with sALS although rare CNVs may contribute to its pathogenesis.

Twin Studies In ALS

One approach to defining the role for genetics in any disease is the analysis of disease concordance in twins. Two studies of twin concor-

Table 3. Genes that modify ALS susceptibility or phenotype

Study	Gene	Susceptibility	Site	Onset	Survival
GWAS	*UNC13A*	+++			+++
	rs3011225-1p34			+++	
Modifier genes	*EphA4*				+++
	EphA3				+++
	MMP9	+++	Spinal MNs		
	Ataxin-2	+++			
	NIPA1	+++			+++
	CX3CR1				+++
	DMT1				+++
	CHGB P412L			+++	
	TMEM106B		FTD	+++	+++
	TREM2				
	ARGEF1	+++			
Known ALS genes	*C9orf72*	+++	Bulbar		
	C9 repeat length	+++		+++	+++
	Profilin-1	+++	Limb		
	SOD1-A4V	+++			+++
	SOD1/SOD1	+++		+++	+++
	FUS-P525L	+++		+++	+++

dance in ALS are instructive in this regard (Graham et al. 1997; Al-Chalabi et al. 2010). A British motor neuron disease (MND) study of 76 twin pairs found ALS heritability to be between 38% and 85% (Graham et al. 1997). A more recent study examined 171 twin pairs identified by combining data from a British MND twin cohort, the National Swedish Twin Registry, and the Kings College Motor Neuron Clinic Registry. The combined data set encompassed 10,872 MND death certificates over a 10-year period, a country-wide study of 86,441 twin pairs, and a clinic-based study of 4982 individuals with ALS or their family members (Al-Chalabi et al. 2010). Of 49 monozygotic twin pairs, five were concordant and 44 were discordant for ALS. Among dizygotic twin pairs studied, all were discordant for ALS. Analysis of different models concluded that ALS heritability is 76% (95% confidence interval, 60%–86%) when including individuals with known ALS in other, nontwin family members. When familial cases were excluded, the heritability of ALS was 61% (95% confidence interval, 38%–78%). Some studies have not identified concordance for ALS among monozygotic twins (Dellefave et al. 2003).

Genes and Gene Variants That Modify Susceptibility and Phenotype in ALS

Genetic variants that are not primary causes of ALS may nonetheless influence ALS phenotype (e.g., age of onset, duration) or susceptibility (Table 3). As noted above, the minor allele of the ALS-related SNP at the locus UNC13A confers enhanced risk for ALS; it also correlates with shorter survival (van Es et al. 2009; Diekstra et al. 2012b; Chio et al. 2013). More recently, SNP rs3011225 on chromosome 1p has also been shown to correlate with survival (Ahmeti et al. 2013). The biological basis for these associations is poorly understood.

Ephrin A4, Ephrin A3

Several genes have now been reported to modify the phenotype of ALS. Perhaps the best example is ephrinA4, a molecule whose normal functions include interacting with tyrosine kinase

receptors on the distal terminals of growth cones to attenuate axon extension during neural development. Beginning with a screen to suppress toxicity of mutant SOD1 in zebrafish, Robberecht and colleagues have shown that transgenic SOD1 ALS mice and rats with reduced levels of ephrinA4 have improved survival; analogously, patients with lower circulating ephrinA4 levels have slower disease progression (Van Hoecke et al. 2012). This observation raises the prospect that compounds that selectively inhibit the EphA4 tyrosine kinase receptor or its signaling might therefore be beneficial in ALS. Within the last two years, data from an ALS cohort in Turkey have suggested that genetic variants in the Eph3A gene may also affect neuroprotection and ALS survival (Uyan et al. 2013).

Matrix Metalloprotease 9

Matrix metalloprotease 9 (MMP9) is a zinc metalloenzyme that mediates proteolysis of the extracellular matrix for critical cellular events such as cell migration, development, and angiogenesis. Experiments by Henderson and colleagues using transgenic $SOD1^{G93A}$ mice determined that MMP9 is expressed shortly after birth in mice and is selectively found in fast-fatigable spinal motor neurons but not in oculomotor or sphincteric neurons (Kaplan et al. 2014). This expression pattern mirrors that of motor neuron susceptibility in ALS, implicating MMP9 as a determinant of motor neuron degeneration. This possibility was underscored by the finding that genetic ablation or enzyme inhibition of MMP slowed endplate denervation and prolonged survival in the mice. Moreover, these observations suggest that interventions that block MMP9 activity may be therapeutic in ALS.

Ataxin 2

Expanded polyglutamate domains in the ataxin 2 gene cause spinocerebellar atrophy type 2 (SCA2). Under normal circumstances, there are less than 22 repeats in the gene; in SCA2 there are usually more than 35 repeats. It was therefore of considerable interest that expansions of intermediate length (27–33) enhance susceptibility to ALS (Elden et al. 2010; Lee et al. 2011). This discovery aligned well with a long-standing clinical observation that SCA2 patients sometimes presented with elements of lower motor neuron degeneration. The finding of the association of intermediate length repeats with ALS was robustly confirmed.

Non-Imprinted in Prader–Willi/Angelman Syndrome

Loss-of-function mutations in non-imprinted in Prader–Willi/Angelman Syndrome (NIPA1) cause juvenile-onset hereditary spastic paraparesis. It now appears that loss of NIPA1 function and expansions of a polyalanine repeat within the protein both increase susceptibility to ALS; furthermore, long (>10) repeats also correlate with earlier age of onset and shorter survival in ALS. Moreover, rare ALS cases harbor potentially damaging missense mutations in the NIPA1 gene (Blauw et al. 2012b). These effects may reflect impairment of the activity in NIPA1 in vesicular trafficking necessary for synaptic function.

CX3CR1

In a Spanish study of 187 cases versus 378 controls, the isoleucine allele of a polymorphic variant in the fractalkine gene CX3CR1 (valine-249-isoleucine, rs3732379) was associated with a faster rate of progression and shorter survival in sALS but not in fALS. This variant did not influence age or site of onset (Lopez-Lopez et al. 2014). The V249I variant of the fractalkine protein, which is expressed only on microglial cells, is associated with reduction in some microglial functions; blunted microglial activity is therefore a potential explanation for the more rapid rate of progression. It is an intriguing parallel that deletion of the mouse CX3CR1 gene also accelerates the course of $SOD1^{G93A}$ ALS in mice (Cardona et al. 2006).

Divalent Metal Transporter 1

In a French analysis of 579 cases and 517 controls, it was observed that one allele of a metal

transport protein divalent metal transporter 1 (DMT1) was associated with a shorter course in patients with limb-predominant ALS. This association was attributed provisionally to a role for iron transport and metabolism in ALS (Blasco et al. 2011b).

Chromogranin B

It has been reported in a French Canadian cohort that a variant (P413L) of chromogranin B is overrepresented in ALS cases, which also shows earlier onset (Gros-Louis et al. 2009). This was not confirmed in other cohorts (Blasco et al. 2011a).

TMEM106B

This gene, whose protein functions in endosomal trafficking, modifies the phenotype of FTD caused by progranulin mutations. Specifically, the major allele of variant rs1990622 in TMEM106B is associated with both lower levels of serum progranulin and earlier onset of progranulin-mediated FTD. TMEM106B also is a modifier of FTD mediated by C9orf72 expansions but in a manner opposite that in progranulin FTD. In C9orf72 FTD, the major allele of TMEM106B correlated with later ages of both onset and death (but not in C9orf72 ALS) (van Blitterswijk et al. 2014). TMEM106B did not modify the age of onset or death in non-progranulin, non-C9orf72 FTD.

As summarized in Table 3, some alleles of known ALS genes have an influence not only on the risk of developing ALS but also on its phenotype. Thus, in cohorts of C9orf72 ALS, bulbar onset of the disease is more frequent than in other ALS cohorts. Among the many SOD1 mutations, the A4V allele is associated with a particularly devastating time course, with mean survival barely 1 year. Of the FUS mutations, the missense mutation P525L that ablates nuclear localization can be associated with juvenile onset and a remarkably fulminant course.

TREM2

Triggering receptor expressed on myeloid cells 2 (TREM2) is a 25-kDa receptor protein expressed in a complex on the surface of microglial cells. It participates in the immune response by signaling release of inflammatory cytokines. A rare polymorphism in the extracellular domain of TREM2, R47H, confers heightened risk for Parkinson's and Alzheimer's diseases (Rayaprolu et al. 2013), and was recently documented to increase the risk for ALS (Cady et al. 2014). In ALS cases, TREM2 expression in the spinal cord is elevated, and higher levels correlate with shorter survival (Cady et al. 2014).

ARHGEF1

McLaughlin et al. (2015) and colleagues analyzed runs of homozygosity in a single large Irish ALS cohort and identified potential recessive ALS risk loci by mapping specific homozygous haplotypes that are overrepresented in cases compared with controls. In particular, two top results (S3430_7; chr19:42,369,393–43,534,282) and (S4200_8; chr19:42,328,529–43,287,462) mapped to a location that spans the gene *ARHGEF* (McLaughlin et al. 2015). This gene also encodes a ρ GEF factor and therefore is a plausible ALS candidate gene, given the emerging importance of GEFs in neuronal homeostasis (as in alsin and C9orf72) (Droppelmann et al. 2013, 2014). The importance of GEF biology in this theme in ALS pathogenesis is further underscored by the observations that (1) a heterozygous frameshift mutation in the *ARHGEF28* gene was identified as a possible causative factor in one pedigree with fALS (Droppelmann et al. 2013), and (2) dysfunction of multiple GEFs plays a role in the pathogenesis of many neurodegenerative disorders (reviewed in Droppelmann et al. 2014).

CONCLUSION

In the last 10 years, progress in understanding the genetic architecture of ALS can only be described as remarkable. With the wide availability of affordable high-throughput genetic sequencing, the rate of discovery of genetic variables that play a role in ALS pathogenesis will only increase. Full exome sequencing is

now the standard in most gene searches. Furthermore, we anticipate that more than 1000 ALS genomes will be sequenced in full in the next 18–24 months. This permits several predictions. First, the remaining, rare causes of Mendelian ALS will likely all be defined. Second, for the first time we will be able to systematically screen noncoding DNA for disease-related genetic variations (e.g., in long noncoding RNAs or microRNA). Third, it will be feasible to study the role in ALS of epistasis—the interactions between multiple genes that each heighten disease susceptibility but cannot initiate pathogenesis alone. There are already several reports of oligogenic ALS cases, in which more than one ALS-related gene variant has been detected in the same individual (Luigetti et al. 2011; Chio et al. 2012; van Blitterswijk et al. 2012; Dekker et al. 2015; Pottier et al. 2015). As a corollary, it seems likely that an increasing fraction of apparently sALS cases will prove to be a consequence of multiple gene variants that are not individually penetrant. It is already apparent that first-degree relatives of ALS patients have an increased incidence compared with unrelated ones (Fallis and Hardiman 2009; Fang et al. 2009). Finally, improved knowledge of genetic variants and their related pathways to neurodegeneration will improve our capacity to define interactions between genetic topology and the environment (e.g., environmental toxins or elements within the microbiome). Perhaps most important, as the understanding of ALS genetics is refined, opportunities for developing meaningful therapies will improve. In part, this may be a direct consequence of improved methods to attenuate expression and toxicity of mutant ALS genes. Indirectly, one anticipates that it will be increasingly possible to stratify and personalize therapeutic approaches and trials based on molecular distinctions between subsets of ALS cases.

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Mehdi Ghasemi and Robert H. Brown, Jr.

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