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Genetics of familial amyotrophic lateral sclerosis

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a late onset, rapidly progressive and ultimately fatal neurodegenerative disease, caused by the loss of motor neurons in the brain and spinal cord. About 10% of all ALS cases are familial (FALS), and constitute a clinically and genetically heterogeneous entity. To date, FALS has been linked to mutations in 10 different genes and to four additional chromosomal loci. Research on FALS genetics, and in particular the discoveries of mutations in the SOD1, TARDBP, and FUS genes, has provided essential information toward the understanding of the pathogenesis of ALS in general. This review presents a tentative classification of all FALS-associated genes identified so far.

Key words
Amyotrophic lateral sclerosis • Genetics • SOD1 • TARDBP • FUS

Introduction

Amyotrophic lateral sclerosis (ALS) is an adult-onset, rapidly progressive neurodegenerative disorder, caused by the selective loss of upper and lower motor neurons in the cerebral cortex, brainstem and spinal cord. Neuronal degeneration leads to weakness, muscular atrophy, and spasticity that evolve to paralysis. The typical age at onset is between 50 and 60 years, and the global incidence is 1-2 new cases per 100.000 individuals every year. The disease is fatal within 2-5 years of onset, generally due to respiratory failure. As the cause of ALS is still unknown, there is presently no effective treatment for it (Rowland et al., 2001). Although the majority of ALS cases are sporadic (sporadic ALS, SALS), 10% of patients have a positive familial anamnesis for motor neuron disease, generally with an autosomal-dominant inheritance pattern, although recessive pedigrees have been described (familial ALS, FALS) (Mulder et al., 1986). The clinical phenotype of FALS cases is usually indistinguishable from SALS. However, in comparison to SALS, FALS is characterized by an equal male:female sex ratio, an earlier age at onset, and, generally a longer disease duration. The first signs of the disease often occur in the lumbosacral segment, and atypical symptoms may be present at onset (Li et al., 1988; Strong et al., 1991; de Belleroche et al., 1995; Majoor-Krakauer et al., 2003). To date, 12 disease loci have been reported to be associated with typical ALS or atypical motor neuron diseases and two loci with ALS with frontotemporal dementia (ALS-FTD) (Table I). The first major breakthrough in our understanding of the genetic basis of FALS came in 1993 with the discovery of pathogenic mutations in the *superoxide dismutase 1* (*SOD1*) gene (Rosen et al., 1993). *SOD1* mutations are the most frequently identified cause of FALS, accounting for ~20% of all patients. An exciting step forward in ALS genetics is represented

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Table I. - Different subtypes of FALS and their genetic determinants.

ALS type	Onset	Inheritance	Locus	Gene	Protein
ALS1	Adult	AD[1]	21q22.1	*SOD1*	Cu/Zn superoxide dismutase
ALS2	Juvenile	AR	2q33-35	*ALS2*	Alsin
ALS3	Adult	AD	18q21	unknown	
ALS4	Juvenile	AD	9q34	*SETX*	Senataxin
ALS5	Juvenile	AR	15q15-21	*SPG11*	Spatacsin
ALS6	Adult	AD[2]	16p11.2	*FUS*	Fused in sarcoma
ALS7	Adult	AD	20p13	unknown	
ALS8	Adult	AD	20q13.33	*VAPB*	VAMP-associated protein B
ALS9	Adult	AD	14q11	*ANG*	Angiogenin
ALS10	Adult	AD	1q36	*TARDBP*	TAR DNA-binding protein
ALS11	Adult	AD	6q21	*FIG4*	PI(3,5)P(2)5-phosphatase
ALS12	Adult	AR/AD	10p15-p14	*OPTN*	Optineurin
ALS-FTD1	Adult	AD	9q21-22	unknown	
ALS-FTD2	Juvenile	AD	9p13.2-21.3	unknown	

by the recent discovery of mutations in the *43-KDa TAR DNA binding protein* (*TARDBP*) (Kabashi et al., 2008; Sreedharan et al., 2008; Van Deerlin et al., 2008) and *fused in sarcoma/translocated in liposarcoma* (*FUS*) genes (Kwiatkowski et al., 2009; Vance et al., 2009). Mutations in *TARDBP* and *FUS* represent ~5% of all FALS cases each. Pathogenic mutations in seven other genes (*ALS2*, *SETX*, *SPG11*, *VAPB*, *ANG*, *FIG4*, and *OPTN*) account for < 5% of families. Usually those mutations are found in isolated pedigrees, often with atypical ALS phenotypes (Hadano et al., 2001; Yang et al., 2001; Chen et al., 2004; Nishimura et al., 2004; Greenway et al., 2006; Chow et al., 2009; Maruyama et al., 2010; Orlacchio et al., 2010). Lastly, variants in several other genes have been suggested to be associated with FALS, although the data are still inconclusive.

ALS1 - Cu/Zn Superoxide Dismutase

The linkage of ALS1 to chromosome 21q22.1 was initially described in 1991 (Siddique et al., 1991). Two years later, Rosen et al. (1993) described eleven disease-associated mutations in the *SOD1* gene, encoding for the Cu/Zn superoxide dismutase, a cytoplasmic enzyme responsible for the catabolism of superoxide radicals to hydrogen peroxide and molecular oxygen (McCord et al., 1969). The gene, spanning 9.3 kb, is composed of five exons and encodes for a 153 residues long protein. SOD1 is a 32 kDa omodimeric metalloenzyme, where each monomer consists of an eight-stranded beta-barrel and binds a copper and a zinc ion (Getzoff et al., 1989). SOD1 is ubiquitously expressed, highly conserved, and represent ~1% of all cytoplasmic proteins. To date, more than 140 different *SOD1* mutations have been reported (http://alsod.iop.kcl.ac.uk), the vast majority of which are missense substitutions distributed throughout the five exons of the gene. Eight frameshift deletions and five insertions, all clustered in exon 4 and 5, which lead to a premature truncation of the protein have also been described. Collectively, *SOD1* mutations are found in ~20% of all FALS patients, and in ~3% of SALS cases (Andersen et al., 2003). *SOD1* mutations are characterized by a considerable interfamilial and intrafamilial variability of the phenotype with regards to the age at onset, site of onset, and disease duration (Cudkowicz et al., 1997). A notable exception is represented by A4V, the mutation most frequently observed in ALS1 pedigrees, which is consistently associated with a high penetrance, younger age at onset, prevalence of lower motor neuron signs, and a very rapid disease course, usually less than 12 months (Cudkowicz et al., 1997; Juneja et al., 1997). Interestingly, the same aggressive phenotype is shared by other less common mutations in the same

region of exon 1, such as A4T, C6F, C6G, and G10V (Morita et al., 1996; Kohno et al., 1999; Aksoy et al., 2003; Kim et al., 2003). Conversely, other mutations, such as G41D, H46R and G93D, display a very mild phenotype, often with carriers surviving more than 20 years after the onset of the disease (Aoki et al., 1993; Cudkowicz et al., 1997; Luigetti et al., 2008). The penetrance of *SOD1* mutations is also variable, being almost complete for A4V, and less than 30% at 70 years for I113T (Orrell et al., 1999). It must be noticed, however, that the majority of the *SOD1* variants described so far are private mutations. Thus, conclusive genotype-phenotype correlation can be safely drawn only for a handful of them. Moreover, although *SOD1* mutations have never been observed in the general population, the pathogenic role of private variants has recently started to be questioned. For instance, Felbecker et al. (2010) described four families in which the E100K and D90A mutations are present in some affected individuals, but do not segregate with the disease within the pedigree. These findings must be taken into serious consideration with regards to genetic testing and counselling in the clinical practice. All *SOD1* mutations are inherited as dominant traits, with the exception of the D90A variant, that is observed both in recessive pedigrees in Scandinavia, and in dominant pedigrees in the rest of the world (Andersen et al., 1996; Robberecht et al., 1996). D90A homozygous families display a milder phenotype, characterized by upper motor neuron signs and prolonged survival, compared to heterozygous individuals that develop classic ALS. That, and the finding that recessive families share a common ancestor suggest the existence of a protective genetic factor in linkage with D90A in the Swedish population (Al-Chalabi et al., 1998).

To date, there is no conclusive explanation on how mutations in the *SOD1* gene cause ALS. Initially, it was hypothesized that mutations would impair the enzymatic activity of the protein, thus resulting in increased cellular levels of reactive oxygen species, oxidative stress, and neuronal death (Deng et al., 1993). However, it has subsequently been shown that some mutants retain full catalytic activity, and that there is no correlation between residual enzymatic activity, clinical progression, and disease phenotype (Radunovic et al., 1997). Additional evidence against a loss-of-function hypothesis comes from animal models: *SOD1* knockout mice do not develop motor neuron disease (Reaume et al., 1996), while transgenic mice (Gurney et al., 1994) and rats (Nagai et al., 2001) overexpressing the human mutant *SOD1* gene do. Also, the expression of mutant *SOD1* alleles in cell culture models induces apoptosis in neurons (Pasinelli et al., 1998). In both *in vitro* and *in vivo* models, dismutase activity appears to be normal or elevated, suggesting that *SOD1* mutations may result into the acquisition of a novel function, toxic to motor neurons (gain-of-function hypothesis). Several studies showed that mutant SOD1 is prone to misfolding and forms cytoplasmic aggregates. In turn, aggregates may lead to cell death by sequestering other cytoplasmic proteins essential for neuronal survival, by clogging the ubiquitin/proteasome system, by chaperones depletion, or by disrupting mitochondria, cytoskeleton and/or axonal transport. For a comprehensive overview on the proposed toxic properties of mutant SOD1 we suggest reading the reviews of Pasinelli et al. (2006) and Boillee et al. (2006).

ALS2 - Alsin

ALS2 is a rare autosomal-recessive, juvenile-onset motor neuron disease characterized by distal muscle atrophy and weakness, and spasticity progressively ascending from the lower limbs to the cervical and bulbar segments, originally described in Tunisia (Ben Hamida et al., 1990). The disease locus was mapped on chromosome 2q33-35 in 1994 (Hentati et al., 1994), and seven years later mutations in the *ALS2* gene were discovered in families of North African and Middle Eastern origin (Hadano et al., 2001; Yang et al., 2001). The *ALS2* gene, encoding for the protein alsin, spans 83 kb of genomic DNA and is composed of 34 exons. Through alternative splicing after exon 4, it gives rise to two different transcripts of 6.5 and 2.6 kb, resulting respectively in a 1657-residue (long form), and a 396-residue (short form) of alsin. At least 13 different *ALS2* mutations have been described so far, the majority of which are frameshift deletions resulting in a prematurely truncated protein, or nonsense mutations. Interestingly, it has been observed that the mutations that affect both the long and short form of the protein result in the juvenile ALS phenotype, while those that alter only the long form are responsible for the milder

phenotypes of juvenile primary lateral sclerosis or infantile-onset ascending hereditary spastic paraparesis, both characterized by an isolated involvement of the corticospinal tracts (Hadano et al., 2001; Yang et al., 2001; Devon et al., 2003; Gros-Louis et al., 2003; Kress et al., 2005; Eymard-Pierre et al., 2006; Panzeri et al., 2006). Alsin localizes to the cytosolic face of endosomal membranes and acts as a guanine nucleotide exchange factor for Rab5 and other small GTPases (Otomo et al., 2003). As such, it is thought to be involved in vescicular trafficking, cytoskeletal organization, and endosomal dynamics. The fact that all ALS2 pedigrees are homozygous, and the observation that mutations lead to protein instability suggest that the loss of the physiological functions of alsin lead to ALS (Yamanaka et al., 2003). It must however be noticed that *ALS2* knockout mice do not develop motor neuron disease (Cai et al., 2005).

ALS4 - Senataxin

ALS4 is a rare autosomal-recessive, juvenile onset, slow progressive motor neuron disease. Patients usually develop symmetrical weakness and atrophy of the distal muscles of the limbs in their second decade. The phenotype is characterized by the involvement of the corticospinal tracts, while bulbar muscles are consistently spared (Myrianthopoulos et al., 1964; Chance et al., 1998). ALS4, initially mapped to chromosome 9q34, has subsequently been found to be caused by mutations in the *SETX* gene (Chen et al., 2004). The gene is composed of 26 exons and encodes for the 303 kDa, 2.677-residue long protein senataxin. To date, only four *SETX* mutations have been identified in ALS pedigrees, and all of them are missense substitutions (Chen et al., 2004; Zhao et al., 2009). Interestingly homozygous or compound heterozygous *SETX* mutations, mostly leading to a premature termination of the protein product, have been identified in patients with ataxia-ocular apraxia-2 (AOA2) (Moreira et al., 2004). Senataxin is a ubiquitously expressed DNA/RNA helicase, possibly involved in repairing DNA double-strand breaks following oxidative stress (Suraweera et al., 2007). Moreover, it has been shown to bind RNA polymerase II and other proteins involved in mRNA transcription and processing, suggesting that it is involved in transcriptional regulation (Suraweera et al., 2009). *SETX* is also highly homologous to the *IGHMBP2* gene, encoding for the immunuglobulin-μ binding protein 2, which is also involved in RNA processing and is mutated in spinal muscular atrophy with respiratory distress type 1, a rare lower motor neuron disease (Grohmann et al., 2001).

ALS5 - Spatacsin

ALS5 is an autosomal-recessive, juvenile-onset motor neuron disease characterized by distal muscle atrophy and weakness associated with pyramidal signs and involvement of bulbar muscles. The disease has been identified in European, North African, South American and Asian pedigrees, and has been mapped to a locus on chromosome 15q15-21 (Hentati et al., 1998). Recently, Orlacchio et al. identified 12 homozygous or compound heterozygous mutations in the *SPG11* gene in 10 unrelated pedigrees (Orlacchio et al., 2010). The majority of the identified variants are frameshift mutations or nonsense substitutions. Homozygous mutations in the same gene have been previously been described in patients with autosomal recessive hereditary spastic paraplegia with thin corpus callosum (ARHSP-TCC) (Stevanin et al., 2007). However, none of the ALS5 patients displayed features suggestive of a concomitant ARHSP-TCC. In a single case, a *post-mortem* examination revealed pathological evidence of neurodegeneration in lower motor neurons, although Bunina bodies and ubiquitinated inclusions seem to be absent. *SPG11* is composed of 40 exons and encodes for the 2443-residue long protein spatacsin. The protein contains four putative transmembrane domains, suggesting that it may be a receptor or a transporter, a leucine zipper and a coiled-coil domain. The physiological role of spatacsin is still unknown, although it is reputed to be involved in axonal transport (Salinas et al., 2008).

ALS6 - Fused in sarcoma

The ALS6 locus on chromosome 16p12.1-q21 has been independently reported by three studies on six European and North American pedigrees with autosomal dominant classic ALS (Abalkhail et al., 2003;

Ruddy et al., 2003; Sapp et al., 2003). Recently, Kwiatkowski et al. (2009), and Vance et al. (2009) identified novel variants in the *FUS* gene as the disease-causing mutations in ALS6 families. Following the original reports, several other groups identified additional variants in ALS cohorts of different ethnicities, proposing an overall mutational frequency of ~4% in FALS and ~1% in SALS (Belzil et al., 2009; Blair et al., 2009; Chiò et al., 2009; Corrado et al., 2009; Damme et al., 2009; Drepper et al., 2009; Ticozzi et al., 2009; Baumer et al., 2010; Hewitt et al., 2010; Millecamps et al., 2010; Rademakers et al., 2010; Robertson et al., 2010; Tsai et al., 2010; Waibel et al., 2010; Yamamoto-Watanabe et al., 2010; Yan et al., 2010). To date more than 30 different mutations have been described, the vast majority of which are missense substitutions and the rest are frameshift or nonsense mutations. Although genotype-phenotype correlations are not possible for the majority of *FUS* mutations, it has been suggested that mutations of arginine 521, in particular R521C, may result in an uncommon phenotype characterized by a symmetrical proximal spinal onset, with early involvement of the axial muscles (Ticozzi et al., 2009). The *FUS* gene, spanning 9 kb and comprising 15 exons, encodes for a DNA/RNA binding protein initially isolated in cancer-associated fusion genes in malignant mesenchymal tumors (Crozat et al., 1993). FUS, which normally localizes to the cell nucleus, is involved in several cellular pathways, including transcriptional regulation, maintenance of genomic stability, and splicing, nucleo-cytoplasmic shuttling, transport, and maturation of mRNAs (Law et al., 2006). In the central nervous system, the protein is involved in regulating mRNA transport towards the dendrites and synaptic plasticity upon the activation of glutamate receptors (Fujii et al., 2005). The FUS protein is composed of an N-terminal transactivating domain rich in serine, tyrosine, glutamine and glycine, a central domain that contains a RNA recognition motif and a Cys_2/ Cys_2-type zinc finger motif, and a C-terminal region rich in arginine and glycine (Morohoshi et al., 1998). The last 18 C-terminal residues constitute the nuclear localization signal (NLS) of the protein (Zakaryan et al., 2006), and the majority of the mutations identified so far are clustered in this region. Interestingly, neuropathological examinations of tissues from patients harboring FUS/TLS mutations showed an increased cytoplasmic FUS-staining, FUS-immunoreactive dystrophic neurites, and cytoplasmic inclusions in lower motor neurons, in the absence of TDP-43 pathology (Kwiatkowski et al., 2009; Vance et al., 2009). Data from *in vitro* experiments further support the notion that the nucleo-cytoplasmic redistribution of FUS is a key event in determining inclusion formation and motor neuron toxicity: transfection of GFP-tagged FUS mutant constructs resulted in increased cytoplasmic localization compared to wild-type FUS in different cell lines. Compartmental fractionation of SKNAS cells transfected with R521G-*FUS* and wt-*FUS* constructs showed higher ratios of soluble cytosolic to soluble nuclear and total insoluble to soluble nuclear FUS for the mutant compared to the wild-type protein, reflecting both an increase in insoluble FUS and a decrease in soluble nuclear FUS. Conversely, mutations do not seem to affect the binding between FUS and its specific RNA targets (Kwiatkowski et al., 2009). Thus, it has been hypothesized that *FUS* mutations may contribute to ALS pathogenesis through the formation of cytoplasmic inclusions and/or the loss of the physiological nuclear functions of the protein. FUS immunoreactive cytoplasmic inclusions have now been found to be a common finding not only in ALS6, but also in atypical subtypes of FTD (Munoz et al., 2009; Neumann et al., 2009; Seelaar et al., 2010). These observations, and the reports of *FUS* mutations in FTD patients, with or without ALS (Blair et al., 2010; Ticozzi et al., 2009; Van Langenhove et al., 2010), have led to the identification of a novel FUS proteinopathy family.

ALS8 - VAMP-associated protein B

ALS8, in linkage with a locus on chromosome 20q13.33, has been initially described in a Brazilian family of Portuguese descent that manifested an autosomal-dominant, slow progressive ALS (Nishimura et al., 2004). All 12 affected individuals studied showed lower motor neuron symptoms, predominantly in the limb muscles. A single patient also presented upper motor neuron signs. Postural tremor, fasciculations, and painful cramps were also observed in the majority of patients. ALS8 was subsequently found to be caused by a single P56S mutation in the *VAPB* gene, which encodes for the VAMP (Vescicle-associated membrane

protein)-associated protein B. The same mutation was observed in seven additional Brazilian families, presenting with slow-progressive ALS, late onset spinal muscular atrophy, or typical ALS with rapid progression (Nishimura et al., 2004; Landers et al., 2008). Although for the original families haplotype analysis suggests a founder effect, P56S has been recently identified in a German pedigree (Funke et al., 2010). The *VAPB* gene spans 57.7 kb of genomic DNA and is composed of six exons. Through alternative splicing, it gives rise to two proteins of 243 (VAPB) and 99 residues (VAPC). VAPB/C are ubiquitously expressed, localize to the endoplasmic reticulum and associate with microtubules, suggesting a role in vesicle trafficking (Nishimura et al., 2004; Kanekura et al., 2006). Since VAPB/C are present in a homo- or heterodimeric state, it is presumed that the P56S mutation exerts a dominant effect on the wild-type subunit. In fact, it has been shown that P56S-VAPB can form insoluble cytoplasmic inclusions in neural and non-neural cell lines, sequestering and co-precipiting wt-VAPB (Suzuki et al., 2009). The aggregates and/or the loss of the physiological functions of VAPB may in turn lead to motor neuron degeneration. Supporting the latter hypothesis is the observation that the silencing of the *Drosophila melanogaster Dvap33* gene, homologous to *VAPB*, leads to progressive paralysis of the larvae, formation of cytoplasmic inclusions and neurodegeneration. The phenotype is rescued by overexpressing the human *VAPB* gene (Chai et al., 2008).

ALS9 - Angiogenin

Increasing evidence supports the hypothesis that angiogenic factors may be involved in the pathogenesis of ALS. A screening of a large cohort of North European patients reported an association between two haplotypes in the vascular endothelial growth factor gene (*VEGF*) promoter and susceptibility to SALS (Lambrechts et al., 2003). The deletion of the hypoxia response element in the gene promoter and the consequent down-regulation of VEGF expression lead to a progressive motor neuron degeneration in mice (Lambrechts et al., 2003). Conversely, the administration of VEGF to $hSOD^{G93A}$ transgenic mice has neuroprotective effects and ameliorates the disease phenotype (Azzouz et al., 2004). In 2004, the rs11701 single nucleotide polymorphism (SNP) in the ANG gene, encoding for angiogenin, a downstream effector of VEGF, was shown to be associated to SALS susceptibility in the Irish and Scottish populations (Greenway et al., 2004). Direct sequencing of the coding regions of *ANG* in 1629 ALS patients and 1264 controls identified seven missense mutations in 15 individuals, of which four were FALS and 11 SALS (Greenway et al., 2006). Additional mutations have been subsequently found in European and North American cohorts, with a mutational frequency higher in FALS (2.3%) than SALS (1.0%) (Wu et al., 2007; Conforti et al., 2008; Gellera et al., 2008; Paubel et al., 2008; Millecamps et al., 2010). The association with rs11701, however, could not be replicated. The identification of a large Dutch ALS-FTD family in which the K17I mutation segregates with the disease led to the designation of all *ANG*-associated FALS as ALS9 (van Es et al., 2009). The *ANG* gene, spanning 5.4 kb on chromosome 14q11, is composed of two exons, of which only one coding. Angiogenin, a member of the pancreatic ribonuclease superfamily, is expressed mainly in hepatocytes and secreted into the serum and the extracellular matrix. After uptaking by still unidentified endothelial receptors, the protein is translocated into the nucleolus where it stimulates tRNA transcription, ribosome biogenesis, protein translation, and cell proliferation (Moroianu et al., 1994; Smith et al., 2006). Mature angiogenin has 123 amino acids and is obtained after the cleavage of a signal peptide 24-residue long, which is responsible for the correct secretion of the protein. Three amino acids (H13, K40, and H114) compose the catalytic site, while the residues 31-35 constitute the nuclear import signal. The majority of the 15 mutations described so far are clustered in these regions and are consequently predicted to disrupt angiogenin secretion, ribonucleolytic activity and/or nuclear translocation, ultimately resulting in impairment angiogenesis (Wu et al., 2007). The loss of angiogenin physiological functions may thus lead to motor neuron degeneration. In fact, wild-type angiogenin has been shown to protect primary motor neurons from hypoxia-induced cell death, while ALS-associated *ANG* mutants do not. Moreover, the mutant protein impairs neurite growth and pathfinding in murine cell lines and appears to be toxic on

motor neurons *in vitro* (Subramanian et al., 2007). Lastly, administration of human recombinant angiogenin prolongs lifespan in hSOD1^{G93A} mice (Sebastia et al., 2009).

ALS10 - TDP-43

Using a combined biochemical and immunoistochemical approach, Neumann et al. (2006) identified the 43-kDa TAR-DNA binding protein (TDP-43) as the main component of ubiquitinated cytoplasmic inclusions in ALS. In aggregates, TDP-43 is hyperphosphorylated and cleaved to generate abnormal C-terminal fragments. Moreover, while in unaffected neurons TDP-43 localizes in the cell nucleus, it is absent from the nuclei of neurons with ubiquitinated inclusions, suggesting a nucleo-cytoplasmic redistribution of the protein. These observations lead to intense speculations on the pathogenic role of TDP-43 in ALS: toxicity might be caused by aggregating TDP-43 being sequestered away from its normal nuclear function or, conversely, TDP-43 aggregates might have a toxic gain-of-function independent of the protein's physiological cellular activities (Lagier-Tourenne et al., 2010; Strong, 2010; Ticozzi et al., 2010; van Blitterswijk et al., 2010). Mutations in the *TARDBP* gene, encoding for TDP-43, have been subsequently found to be a major cause of FALS, having been identified in several populations of different geographic origin (Gitcho et al., 2008; Kabashi et al., 2008; Kuhnlein et al., 2008; Rutherford et al., 2008; Sreedharan et al., 2008; Van Deerlin et al., 2008; Corrado et al., 2009; Del Bo et al., 2009; Kamada et al., 2009; Lemmens et al., 2009; Ticozzi et al., 2009; Iida et al., 2010 epub; Millecamps et al., 2010; Tsai et al., 2010). The proposed mutational frequency is ~5% for FALS and 0.5-2% for SALS. TDP-43 is a 414 amino-acids multifunctional DNA/RNA binding protein belonging to the heterogeneous ribonucleoprotein (hnRNP) family that was originally identified as a binding protein of the HIV-1 virus TAR DNA element (Ou et al., 1995). Although the specific functions of TDP-43 in neuronal cells remain to be evaluated, the protein has been demonstrated to play a role in several biological processes, including gene transcription, splicing regulation, transport and stabilization of mRNA molecules (Buratti et al., 2008). The protein is constituted by two highly conserved RNA recognition motifs, flanked by an N-terminal domain and a C-terminal tail. The latter element contains a glycine-rich region that is reputed to mediate protein-protein interactions, mainly with others hnRNPs (Buratti et al., 2005). To date, more than 30 different *TARDBP* mutations have been described, all of which are missense substitutions. With a single exception, all of them are clustered in the C-terminal glycine-rich region encoded by exon 6. Since most *TARDBP* mutations are private, it is difficult to establish clear genotype-phenotype correlation. It has been suggested that A382T, which is the variant most commonly observed, may be associated with a predominantly lower motor neuron disease with an asymmetrical onset in the distal muscles of the limbs, subsequently spreading to proximal muscles, with relative sparing of the bulbar muscles (Corrado et al., 2009).

ALS11 - PI(3,5)P(2)5-phosphatase

Recently, Chow et al. (2009) identified ten heterozygous mutations in the *FIG4* gene in six SALS and three FALS patients. The identified variants include missense and nonsense substitutions, mutations at consensus splice sites and frameshift insertions and deletions. Interestingly, two patients had been diagnosed with primary lateral sclerosis, and the majority of the others had prominent signs of corticospinal tracts degeneration. The compound heterozygosity of the I41T *FIG4* mutation with a nonsense or frameshift mutation had been previously been identified as the genetic cause of the Charcot-Marie-Tooth disease type IV, an autosomal recessive demyelinating neuropathy characterized by an infantile onset and rapid progression (Chow et al., 2007). *FIG4*, composed of 23 exons, encodes for a 907-residue long phosphoinositide phosphatase that regulates the synthesis and turnover of phosphatidylinositol-3,5-bisphosphate, a signalling lipid that mediates the retrograde transport of endosomal vescicles to the *trans*-Golgi network (Rutherford et al., 2006). Interestingly, pale tremor mice, which are homozygous for null mutations of *FIG4*, display widespread neuronal degeneration in sensory and autonomic ganglia, motor cortex, striatum, and cerebellum (Chow et al., 2007). A similar phenotype is observed also in mice knockout for *Vac14*, a gene

encoding for a protein associated to the FIG4 complex (Zhang et al., 2007).

Other FALS-associated genes

Mutations in several genes involved in cytoskeletal stability and axonal transport have been suggested to play a role in ALS pathogenesis. In particular, six deletions within the C-terminal lysine-serine-proline (KSP) phosphorylation domain of the *NEFH* gene, encoding for the neurofilament heavy subunit, have been found in several SALS patients and in a pedigree with autosomal dominant FALS (Figlewicz et al., 1994; Al-Chalabi et al., 1999). However, another study on 117 unrelated FALS cases did not identify any mutation in the KSP region of *NEFH* (Rooke et al., 1996). Peripherin is a neuronal intermediate filament protein involved in axonal outgrowth, and commonly detected within ubiquitinated inclusions in motor neurons of ALS patients (He et al., 2004). Mice overexpressing wild-type peripherin develop over time a selective degeneration of spinal ventral roots and motor axons, as well as intermediate filament inclusions within motor neurons (Beaulieu et al., 1999). A homozygous missense mutation and a heterozygous frameshift deletion in the *PRPH* gene, encoding for peripherin, have been identified in ALS patients (Gros-Louis et al., 2004; Leung et al., 2004). Dynactin is a multi-subunit protein that modulates binding between cytoplasmic dynein and microtubules, thus being essential for retrograde transport. The dynactin complex is composed of ten different subunits, of which the largest one is p150, encoded by the *DCTN1* gene. Overexpression of dynamitin in transgenic mice leads to a disassembling of the dynein-dynactin complex, resulting in an inhibition of retrograde axonal transport and in a late onset, slowly progressive lower motor neuron disease (LaMonte et al., 2002). A single G59S mutation in the p150 domain of *DCTN1* has been observed in a family with autosomal dominant distal lower motor neuron disease with vocal cord paralysis (Puls et al., 2003). Subsequently, three additional heterozygous missense mutations have been found in sporadic and familial patients with classic ALS (Munch et al., 2004). Interestingly, heterozygous mutations in *DCTN1* have also been identified in Perry syndrome, a rapidly progressive, autosomal dominant atypical parkinsonism characterized by weight loss, depression, nocturnal hypoventilation, and the presence in the extrapyramidal system of TDP-43 immunoreactive inclusions indistinguishable from those found in ALS (Farrer et al., 2009). A second group of candidate genes suggested to play a role in FALS pathogenesis are those involved in the metabolism of xenobiotics. Organophosphorous compounds (OP) are chemicals widely employed in agricultural and industrial settings that can disrupt the cholinergic transmission in the central nervous system and at neuromuscular junctions. The covalent binding of OP to the neuropathy target esterase protein (NTE) leads to axonal degeneration in the spinal cord and peripheral nerves, resulting in delayed neuropathy. Homozygous mutations in the *NTE* gene have been associated with a slowly progressive motor neuron disease with spastic paraplegia and distal muscle atrophy in two pedigrees (Rainier et al., 2008). A major detoxifying system for OP is represented by the paraoxonase enzymes (PON1, PON2 and PON3), which are also involved in protecting cells against oxidative damage. Several studies have suggested an association between common haplotypes in the *PON* cluster and SALS susceptibility (Saeed et al., 2006; Slowik et al., 2006; Cronin et al., 2007; Morahan et al., 2007; Landers et al., 2008; Valdmanis et al., 2008), although published genome-wide association studies and a metanalysis of the literature failed to replicate these results (Wills et al., 2009). A recent study, however, identified eight mutations in the *PON* cluster in 12 patients, of which nine were FALS and three SALS. All were heterozygous missense or splicing mutations, with the exception of a single homozygous mutation in *PON2*, identified in an autosomal recessive pedigree (Ticozzi et al., 2010). Since, however, the segregation of the mutations with the disease could not be proven, nor functional data are available, further studies will be needed to validate the role of *PON* mutations in ALS pathogenesis.

Summary

The information provided by genetic studies toward the understanding of the pathogenesis of both FALS and SALS has been invaluable. It has led over the years to the identification of novel cellular pathways involved in motor neuron degeneration, has

provided potential therapeutic targets, and made possible the engineering of animal models of ALS, providing essential tools for validating drugs efficacy. Undoubtedly, the discovery of novel FALS-associated genes, will dramatically further our knowledge of ALS pathogenesis; unfortunately, only one third of the genetic variability in FALS has been explained so far. To date, two strategies have typically been used to identify new genes in monogenic diseases: linkage analysis and the candidate gene approach. Linkage analysis requires large pedigrees composed of many affected individuals in different generations: this task is considerably difficult in ALS, a disease of adult life with a rapid disease course. On the other hand, the latter approach, while feasible in cohorts of unrelated FALS cases, is flawed by a selection bias. Until now, it was not economically feasible to screen for rare variants at a genome-wide scale on large cohorts. However, the recent advances in automated short-read DNA sequencing offer new solutions to this problem: it is now possible to sequence only protein-coding regions of the genome (exomes) in order to reduce costs while enriching for discovery of highly penetrant variant. Using this approach, several groups identified genes implicated in the Freeman-Sheldon syndrome, Miller syndrome, congenital chloride diarrhea, and X-linked mental retardation, proving that the sequencing of exomes in a small number of unrelated affected individuals is a powerful, efficient strategy for identifying genes underlying Mendelian diseases (Choi et al., 2009; Ng et al., 2009a; 2009b; Tarpey et al., 2009). Undoubtedly, the application of these techniques to the study of genetics of neurodegenerative disorders, including ALS, will help identifying novel causative genes, thus providing essential information about pathogenesis and new powerful tools aimed at a treatment of these devastating diseases.

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