Review

Clinical and genetic heterogeneity of amyotrophic lateral sclerosis


Although clinical picture of amyotrophic lateral sclerosis (ALS) is a stereotypical one, resulting from combination of signs secondary to dysfunction of both upper motor neuron (UMN) and lower motor neuron (LMN), clinical heterogeneity is a consistent feature of the disease. Age of onset, relative mix of UMN and LMN signs, duration of the disease and association with other conditions are major factors contributing to variable clinical phenotypes. Genetically, familial forms of ALS are associated with a large number of pleiotropic genes whose mutations impair different biochemical pathways, resulting in overlapping clinical and pathological phenotypes. Over the last few years contribution of large- and low-effect genes to sporadic ALS is increasingly recognized.

Conflict of interest

The authors report no conflicts of interest.

Amyotrophic lateral sclerosis (ALS) is characterized by relentless degeneration of upper motor neuron (UMN) and lower motor neuron (LMN) leading to progressive muscular paralysis. The disease occurs as sporadic amyotrophic lateral sclerosis (SALS) in the majority of cases, while nearly 5% of patients have a positive familial amyotrophic lateral sclerosis (FALS) (1). The question of whether ALS is a single disease with variable phenotypic expression or different diseases with heterogeneous causes has represented a matter of lively debate in the literature over the last few years (2). The discovery of mutations in SOD1 as causative of FALS (3), in 1993, started the molecular genetics era of ALS research and over the last 20 years an increasing number of causative genes have been identified, revealing a high degree of genetic heterogeneity.

Clinical heterogeneity

Clinical picture of ALS is a stereotypical one, resulting from combination of signs secondary to dysfunction of both UMN and LMN (4). However, clinical heterogeneity is a recognized feature of the disease due to the following factors.

Age of onset

Mean age of disease onset is about 65 years in population-based studies (5). ALS affects people of all ages but the age-adjusted incidence rate varies greatly in different age groups. Incidence is very low in the first four decades (1.5/100,000/year), increases abruptly around age 40, reaching its peak between ages 60 and 79 (10–15/100,000/year), and decreases thereafter (5).

The presence of a peak in the age-specific incidence curve suggests that the disease results from a time-dependent exposure to some genetic or environmental risk factors. According to this hypothesis, early onset of the disease might reflect a major exposure to one or more of these risk factors (6). Interestingly, patients with young-onset ALS with onset in the third to fourth decade disclose peculiar clinical features, including predominant UMN signs, male prevalence, less common bulbar onset and more prolonged survival (6).

Juvenile ALS

In rare patients, labeled as juvenile amyotrophic lateral sclerosis (JALS), onset occurs in the first two decades. JALS is an heterogeneous condition distinct from...
clinical ALS, as it usually has a relatively benign course and is familial in the great majority of cases (7, 8). However, an aggressive course may be observed in sporadic JALS and in some of these cases pathological examination showed peculiar cytoplasmic basophilic inclusions in motor neurons (9, 10).

Combination of UMN and LMN signs

The variable mix of UMN and LMN signs represents a major contributor to phenotypical heterogeneity of ALS (2, 4). Clinical manifestations of ALS exist on a continuum, ranging from apparently pure LMN dysfunction to severe pyramidal impairment with minor LMN signs. Classic ALS (Charcot type) is the most frequent form, accounting for about 70–90% of cases (Fig. 1). It is characterized by predominant LMN signs combined with slight to moderate pyramidal signs. Included in this category are also patients with preserved reflexes in atrophic limbs, or central conduction time prolongation at motor evoked potentials. In a minority of ALS patients clinical manifestations are dominated by pyramidal signs, consisting mainly of severe spino-bulbar spasticity, associated with slight LMN signs. This category, termed upper motor neuron-dominant (UMN-D) ALS, shows significant differences in age of onset, sex ratio, pattern of spreading and prognosis with respect to classic ALS, suggesting different disease mechanisms (11). Patients with pure LMN signs without any accompanying clinical or electrophysiological UMN signs are labeled as progressive muscular atrophy (PMA). However, it should be considered that pyramidal signs can be masked by LMN dysfunction at both clinical and electrophysiological level. This observation may explain the demonstration of UMN pathology in half of PMA patients at autopsy (12). The hypothesis that PMA and ALS are distinct entities is unlikely, as they show large clinical and genetic overlapping (13, 14).

About 2–5% of patients with motor neuron disease shows an exclusive involvement of UMN with predominant spino-bulbar spasticity, and this condition has been labeled as primary lateral sclerosis (PLS) (15).

Site of onset

A consistent feature of ALS is that it starts as a focal process involving variable regions in the neuraxis and then spreads through all the motor system (17). On the basis of the clinical pattern in the initial phase of illness, different variants have been described, including bulbar, spinal and pseudoneuritic forms, flail arm syndrome, and Mill’s hemiparetic type (2, 4). Body region onset may prove to be an important tool for assigning nosology. Consistently, the flail arm form is characterized by symmetric, predominantly proximal, wasting and weakness of both arms with relative sparing of lower limbs. This ALS form is prevalent in males, starts after the age of 40 and shows a slightly better clinical course with respect to classic ALS (18). Patients with bulbar onset ALS usually present with dysarthria and dysphagia, limbs symptoms can develop simultaneously with bulbar symptoms or can occur within 1–2 years. Bulbar onset ALS is more frequent in females and has a worse prognosis with respect to the spinal onset form. The term progressive bulbar palsy was historically used to designate this subset of ALS, but evidence that it represents a distinct entity is uncertain.

Survival

Median survival of ALS is approximately 3 years after the onset, but duration of the disease varies widely in individual patients, ranging from few months to over 10 years (6, 19). There is a general consensus in considering older age and bulbar onset as major negative prognostic factors (19). The UMN-D phenotype appears to be a strong independent predictor of long survival (6, 11). Disease duration depends on the timing of involvement of respiratory muscles. Of note, both temporal and spatial pattern of spreading of the disease process in different body regions are highly variable among patients. Respiratory weakness may represent the onset symptom in 5% of patients or can take place later in the course of the disease, as in flail arm ALS and in UMND forms (2, 11, 18).

Association with other conditions

ALS has been generally considered a paradigm of pure motor neuron disorder. Nevertheless, it is currently recognized that pathologic changes are not limited to motor systems. Patients with ALS may exhibit cognitive abnormalities ranging from impaired frontal executive dysfunction to overt fronto-temporal dementia (FTD) (20). A recent population-based study showed that
comorbid dementia occurs in approximately 14% of patients with a new diagnosis of ALS (21). Cognitive impairment, predominantly in the form of executive dysfunction, occurs in more than 40% of ALS patients who have no evidence of dementia. Further support for the concept that FTD and ALS are closely related conditions is the recognition of families with pure FTD, pure ALS and ALS/FTD. Of note, both ALS and FTD show neuronal inclusions positive for the transactive response DNA-binding protein 43 TDP-43 (22) and share many gene defects, including C9ORF72, TARDBP, FUS (fused-in-sarcoma) and VCP mutations.

Association of ALS with Parkinson disease (PD) has been reported as well (23, 24). Supporting the link between ALS and PD is the identification of mutations in TARDBP (25), ATXN2 (26, 27), C9ORF72 (28, 29) and ANG (30) in both conditions.

Genetics of ALS

The Juvenile form of ALS is familial in the great majority of cases, with both autosomal-recessive and autosomal-dominant pattern of inheritance. Mutations in several genes, including ALSIN, SETX, Spatacsin and SIGMAR1 have been shown as the underlying cause of the disease (31).

Regarding the classic adult-onset form, most cases occur as SALS, and only a small proportion of patients have a positive FALS, varying from 1% to 11.6% in large reported series. A recent meta-analysis of data showed that the rate of FALS among prospective population-based registries was 5.1% (1). ALS is considered to be familial if one or more relatives are reported as affected by the same disease, but the number of affected relatives and degree of relationship vary greatly among kindreds. Importantly, in 50–75% of FALS only two affected relatives are reported in families (32, 33). In these instances the pattern of inheritance is unclear and there is an uncertainty as to whether these patients should be classified as FALS (34). In fact, the possibility that a second person among relatives is affected by chance may not be excluded. Criteria were recently proposed for classifying FALS into possible, probable and definite groups (35). Genetic component due to the currently known ALS genes has been shown to vary greatly among these three categories of FALS (33).

Genetics of FALS

To date, a large number of genes causing or predisposing to ALS have been identified (http://alsod.iop.kcl.ac.uk/). Actually, only a limited number of genes, including C9ORF72 (36, 37), SOD1 (3), TARDBP (38, 39) and FUS (40, 41), are responsible for a considerable proportion of FALS cases. A constellation of other genes, each causing very few FALS cases, are increasingly recognized. This latter group includes VAPB, FIG 4, CHMP2B, OPTN, DAO, VCP, UBQLN2 and SQSTM1. Mutations in PFN1, the most recently discovered gene, are very rare (42). About 50–60% of FALS cases have mutations in these genes. (43–45).

C9ORF72

A large hexanucleotide (GGGGCC) repeat expansion in the first intron of C9ORF72 located on chromosome 9p21 is the most common mutation detected in patients with familial ALS (36, 37, 43–49). Mutation frequency varies between different populations, countries and regions, ranging from 0% to 18% in Asian countries to 46% in Finland and France. The percentage of mutated cases raises to 50–72% in families with ALS/FTD phenotype (43, 46). Notably, mutations in C9ORF72 are the most common cause of familial FTD as well, accounting for 11.7–17.8 of cases (36, 47, 48). The associated risk haplotype is shared by most ALS families of European ancestry, suggesting a common founder (37, 46).

The protein encoded by C9ORF72 is unknown. Using fluorescence in situ hybridization technique with a probe targeting the GGGGCC repeat, multiple nuclear RNA foci of an abnormal mRNA have been detected in brain tissues from patients carrying the expanded repeat, suggesting a toxic gain-of-function mechanism. However, reduced expression of one of the transcripts of C9ORF72 has been observed as well, consistent with a loss-of-function mechanism (36, 37). At a neuropathological level, patients with mutations in C9ORF72 show TDP-43-immunoreactive protein aggregates, and presence of ubiquitin-positive, TDP-43-negative inclusions in a variety of neuroanatomical regions (49).

Comparing the phenotype with that of patients carrying mutations in other ALS-related genes and of patients with unidentified genetic defects, those with C9ORF72 expansion show some consistent differences (28, 43–45, 49). The most notable one is a significantly higher frequency of cognitive impairment which affects 40–50% of cases compared with 8–9% of non-C9ORF72 expansion cases. In patients carrying C9ORF72 mutations bulbar onset seems more frequent and median survival consistently lower than in patients carrying TARDBP, SOD1 or unknown mutations. C9ORF72 expansion is more frequent among patients with onset >61 years (45).

SOD1

Mutations in SOD1, the superoxide dismutase 1 encoding gene, have been found in 12–23% of FALS (http://alsod.iop.kcl.ac.uk/). SOD1 is a 153 amino acids protein which is expressed in all cells. Mechanisms by which mutated SOD1 cause the disease remain enigmatic. It is currently considered that mutant SOD1 causes neurodegeneration by an acquired novel cytotoxic activity which is multifactorial, affecting DNA/RNA metabolism, mitochondria, neurofilaments and axonal transport, function of the endoplasmic reticulum, the Golgi apparatus and the proteasome (50).
One hundred sixty-six mutations have been identified so far throughout all five exons, consisting mostly of missense mutations, while non-sense mutations or gene deletions are uncommon. Most SOD1 mutations are autosomal dominant, but the D90A variant can be transmitted in either a dominant or a recessive manner (51, 52). In patients with SOD1 mutations cognitive impairment is very rare (0–2.6%) and bulbar onset is observed less frequently (7–12%) than in non-SOD1 patients (44, 45, 53). Mean age of onset is greater than in FUS patients but it is lower than in other non-SOD1 patients. Specific gene variants, including L106V, G37R and L38V, are associated with young onset (53).

Duration of the disease is quite heterogeneous, varying from few months to decades (44, 45, 53). Some mutations, including D90A, G37R, G41D, G93C and D11Y, have been consistently associated with prolonged survival while others, such as A4V and G85S, with aggressive course. Frequency of individual mutations varies in different countries. The A4V variant is the most frequent mutation in North America, occurring in about 50% of SOD1 FALS cases, while in Europe it is relatively rare.

TARDBP and FUS

The identification of mutations in these two genes in cases of both familial and sporadic ALS was a major breakthrough in the history of ALS research as it represented the convergence of genetic research into earlier neuropathologic studies. In 1988, several studies had shown that motor neurons and glial cells from ALS patients contained abnormal proteinaceous accumulations labeled by antibubiquitin antibodies (54, 55). Content of these inclusions was initially unknown. After the discovery of mutations in SOD1 associated to FALS, it was showed that inclusions were stained with antibodies to SOD1 in patients with mutated SOD1 (56). However, SOD1 was not a component of the inclusions in sporadic ALS and in non-SOD1 familial forms. A landmark was the discovery of TDP-43 as major component of the protein aggregates (22). Notably, all cases of ALS, including sporadic ALS, ALS with dementia and SOD1-negative FALS had neuronal and glial inclusions that were immunoreactive for both ubiquitin and TDP-43, indicating a central role of TDP-43 in disease pathogenesis (57). The absence of TDP-43 pathology in cases with SOD1 mutations suggests that different mechanisms underlie motor neuron degeneration (58). TDP-43 discovery was followed by the identification of mutations in TARDBP, the TDP-43 encoding gene, in a subset of ALS patients (38, 39). Soon after, mutations in FUS, encoding a protein with several structural and functional similarities with TDP-43, were discovered in FALS and SALS patients (40, 41). Also patients with mutated FUS showed cytoplasmic mislocalization of the FUS protein. Whether FUS is involved in the pathogenesis of SALS and other forms of FALS without FUS mutations is currently controversial.

It was recently reported that FUS-immunoreactive inclusions are a common finding in sporadic and in non-SOD1 familial ALS without FUS mutations (59).

TDP43 and FUS are DNA/RNA-binding proteins which are able to shuttle between nucleus and cytoplasm due to their content of a nuclear localization signal and a nuclear export signal (60). They play several functions related to RNA metabolism in both compartments, including transcription, splicing, transport, translation, degradation and microRNA processing. Both proteins are present predominantly in cell nuclei in physiological conditions. In ALS, mislocalization of FUS and TDP-43 in the cytoplasm with inclusion formation has been proposed to cause either a loss of the normal protein function in the nucleus, a gain of toxic function in the cytosol, or both (60).

TDP-43 is a 414 amino acid protein encoded by six exons and containing two RNA recognition motifs and a C-terminal glycine-rich region. Most of the identified mutations are localized in the glycine-rich region encoded by exon 6. Mutations in TARDBP have been identified in 2–5% of FALS (44, 45). Of note, a unique TARDBP missense mutation (p.A382T) has been found in approximately one third of all ALS cases in the island of Sardinia, a genetic isolate phylogenically distinct from other European populations (61). Regarding the clinical phenotype, cognitive impairment has been observed in 31% of patients with mutations in TARDBP (44). The rare occurrence of TARDBP mutations in patients with age of onset >61 years (45) has not been confirmed in other studies (62). Survival is longer than in FUS and C9ORF72 mutated patients.

FUS is a 526 amino acid protein encoded by 15 exons. FUS mutations are found in 1–5% of FALS (40, 41, 44, 45). Most FUS mutations cluster in the C-terminus of the protein, encoded by exon 15, containing a non-classical nuclear localization sequence (40, 41). Impaired transportin-mediated nuclear import of FUS, resulting from disruption of this motif, has been shown as a mechanism for cytoplasmic mislocalization of FUS (63). Clinical phenotype of FUS mutated patients discloses some distinctive characteristics (44, 45, 64–67). Age of onset is consistently lower than in other FALS; FUS mutations are the most common genetic defect in FALS patients with the young onset form, affecting 35% of patients aged <40 years (45). Of importance, specific FUS mutations, including the missense variant P525L, frameshift mutations and gene deletions, have been identified in patients with juvenile onset (<25 years) and very aggressive course and in cases with basophilic inclusions (68–71). Predominant LMN signs, weakness of neck extensor muscles with proximal, nearly symmetric impairment of upper limb muscles are seen at onset in patients with the common R521C variant of FUS (64, 65). Cognitive impairment has been reported in 0–5% of FALS patients with FUS mutations. Survival is significantly shorter than in other FALS, with exceptional patients living more than 4 years.
There is increasing evidence that the dichotomy between familial and apparently sporadic ALS is artificial as genetic factors may play a relevant role also in the pathogenesis of SALS. From a clinical point of view patients with SALS are indistinguishable from those with FALS. Both conditions show similar pathological patterns, namely the presence of ubiquitinated TDP-43 positive inclusions. Heritability of apparently sporadic ALS has been addressed by examining the concordance among ALS patients and their relatives. On the basis of twin data studies, heritability of sporadic ALS was estimated to be 0.61 (0.38–0.78) (72). Other studies have shown a small but definite increased risk to first-degree relatives of patients with sporadic ALS (73).

**High-penetration susceptibility model**

The observation that mutations in large-effect genes associated with FALS may be detected in cases with SALS is a major clue in favor of the genetic basis of SALS. In fact, discoveries of new genes involved in FALS have been invariably followed by the identification of mutations in the same genes in patients with apparently sporadic disease (Table 1). In a clinic based population about 11% of apparently sporadic ALS harbored mutation in major FALS genes (74). Considering that only 50–60% of patients with FALS have mutations in currently known genes, the discovery of new genes in the future is expected to increase the proportion of SALS with proven genetic etiology.

The detection of mutations in large-effect genes in ALS patients with no apparent family history may have several explanations. The first one is inaccuracy in the definition of FALS due to ascertainment bias. Lack of knowledge of family history, misdiagnosis of ALS in family members, early death due to other causes of family members prior to developing motor neuron degeneration are common sources of mistake. Furthermore, a large-effect gene mutation may not appear familial if the family size is small or if a single ALS gene predisposes to multiple neurodegenerative disorders. Indeed, pleiotropy seems to be a consistent feature of several genes, such as C9ORF72, FUS, TARDBP, OPTN, FIG 4 and ANG (75). A second explanation for the occurrence of mutations in FALS genes in sporadic cases is that mutations may be *de novo* or low penetrant. *De novo* FUS mutations have been described in several cases of SALS disclosing early onset and aggressive course (10, 68, 70, 71). It is worth noting that *de novo* mutations are not reported in other genes with the exception of a single case of SOD1 mutation (76). Supporting the hypothesis of incomplete penetrance is the frequent detection of the same mutations found in SALS patients also in unaffected relatives, including very old individuals (74).
Oligogenic model

Patients harboring double mutations in ALS-associated genes are increasingly recognized (74, 77, 78). Evidence for a digenic inheritance in ALS is supported by two studies showing that the frequency of double mutations in both FALS and SALS is higher than that expected on the basis of chance (74, 78).

Low-penetrance susceptibility model

A different model might explain the genetic contribution to SALS. In a polygenic threshold model, SALS might result from summatory effects of a series of low frequency, weakly deleterious mutations in a variety of genes, each conferring a moderate increase in relative risk (79). According to this model, disease develops only once a critical threshold of liability is crossed, due to the cumulative contribution of multiple genetic and environmental factors. Several genes have been proposed but for most of them the role in the etiopathogenesis of SALS remains unclear (reviewed in Ref. (75, 80).

The predisposing role to SALS of CAG expansion in ATXN2 has been consistently suggested by several association studies carried out on large series of patients and controls (26, 81, 82). The normal-size range of the ATXN2 polyglutamine tract extends between 14 and 31 repeats, being 22 or 23 the most frequent ones. Expansions of more than 34 repeats are known to cause spinocerebellar ataxia type 2. Intermediate length of a 27–33 CAG repeat in ATXN2 confers an increased risk for developing ALS. The association is mainly driven by the longer (31–33) polyQ repeats, which have been found in 1.8–3.7% of different series of patients, compared with 0–0.2% of control individuals. Supporting the pathogenic role of ATXN2 is the observation that it is a modifier of TDP-43 and FUS toxicity (81, 83).

ANG was initially considered a Mendelian gene implicated in both FALS and SALS (84). In a recent analysis mutations in ANG were found in 0.46% of 6471 ALS patients, compared with 0.04% of 7668 control individuals (30). Although the frequency of ANG variants is very low, robust data indicate that ANG should be regarded as low-penetrance susceptibility gene for ALS.

Conclusions

There is increasing evidence that the level of clinical and etiological heterogeneity of ALS is far greater than previously assumed. Several clinical phenotypes of ALS may be identified, but no clear boundaries are observed among them, as they represent points on a spectrum. FALS is caused by several pleiotropic genes whose mutations impair different biochemical pathways, resulting in overlapping clinical and pathological phenotypes. A proven genetic etiology affects a considerable proportion of apparently SALS patients, with relevant implications in clinical practice and in a research setting. Future studies will elucidate genetic component of SALS, including factors influencing penetrance.

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References

Sabatelli et al.


Clinical and genetic heterogeneity of ALS


