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MINI-SYMPOSIUM: AMYOTROPHIC LATERAL SCLEROSIS: AN UPDATE ON ITS COMPLEXITY

New ALS-Related Genes Expand the *Spectrum Paradigm* of Amyotrophic Lateral Sclerosis

Mario Sabatelli[1]*; Giuseppe Marangi[2]; Amelia Conte[1]; Giorgio Tasca[3]; Marcella Zollino[2]; Serena Lattante[2]*

[1] Department of Geriatrics, Neurosciences and Orthopedics, Clinic Center NEMO-Roma. Institute of Neurology.
[2] Institute of Medical Genetics, Catholic University School of Medicine, Rome, Italy.
[3] Don Carlo Gnocchi ONLUS Foundation, Italy.

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Corresponding author:
Mario Sabatelli, MD, Centro Clinico NEMO-Roma, Policlinico A. Gemelli. Largo Gemelli, 8. 00168 Rome, Italy
(E-mail: *msabatelli@rm.unicatt.it*)

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*Contributed equally to this work.

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Abstract

Amyotrophic Lateral Sclerosis (ALS) is characterized by the degeneration of upper and lower motor neurons. Clinical heterogeneity is a well-recognized feature of the disease as age of onset, site of onset and the duration of the disease can vary greatly among patients. A number of genes have been identified and associated to familial and sporadic forms of ALS but the majority of cases remains still unexplained. Recent breakthrough discoveries have demonstrated that clinical manifestations associated with ALS-related genes are not circumscribed to motor neurons involvement. In this view, ALS appears to be linked to different conditions over a continuum or spectrum in which overlapping phenotypes may be identified. In this review, we aim to examine the increasing number of *spectra*, including ALS/Frontotemporal Dementia and ALS/Myopathies *spectra*. Considering all these neurodegenerative disorders as different phenotypes of the same *spectrum* can help to identify common pathological pathways and consequently new therapeutic targets in these incurable diseases.

INTRODUCTION

Amyotrophic Lateral Sclerosis (ALS) is a devastating neurodegenerative disease leading to paralysis and respiratory failure within 3–5 years after symptoms begin. The clinical picture of ALS is a stereotypical one, resulting from a combination of signs secondary to dysfunction of upper motor neurons (UMN) in the cerebral cortex and of lower motor neurons (LMN) located in the brainstem and the spinal cord. However, clinical heterogeneity is a well-recognized feature of the disease as age of onset, site of onset and duration of the disease can vary greatly among patients (93, 103). The variable mix of UMN and LMN signs is an additional major contributor to clinical heterogeneity of ALS (39, 91, 92). Classic ALS, in which predominant LMN signs combine with slight to moderate pyramidal syndrome, is the most frequent form. Other less frequent phenotypes include Progressive Muscular Atrophy (PMA), with pure LMN involvement, and Primary Lateral Sclerosis (PLS), with isolated UMN involvement. PMA and PLS are the two extremities of the lower and upper motor neuron involvement *spectrum*, where intermediate phenotypes may be identified, including UMN-Dominant ALS (91, 92, 103) (Figure 1).

Since the discovery of *SOD1* mutations in familial ALS cases (fALS) in 1993 (89), genetic research has revealed that ALS is not a single entity but rather a syndrome in which a constellation of causative genes have been discovered and new ones are expected to be discovered in the next few years, most likely.

A limited number of genes, including *C9orf72*, *SOD1*, *TARDBP*, *FUS* and *TBK1,* are responsible for a significant proportion of familial and sporadic ALS cases. Conversely, several genes are increasingly being recognized, which are responsible for a small number of cases or even for isolated ALS families (93). The etiologic fragmentation of ALS, driven by the genetic discoveries, contrasts with an opposite view in which near all ALS subtypes appear to be unified by a single pathological signature, namely the presence of abnormal accumulation of the transactive response DNA binding protein (TDP-43) in the cytoplasm of neuronal and glial cells (74).

Notably, in the light of the recent genetic discoveries some conceptual aspects regarding ALS are going to be revisited.

ALS: PART OF SEVERAL *SPECTRA* RATHER THAN A SINGLE ENTITY

While ALS has long been considered a paradigm of pure motor neuron disorder, there is now evidence that the majority of ALS-related genes are pleiotropic, as they give raise to disparate clinical manifestations. Importantly, clinical manifestations associated with single gene defects appear to vary over a *continuum* or *spectrum*

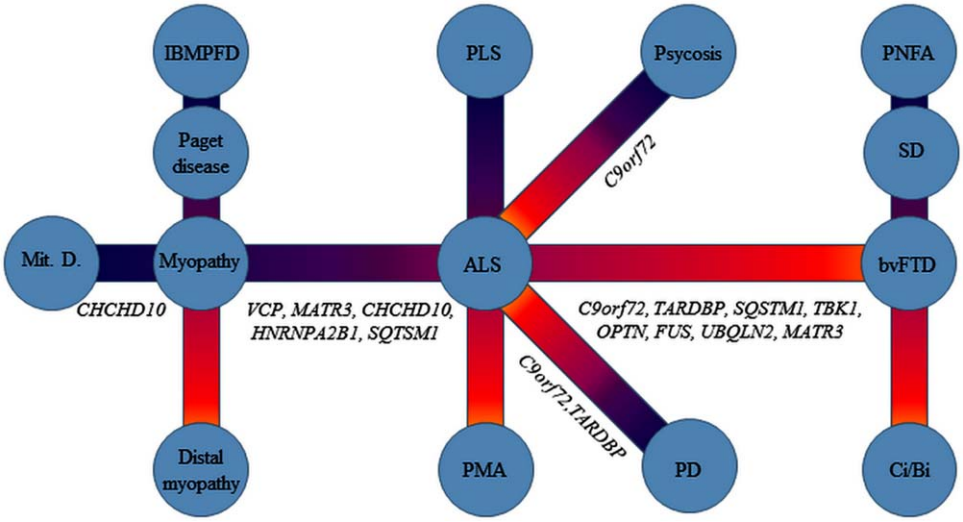

Figure 1. ALS is a part of several clinical *spectra*. Intermediate/overlapping phenotypes may occur in each *spectrum*. IBMPFD = inclusion-body myopathy with Paget disease of the bone and fronto temporal dementia; PD = Parkinson Disease; PLS = primary lateral sclerosis; PMA = progressive muscle atrophy; PNFA = progressive nonfluent aphasia; SD = semantic dementia; Ci/Bi = mild cognitive (language, executive) or behavioral impairment); bvFTD = frontotemporal dementia behavioral variant; Mit.D = mitochondrial diseases.

with frequent overlapping phenotypes. The discovery of new ALS-related genes is expanding the number of conditions linked to ALS, suggesting that the clinical syndrome called ALS should be reconsidered from the perspective of the *"spectrum paradigm."*

ALS/ FRONTOTEMPORAL DEMENTIA SPECTRUM

The most common and well-established *spectrum* is the ALS/Frontotemporal Dementia (FTD) *spectrum*, connecting ALS with FTD, the second cause of presenile dementia due to degeneration of the frontal and temporal lobes in the brain (60).

The link between these two conditions is supported by several clinical, pathological and genetic observations. From a clinical point of view, patients with ALS may exhibit cognitive abnormalities ranging from impaired frontal executive dysfunction to overt FTD. In a population-based study, it was showed that co-morbid dementia occurs in approximately 14% of patients with a new diagnosis of ALS (83). Cognitive impairment without overt dementia, mainly in the form of executive dysfunction, occurs in more than 40% of ALS patients. Conversely, 15% of FTD patients develop a motor neuron disease. The description of ALS patients with relatives affected by pure ALS or pure FTD or ALS/FTD strongly supports the concept that ALS and FTD are closely related conditions (60). On pathological ground, neuronal inclusions positive for TDP-43, detected in ALS are also present in FTD patients (74). Finally, the decisive evidence was the identification of an etiologic link. Many genetic defects, including mutations in *VCP*, *TARDBP*, *SQTSM1*, *FUS* and *UBQLN2* underlay this *spectrum* but the identification of *C9orf72* gene was the biggest advance in this field. The large hexanucleotide (GGGGCC) repeat expansion in the first intron of *C9orf72,* located on chromosome 9p21, is the most common mutation detected so far in patients with familial ALS (50%–72% of fALS) as well as in patients with familial FTD (11.7%–17.8% of cases) (16, 26, 40, 68, 84, 86, 98, 99, 101). Interestingly, the frequency of cognitive impairment is 40%–50% in ALS patients carrying the *C9orf72* expansion, compared with 8%–9% of ALS cases without *C9orf72* mutation (26, 40, 98).

Newly discovered genes have been related to ALS/FTD spectrum. One of these is *MATR3*, encoding a nuclear matrix protein, identified by exome sequencing in 2014. A missense mutation in *MATR3* was found in a family of European ancestry with several members affected by ALS and dementia or cognitive impairment (48). However, no cognitive impairment has been reported in the two other mutated *MATR3* cases described, a Taiwanese patient with A72T mutation (65) and an Italian patient with C85S variant (76). *MATR3* mutations appear to be rare in ALS patients as they were not found in ALS and ALS/FTD series from France (69) and Australia (35). Further cohorts need to be screened to assess the contribution of *MATR3* in ALS/FTD.

In 2014, the *CHCHD10* gene, encoding a mitochondrial protein of the intermembrane space, was first associated to familial ALS/FTD (4). The S59L mutation was identified in a French family in which one member had isolated ALS and the remaining had FTD associated with ALS, ataxia or myopathy (4). Screening of *CHCHD10* in French cohorts, including 26 familial FTD/ALS, 85 sporadic FTD/ALS and 9 familial FTD, resulted in the detection of the previously identified S59L mutation in 1/26 (3.8%) of familial ALS/FTD and of a novel P34S variant in 2/85 (2.3%) of sporadic ALS/FTD (14). Notably, patients with P34S presented with behavior disorders and later developed motor neuron disease. Among an Italian cohort of 224 SALS patients, of whom 31 also had FTD, the P34S variant was detected in two cases with isolated ALS (17). An additional Italian patient with isolated ALS and P34S variant has been described (88). The pathogenicity of P34S is controversial: the variant did not always segregate with the disease, it was found in healthy controls and some bioinformatics prediction programs suggested its nonpathogenicity (1, 30, 67, 113, 124). Other *CHCHD10* variants have been identified later by whole exome

sequencing (WES), consisting of R15L in 2/102 German cases, and of G66V in a Finnish patient out of 26 Nordic FALS subjects (71). All of them had isolated ALS without dementia. The R15L variant was also described in three additional families and in one additional sporadic patient with isolated ALS (47, 56, 125). In addition, the P80L and P12S variants were identified in patients with classic ALS, and the P23T, A35D and Q82X variants in patients with sporadic FTD (31, 125).

Thus, *CHCHD10* mutations may be associated with different phenotypes and variable age of onset and disease duration. The higher frequency of *CHCHD10* mutations in sporadic than in familial cases may be explained by incomplete penetrance.

Another new gene linking ALS to FTD is *TUBA4A*, encoding a component of microtubules, namely an α-tubulin (100). By analyzing exomes of patients and controls, an excess of rare damaging variants in *TUBA4A* was found in fALS patients. Data were supported by functional studies, demonstrating that some of these variants disrupt microtubule dynamics and stability, at least in *in vitro* models (100). The screening was extended to a large cohort of sporadic cases with different geographic origin and to a smaller cohort of ALS patients with concomitant FTD. Further mutations, with predicted deleterious effects, were described, supporting the role of this gene in ALS and the hypothesis that defects in neuronal cytoskeleton architecture can lead to neurodegeneration (80). At least in three cases, *TUBA4A* mutations were associated to ALS/FTD: a patient with ALS/FTD carried the R215C (and his mother, not available for the test, had FTD as well), an ALS patient carried the T145P variant and reported a family history of FTD (100) and finally an ALS patient with D438N had also cognitive impairment (80). Further studies are needed to assess the impact of *TUBA4A* in these disorders, especially in ALS/FTD, to test the segregation of the mutations and to clarify the presence of variants in healthy controls. Conversely, all the mutations found only in patients affect a conserved region in exon 4, suggesting that they can cause the disruption of a crucial functional domain of the protein, as suggested by in vitro experiments (100).

The most recent ALS-related gene is TANK-binding kinase 1 (*TBK1*) (18, 36). Frequency of dominant *TBK1* loss-of-function mutations was 4% in a cohort of 252 genetically unexplained fALS patients (18). Importantly, 50% of patients with *TBK1* mutations showed cognitive impairment or overt FTD, a proportion that is similar to that observed in the affected carriers of *C9orf72* expansion (18, 26, 40, 98). Eight different loss-of-function mutations in *TBK1* were described in familial ALS/FTD patients of European origin (36). Two additional studies confirmed the role of *TBK1* in ALS/FTD. Among 104 pathologically confirmed Frontotemporal Lobar Degeneration-TDP patients, negative for *C9orf72* and *GRN* mutations, three were found to carry a heterozygous missense mutation in *TBK1* and one a loss-of-function mutation (R117*) in *TBK1* associated with deletion of exons 13–15 of *OPTN* (G538Efs*27) (82). Of interest, an additional patient had a compound heterozygous mutations in *OPTN* (82). More recently, a frameshift mutation (L399fs), likely resulting in a truncated protein, has been found in a fALS patient of Chinese origin (121).

These findings make *TBK1* a major gene causing ALS/FTD *spectrum*, add *OPTN* to the list of genes underlying this *spectrum* and stress the involvement of the autophagy pathway in the pathogenesis of these diseases.

ALS/ MYOPATHY *SPECTRUM*

Genetic findings along with clinical and pathological observations indicate that ALS may be linked to different forms of muscular disorders.

ALS/inclusion body myopathies *spectrum*

Inclusion Body Myopathies (IBMs) are a heterogeneous group of muscular disorders including two main categories: sporadic inclusion body myositis (sIBM) and hereditary inclusion body myopathy (hIBM). sIBM occurs sporadically in families while hIBM shows Mendelian inheritance with autosomal recessive or dominant transmission (29).

Muscle biopsy in both entities reveals distinctive abnormalities, including (i) myopathic changes such as variability of muscle fiber diameter and central nuclei, (ii) rimmed vacuoles, (iii) cytoplasmic inclusions. Endomysial inflammatory exudates surrounding and invading non-necrotic muscle fibers are present in sIBM while are not a feature of hIBM.

However the distinction between the "inflammatory" and the "degenerative" components in IBMs is not well established. Clinically, sIBM is usually refractory to immunotherapies and shows a slowly progressive, degenerative-like course leading to disability, though life expectancy is not affected. The degenerative pathophysiology of sIBM is further supported by the identification in muscle fibers of protein aggregates generally associated with other neurodegenerative diseases, including TDP-43 and p62, amyloid-β, hyperphosphorylated tau, ubiquitin, neurofilament heavy chain, presenilin and parkin (7, 29).

ALS/sIBM

sIBM is the most frequent acquired myopathy among the elderly. It is characterized by weakness and atrophy involving mainly the quadriceps and deep finger flexor muscles in the forearm, followed by involvement of more proximal muscle groups. ALS and sIBM may resemble each other in several aspects as to make differential diagnosis challenging. Clinically, some features of sIBM overlap to a large extent with those observed in ALS. In fact, in sIBM patients muscular involvement may be asymmetric, dysphagia occurs in 50%–70% of cases and the course of the disease is progressive. Importantly, EMG examination in sIBM demonstrates fibrillation potentials in the majority of cases, and motor unit action potentials show mixed myopathic and neuropathic changes, with the latter being likely due to reinnervation of denervated and split muscle fibers. In some cases, the neurogenic pattern may overshadow the myopathic changes leading to misdiagnosis of ALS. Muscle biopsy and imaging are useful tools to diagnosis (105). Though ALS and sIBM remain separate entities, TDP-43 accumulation represents an important link between these conditions but its significance remains to be elucidated (7, 29). Interestingly, genetic components in sIBM are starting to be explored more systematically. Besides the known association with the major histocompatibility loci, rare variants in *VCP* and *SQSTM1* genes as well as in genes involved in mitochondrial DNA maintenance have been reported in sIBM patients (37, 119).

ALS/hIBM

Three main clinical-genetic conditions are included in the hIBM group: hereditary inclusion-body myopathy with Paget's disease of the bone (PDB) and frontotemporal dementia (IBMPFD); GNE myopathy caused by mutations in the UDP-Nacetylglucosamine 2-epimerase/N-acetylmannosamine kinase (*GNE*); hereditary inclusion-body myopathy with congenital joint contractures and external ophthalmoplegia associated with mutations in Myosin Heavy Chain IIa gene (MyHC-IIa).

Mutations in valosin-containing protein (*VCP*) were identified in a proportion of IBMPFD (52, 115, 116, 118). The gene was identified using a candidate gene approach in 13 families linked to chromosome 9, where 82% of individuals had myopathy, 49% had PDB and 30% had early-onset FTD (115). The majority of mutations were found within the N-terminal domain of the protein, with a mutational hot-spot at codon 155, where different amino acid changes were described (R155H, R155P, R155C) (115). The recurrent R155H missense variant was then identified in an Italian family where three carriers presented with progressive IBM and FTD and only one developed PDB. Muscle biopsy showed atrophic fibres, rimmed vacuoles and congophilic amyloid deposits, which were described also in a VCP mouse model (114). In a review of 122 VCP related IBMPFD cases, approximately 50% showed osteolytic bone lesions consistent with PDB and only one third developed FTD. Muscular weakness was present in about 90% of patients, with a mean onset of 45 years of age, and it was the presenting symptom in more than 50% of patients. Muscle involvement was an isolated symptom in 30% of patients (52).

The clinical phenotypes of VCP-related myopathy are highly variable, including the limb girdle muscle phenotype, a scapulohumeral form, a distal myopathy and an axial form with head drop. The pattern of muscle involvement is frequently focal, while symmetric distribution is less common. The characteristic rimmed vacuoles are described in 39% of muscle biopsies (51, 52).

Patients carrying the same *VCP* mutation show different clinical phenotypes. For example, the missense variant R159H was reported in members of a Belgian family presenting with pure FTD, while patients from another unrelated Belgian family had FTD and PDB; no signs of IBM was observed in these families (111).

The identification of *VCP* mutations in a subset of sporadic and familial ALS cases (46) has established a consistent link between ALS and IBMPFD. Actually, in the original family with IBMPFD, in which a *VCP* mutation was later identified (52, 109), the disease was described as a "familial disorder of combined lower motor neuron degeneration and skeletal muscle disorganization," suggesting coexistence of both a primarily neurogenic process and myopathy. The concept that ALS and IBMPFD are part of a spectrum of VCP proteinopathies is supported by the description of *VCP* mutated individuals from the same kindred presenting with different phenotypes, including ALS, IBM, FTD or myopathy (5, 38, 52, 55). Some specific *VCP* mutations have been reported to cause ALS, others only IBMPFD, while others have been described in association with both conditions (38). *VCP* mutations are rare in ALS patients, accounting for $<1\%$ of fALS and are more frequently associated with ALS/FTD than with a pure ALS phenotype (2, 44, 53, 59, 70, 107, 120, 126).

The presence of TDP-43-positive aggregates is a common phenomenon in ALS and myopathies associated with *VCP* mutation as well as in other forms of hereditary IBM (8, 24, 57, 75, 94, 117).

Importantly, ALS/IBMPFD *spectrum* is associated with other, though less frequent, genetic defects.

Mutations in *HNRNPA2B1*and *HNRNPA1* have been identified in ALS and IBMPFD (50). In a family with IBMPFD, with no *VCP* mutations, WES allowed for detection of a variant in *HNRNPA2B1* gene (50). Two affected members had myopathy and PDB, one had myopathy, PDB and cognitive impairment and two had motor neuron disease, associated with myopathy, PDB and cognitive impairment. Muscle biopsies from one patient with motor neuron dysfunction showed atrophic fibres, central nuclei and rimmed vacuoles with TDP-43 accumulation. Conversely, a variant in *HNRNPA1* was identified in a German family with PDB associated with a late-onset autosomal dominant myopathy starting in the pelvic girdle at about age 40 years, with rapidly-progressing course. Histology showed myopathic changes with rimmed vacuoles and inclusion bodies on muscle biopsy (50). Finally, a mutation in *HNRNPA1* has been detected in cases with autosomal dominant ALS (50).

In another family with *HNRNPA2B1* mutation, clinical manifestations were characterized by myopathy and PD in all four members, with associated neurogenic weakness in one individual and cognitive impairment in the remaining three (5).

The absence of known disease-causing mutations in several sporadic and familial cases with the ALS/IBMPFD *spectrum* indicates that genetic heterogeneity of this condition is likely to expand over the next years (5, 63, 95).

Some authors proposed the term "Multisystem Proteinopathy (MSP)" to define the ALS/IBM *spectrum* (5, 106) where a combination of two or more signs among IBM, PDB and ALS/FTD was considered sufficient for the diagnosis of MSP.

ALS/DISTAL MYOPATHIES

Distal myopathies are a group of muscle diseases characterized by predominant weakness in the feet and/or hands. More than 20 distinct subtypes have been identified, with both autosomal dominant and recessive inheritance and many of them yet lack genetic characterization (110). On muscle pathology, several forms of distal myopathies show the presence of vacuoles that are lined (rimmed) by red granules on modified Gomori trichrome staining called "rimmed vacuoles." They represent accumulation of autophagic vacuoles, due to lysosomal dysfunction or to proteins accumulation (66).

At least three ALS-related genes, including *VCP*, *MATR3* and *SQSTM1*, have been also associated with distal myopathies. These genetic data, along with the observation of combined ALS/distal myopathy phenotypes in some individuals and the evidence of rimmed vacuoles with TDP-43 inclusion on muscle biopsy, support the notion that ALS/distal myopathy with rimmed vacuoles may represent a subgroup of the ALS/myopathy spectrum.

Distal myopathy and *VCP*

As previously mentioned, almost 90% of reported patients with *VCP* mutations show proximal limb-girdle myopathy. A new phenotype has been described in a Finnish family harboring the P137L

mutation, where nine affected members had an autosomal dominant distal myopathy with onset after age 35 (77). Three patients developed overt FTD several years after the onset of muscle weakness, but none of them had PDB. Muscle biopsy showed rimmed vacuoles with TDP-43 and p62 positive inclusions. The same mutation has been detected in patients in whom weakness in hands or feet was the presenting symptom with later progression to proximal muscles (102).

Distal myopathy and *MATR3*

A mutation in *MATR3* (S85C) was first identified as the cause of an autosomal dominant, distal, asymmetrical myopathy with vocal cord paralysis and pharyngeal weakness in a large North American multigenerational family (34) and in an unrelated Bulgarian family (96). Muscle biopsy disclosed a chronic noninflammatory myopathy with rimmed vacuoles, but features suggesting denervation were also detected, including presence of atrophic fibers. EMG examination detected myopathic changes in some members and neurogenic alteration in other relatives.

As mentioned before, in 2014 mutations in *MATR3* were identified in ALS patients as well. The F115C variant in *MATR3* was detected in a large ALS pedigree of European ancestry (48) and WES on additional 108 fALS cases revealed a T622A (exon11) mutation in one patient, corresponding to a cumulative frequency of *MATR3* mutations in 2.75% of fALS. In addition, custom resequencing of genes linked to neurodegeneration in 96 British ALS cases identified a P154S missense variant in *MATR3* in an individual diagnosed with sporadic disease (48). In the light of these findings, the American family with distal myopathy associated with S85C *MATR3* mutation (34, 96) was re-evaluated, leading to a reclassification of these cases as a form of slowly progressive ALS (48). The presence of the "split-hand" sign, commonly observed in ALS patients (32) and of brisk reflexes in most cases was considered sufficient to diagnosis.

However, two recently published papers confirmed that the S85C *MATR3* mutation is associated with a distal myopathy. In 16 German patients from 6 families, harboring the S85C *MATR3* mutation, muscle biopsy showed only myopathic changes, including variation of fiber size, internal nuclei, minor fatty replacement of muscle fibers, rimmed vacuoles or end stage myopathic changes (eg, major fatty replacement of muscle tissue) (72). In an American family with the S85C *MATR3* mutation no changes compatible with a neurogenic process were observed on both muscle pathology and electromyography (78). Muscle biopsy showed numerous rimmed-vacuolated fibers with no neurogenic changes. Authors raised the possibility of minor upper motor neuron signs in four patients. Conversely, in a recently described Asian family with S85C *MATR3* mutation a mixed myogenic and neurogenic pattern was detected on electrodiagnostic and muscle biopsy studies (122). Based on these results, there is evidence that the S85C *MATR3* mutation is associated with both a pure distal myopathy phenotype and ALS/distal myopathy phenotype, in which combined myopathic and neurogenic features are present at clinical, EMG and muscle biopsy examination.

Mutations in *MATR3* appear to be very rare in ALS, as only a few additional patients have been reported so far. One mutation (A72T) was found in one sporadic case from a cohort of 207 Taiwanese ALS patients, including 30 fALS and 177 sALS (65). The patient had a classic phenotype with bulbar onset and disease duration of 11 years. Exons 2, 11 and 12 of the *MATR3* gene were studied in 200 Italian ALS patients, showing a R147W variant in one sporadic case with a slowly progressive disease with predominant lower motor neurons involvement (76). Three further variations have been identified in 3 sALS cases, 1 missense (V394M) and 2 spicing (c.48 + 1G>T, c.−339 + 2T>A), studying a total of 83 fALS and 164 SALS of French-Canadian and European origins (64).

Importantly, *MATR3* encodes an RNA- and DNA-binding protein that interacts with TDP-43 (48). TDP-43 and other autophagic markers (p62 and SMI-31) were components of the rimmed-vacuolar pathology in S85C *MATR3* myopathy (122).

These results indicate that the *spectrum* of diseases associated with *MATR3* mutations includes ALS, distal myopathy with rimmed vacuoles, combined ALS/distal myopathy and possibly FTD.

Distal myopathy and *SQSTM1*

SQSTM1 mutations were already known to be associated with PDB (45, 61), accounting for 25%–50% of familial and 5%–10% of sporadic PDB patients (85). In the last few years *SQSTM1* mutations have been found in patients with sALS and fALS (33), ALS/FTD, FTD/PD and pure FTD (9, 43, 54, 58, 62, 90, 97, 108, 112, 115). The latest discovery that *SQSTM1* mutations can be associated with a distal myopathy with rimmed vacuoles indicates that *SQSTM1* mutations contribute to the aetiology of ALS/IBMPFD *spectrum* (10). Interestingly, the variant associated to distal myopathy is a splice donor mutation (c.1165 + 1 G>A), resulting in the expression of two different isoforms of SQSTM1. This variant was found in one family and in one sporadic patient, all carriers having late-onset distal lower extremity weakness with rimmed vacuoles with TDP-43 and SQSTM1 inclusions on muscle biopsy (10).

ALS/MITOCHONDRIAL DISEASE *SPECTRUM*?

There are several works in literature supporting the role of mitochondrial dysfunction in the pathogenesis of ALS (for comprehensive reviews, see (6, 21, 22, 104)). The evidence of swollen mitochondria in the early stages of disease in transgenic mice model carrying *SOD1* mutations, indicates that mutant SOD1 toxicity may be mediated by damage to mitochondria in motor neurons (6, 25, 123).

However, pure motor neuron impairment is not included among clinical manifestations of Mitochondrial Diseases, although rare cases with motor neuron pathology have been described in patients with mutations in genes encoding for mitochondrial proteins (19, 27, 42, 87).

Mitochondrial diseases are phenotypically and genetically heterogeneous disorders caused by mutations in either the mitochondrial DNA (mtDNA) or the nuclear DNA (nDNA). nDNA encodes most of the respiratory-chain proteins, which are needed for the proper assembly and function of respiratory chain complexes, for mtDNA maintenance and translation, phospholipid composition of the inner mitochondrial membrane or mitochondrial dynamics. Mutations in these factors affect mtDNA directly, either quantitatively (mtDNA-depletion syndromes) or qualitatively (multiple deletion syndromes) (27, 28).

The recent identification of *CHCHD10* mutations in a subset of ALS patients opened a new window on the relationship between ALS and Mitochondrial diseases (4). As previously mentioned, *CHCHD10* encodes a coiled-coil helix coiled-coil helix protein, located in the intermembrane space of mitochondria, whose function is unknown. Patients in the pedigree reported by Bannwarth *et al* had reliable clinical, morphological and biochemical evidence of mitochondrial disease.

Clinically, affected individuals showed variable combinations of proximal myopathy, palpebral ptosis, ataxia, dementia and sensorineural deafness, all representing canonical signs of Mitochondrial Diseases. Muscle pathology showed typical mitochondrial alterations, consisting of cytochrome c oxidase (COX)-negative fibres, ultrastructural mitochondrial abnormalities, impaired respiratory chain activity, and multiple deletions consistent with altered mtDNA maintenance.

The discovery of *CHCHD10* mutations in a proportion of familial and sporadic cases of ALS/FTD-ALS reinforces the hypothesis of a mitochondrial dysfunction in ALS and suggests the possibility of an ALS/mitochondrial diseases spectrum.

However, clinical signs reminiscent of Mitochondrial Diseases were never reported in all *CHCHD10* mutated ALS cases reported so far. Furthermore, only few cases with *CHCHD10* mutations underwent muscle pathology investigation. Severe histochemical COX deficiency with no evidence of mtDNA deletion or depletion was detected on muscle biopsy in a classic ALS patient harboring the P80L *CHCHD10* mutation (23, 87). The same authors detected COX deficiency in additional 6 patients from a group of 50 sALS in which muscle biopsy was performed. Besides *CHCHD10* mutation, genetic analysis showed *SOD1* mutation in one patient and *TARDBP* mutation in another case (23). The association of *CHCHD10* with mitochondrial disease was reinforced by the detection of a double missense mutation in *CHCHD10* in cis (R15S and G58R) in a large Puerto Rican kindred with an autosomal dominant mitochondrial myopathy without sign of motor neuron involvement. Affected members had severe exercise intolerance, progressive proximal weakness and lactic academia presenting in the first decade of life (3). Muscle histochemistry showed ragged-red fibers and electron microscopy showed globular mitochondrial inclusions (41).

Of note, *CHCHD10* is involved in another group of motor neuron diseases. The missense G66V mutation was reported in 55 patients from 17 unrelated Finnish families with late onset spinal motor neuronopathy (LOSMoN/SMAJ) (81). On muscle pathology, chronic myopathic changes were found in SMAJ, with no mitochondrial abnormalities (49). Conversely, no mutations were detected in a cohort of 72 French Spinal Muscular Atrophy patients (73). Furthermore, the same G66V variant was observed to cause variable phenotypes within a Finnish family, ranging from axonal Charcot Marie Tooth neuropathy to spinal muscular atrophy with clinical signs leading to an initial diagnosis of ALS (79).

ALS/OTHER NEUROLOGICAL DISORDERS

Extrapyramidal, cerebellar, sensory or autonomic systems dysfunction may be sometimes identified in ALS patients [for review see (103)]. Though these findings confirm the view that ALS may be part of a multisystem neurological disease, definite clinical, genetic and pathological data linking ALS with these conditions are still to be addressed.

CONCLUSIONS

ALS is a heterogeneous disorder. Genetic and pathological studies in familial forms of the disease are revealing that ALS is mechanistically linked with an increasing number of disorders. The *spectrum* paradigm represents a conceptual shift of our thinking about ALS, indicating that ALS should be considered as one of the different manifestations associated with defects in several pleiotropic genes.

The relevance of these considerations for the most common sporadic ALS remains to be elucidated. However, it should be considered that many clinical and genetic observations support the view that also fALS and sALS are linked over a spectrum. Family histories indicate that heritability of ALS varies over a continuum ranging from fALS with Mendelian segregation to sALS. In fact, different categories of fALS have been delineated, including definite, probable A, probable B and possible fALS (11–13, 20). Genetic information strongly supports this view as the frequency of mutations in currently known ALS genes decreases over a continuum from 60%–80% in definite fALS to 11%–28% in sALS (Figure 2) (13, 20). In this spectrum the same genetic variants may contribute to Mendelian forms and to apparently sporadic forms.

The complexity of ALS is increasing but also our understanding of ALS pathogenesis is rapidly expanding. Genetic research is providing important insights into the cellular pathways and mechanisms underlying motor neuron degeneration. The identification of pathways involved in the disease and pathophysiological mechanisms will open new therapeutic perspectives for this spectrum of currently incurable disorders.

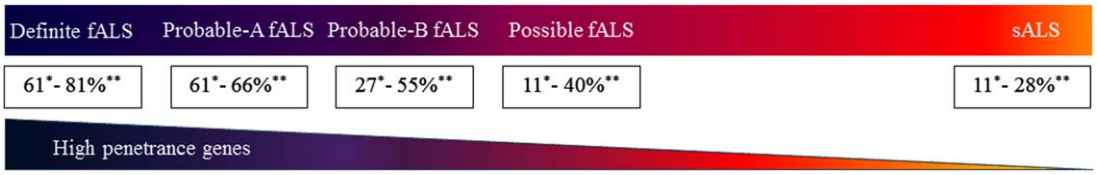

Figure 2. The sporadic/familial *spectrum*. Definite fALS: patients with at least two first- or second-degree relatives with ALS; Probable A fALS: patients with one affected direct-line first-degree-relative (father/mother–child); Probable B fALS, patients with one affected sibling or second-degree relative; Possible fALS: ALS patient with a distant relative with ALS (more distant than first- or second-degree) or sporadic ALS with a relative affected by one of the other disorders of ALS *spectra*; sALS: sporadic ALS. Frequencies of mutation in major ALS genes in each category are described (*See Ref. 11; **See Ref. 20).

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