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Familial amyotrophic lateral sclerosis with a mutation in exon 4 of the Cu/Zn superoxide dismutase gene: pathological and immunocytochemical changes

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Abstract Detailed molecular pathology studies and clinicopathological phenotyping of familial amyotrophic lateral sclerosis (FALS) with characterised mutations in the gene encoding Cu/Zn superoxide dismutase (SOD1) will yield important insights into the pathogenesis of motor neuron degeneration. An autopsy case is described with the mutation E100G (exon 4) of the SOD1 gene in which full neuropathological examinaton including immunocytochemistry of ubiquitin and neurofilament epitopes was performed. The case falls into the category of "amyotrophic lateral sclerosis (ALS) with posterior column involvement." Critical analysis of the findings indicates a truly multisystem disorder in which ascending sensory pathways and components of the efferent cerebellar pathways are at least as severely affected as the motor system. Abnormal neurofilament phosphorylation was not a prominent feature. Ubiquitinated neuronal inclusions were infrequent except in the hippocampal denate granule cells where they were indistinguishable from sporadic cases of ALS-dementia. The motor cortex was preserved despite severe distal axonal loss in the corticospinal tract. These findings suggest a primary failure of axonal maintainance affecting several neuronal groups with long projecting axons. The differences and similarities compared to previously reported case with I113T (exon 4) and A4T (exon 1) mutations are discussed. Findings related to inflammatory cell infiltration, ubiquitination and neurofilament phosphorylation are discussed with reference to the pathogenesis of sporadic ALS.

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Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease in which there is selective injury and cell death of motor neurons in the brain and spinal cord. The familial form of the disease accounts for approximately 10% of cases and is transmitted in an autosomal dominant manner [12]. In 15–20% of familial ALS pedigrees there is a missense mutation within the gene on chromosome 21 encoding Cu/Zn superoxide dismutase (SOD1) [4, 20]. Mutations within exons 1, 2, 4 and 5 of the SOD1 gene have been described [1, 4, 5, 8, 12, 13, 17, 18, 20, 24]. Most of the mutations are localised in sites believed to be relevant either for correct folding of the SOD1 protein or for dimer contact, the exception being the H46R mutation which involves one of the residues binding catalytic copper at the active site [13].

The clinical features of patients with familial ALS appear to be similar, if not identical, to those present in patients with the sporadic form of the disease [11]. Pathological studies in familial and sporadic ALS have also shown similar findings. In addition, familial ALS (FALS) cases are frequently described with degeneration of the spinal dorsal columns [28]. However, there have been few detailed neuropathological studies of patients with defined SOD1 mutations [16, 21, 25]. Such studies are needed to establish the correlation between the molecular defects and clinical and pathological features in this important subgroup of ALS cases. Molecular/pathological correlations in familial ALS may provide important insights into the degenerative process in the commoner sporadic form of the disease.

The aim of this study is to describe the neuropathological features in case of FALS with a Glu100Gly (E100G) mutation in exon 4 of the SOD1 gene. Several unusual histopathological features have been identified.

Case report

The patient was a Caucasian female who presented in 1988 at the age of 36 years with a 3-month history of progressive weakness and wasting of the left leg, and left-sided foot drop. She had noticed slight wasting of the left hand and forearm with weakness of grip strength, and twitching of the left quadriceps and the intrinsic muscles of the left hand. A pregnancy in 1973 had resulted in a stillbirth, but her previous history was otherwise unremarkable. She had five living children who were fit and well. There were four relatives known to have suffered and died from a progressive motor disorder with symptoms similar to those of the patient. A maternal aunt had died at the age of 46 years, the mother at the age of 42, the maternal grandmother at the age of 52 and the maternal great-grandfather at an uncertain age. The pattern of disease indicated autosomal dominant inheritance with similar phenotype in affected individuals. The patient had three other siblings, including a twin brother, who are presently well.

On examination at the time of presentation there were no significant abnormalities on general medical examination. On neurological examination there was mild weakness of neck flexion but no other abnormalities in the cranial nerve territory. There was significant muscle wasting of the forearm, hand, quadriceps and calf muscles on the left side and fasciculations were evident in the left quadriceps. There was weakness in a pyramidal distribution in the left upper and lower limbs as well as the intrinsic muscles of both hands. The tendon reflexes were all pathologically brisk and both plantar responses were extensor.

Routine screening investigations including full blood count, erythrocyte sedimentation rate, urea and electrolytes, bone chemistry, liver function tests, blood glucose, and thyroid function tests were normal. Auto-antibody screen, VDRL, borrelia and HIV antibody titres were negative, and serum protein electrophoresis showed no evidence of a paraprotein band. The cerebrospinal fluid was normal with less than 1 white blood cell/μl and a protein level of 0.22 g/l. Radiographs of the cervical and lumbosacral spine were normal. A neurophysiological assessment showed normal motor and sensory conduction velocities, and widespread chronic active neurogenic changes in the muscles of all four limbs.

DNA from the patient was subsequently screened for the presence of mutations in the SOD1 gene. Polymerase chain reaction single-strand conformation polymorphisms (PCR-SSCP) was employed for screening all five exons of the SOD1 gene for mutations [15]. Single-stranded, non-radioactive PCR products were visualised on non-denaturing polyacrylamide gels by a sensitive silver-staining technique. A mobility shift was detected in the exon 4 PCR product and direct PCR sequencing revealed a Glu→Gly nucleotide transition at codon 100. This exon 4 mutation has been previously described in familial ALS cases [8, 20].

The subsequent clinical course was one of progressive deterioration. By early 1992 she was severely tetraparetic but had only mild bulbar dysfunction. She died in 1992, 39 months after presentation, from respiratory failure.

Autopsy findings

The body was poorly nourished, with generalised limb muscle wasting, and weighed 42 kg. The lungs showed acute congestion without evidence of bronchopneumonic consolidation. There was a mild degree of hepatic congestion and minimal atheroma in the distal aorta and coronary arteries, but otherwise there were no abnormalities outside the nervous system. The general findings were consistent with the cause of death being respiratory failure. External examination of the brain and spinal cord showed only the presence of anterior spinal nerve root atrophy affecting both the cervical and lumbo-sacral limb enlargements.

Materials and methods

At autopsy the brain, spinal cord, sympathetic and dorsal root ganglia and multiple skeletal muscle samples were retained. The brain and spinal cord were subdissected while fresh to provide tissue for fixation and snap freezing. The spinal cord was exposed on its ventral surface by incision of the dura. The ventral roots were carefully identified with reference to the T1 root identified previously at removal. The following segments were retained for processing to paraffin wax following formalin fixation: C4, C6, C8, T2, T6, T8, T12, L2, L4, S1 and S2.

The brain was dissected as follows. The upper pons was transected horizontally at right angles to the brain stem axis and the brain stem and cerebellum fixed intact. The brain was then bisected in the sagittal plane, the right diencephalon and hemisphere being retained for fixation. Prior to the selection of tissue blocks for paraffin wax impregnantion the brain stem was sliced in the horizontal plane, the cerebellum parasagitally, and the cerebral hemisphere in the coronal plane.

The whole brain stem and vermis were processed for histology. Samples from the cerebellar cortex and dentate nucleus, neocortical lobes, hippocampus and motor cortex (four levels) were taken. The basal ganglia and thalamus were processed as intact serial blocks including hypothalamus, insular cortex, substantia innominata, amygdala and entorhinal cortex. Routine sections were prepared from all these blocks stained with haematoxylin and eosin (H&E), cresyl fast violet (with and without Luxol fast blue) and the Cross modification of the Palmgren technique. Segments from the cervicomedullary junction, C3 and T2 were processed by the Marchi impregnation method.

Extensive immunocytochemical (ICC) examination was performed using the following antibodies: unbiquitin (Dako, 1:1000), glial fibrillary acidic protein (GFAP; DAKO, 1:4000), CD68 (pan-macrophage; DAKO, 1:50), leucocyte common antigen (LCA; pan-lymphocyte; DAKO, 1:50), SMI31 (phosphorylated neurofilaments; Sternberger Monoclonals, 1:10,000) and SMI32 (non-phosphorylated neurofilaments; Sternberger monoclonals, 1:800). An avidin-biotin complex system was used for the secondary antibody step (Vectastain, Elite, Vector Labs). Microwave antigen retrieval was employed for GFAP and SMI32.

Results

The findings were broadly consistent with the pathological features of "ALS with posterior column involvement". In addition a number of observations were made which differ from previous findings or potentially illuminate pathogenesis.

Spinal cord and motor system

At all levels of the spinal cord there were prominent white matter changes. There was demyelination of tracts in lateral, ventral and dorsal funiculi; the dorsal and ventral spinocerebellar tracts were more severely affected than the lateral corticospinal tract. In the posterior columns the area of myelin loss involved the central part of the funiculus gracilis and, at higher levels, the centre of funiculus cuneatus. These long-tract changes were symmetrical and correlated closely with the distribution of Marchi reaction product (Fig. 1). The anterior horns showed severe gliosis and loss of lower motor neurons (LMN). Remaining LMN were either normal or atrophic in H&E sections, ex-

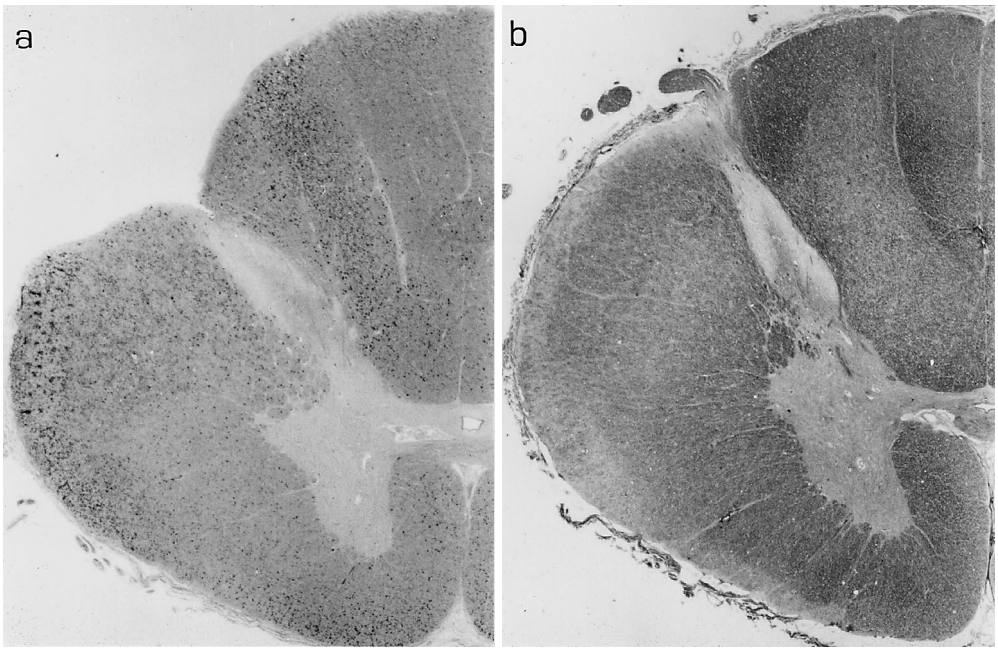

Fig. 1 **a** Marchi reaction product is diffusely distributed in dorsal, lateral and ventral funiculi at the T2 level. There is focal concentration in certain regions such as the central funiculus cuneatus. **b** Myelin pallor is distributed in the same pattern as the Marchi reaction (Cresyl violet/Luxol fast blue; C4 segment)

cept for an occassional cell showing central chromatolysis. Inclusion bodies were not present in conventionally stained sections. Clarke's nucleus was severely gliotic and depleted of neurons but the intermediolateral, sacral sympathetic, and Onuf's nucleus neurons appeared normal.

The use of ICC for GFAP, CD68 and LCA highlights the extent of degeneration. Large stellate astrocytes were present throughout the cord in all areas of grey and white matter, including the dorsal horn and substantia gelatinosa. Reactivity to CD68 showed three patterns: increased globular and dendritic profiles of perivascular cells, parenchymal permeation by globular and foamy cells, and a diffuse increase in parenchymal dendritic cells. The former two changes were restricted to areas of long-tract degeneration in white matter (Fig. 2 a). The third appearance was also present in affected areas of grey matter and, together with GFAP, provided a sensitive marker of pathological involvement in areas of equivocal or normal appearance in conventional stains (Fig. 2 c). Thus increased macrophage densities were present in the ventral horn, Clarke's column, and mid-dorsal horn (Rexed laminae III–V) but were much less prominent in the substantia gelatinosa and intermediolateral (thoracic) areas. Infiltration by LCA-immunoreactive lymphocytes was present with focal perivascular cuffing in the areas of myelin degeneration (posterior and lateral columns; Fig. 2 b). Nerve root glial bundles were present in both anterior and dorsal nerve roots.

In the medulla oblongata and pons there was moderate neuronal depletion, gliosis and CD68 immunoreactivity affecting the motor nuclei of the trigeminal, facial and hypoglossal nerves. In the hypoglossal nucleus collections of macrophages associated with neuronophagia were seen. In the lower pons the extent of CD68 immunoreactivity in the depleted facial motor nucleus contrasted with the paucity of staining in the intact abducens nucleus in the same section (Fig. 2 c, d). The nerve roots of the fifth and seventh nerves also showed frequent CD68-staining cells. The pyramids showed myelin loss, were gliotic, and permeated by CD68-staining cells.

In the midbrain the oculomotor nucleus was normal and showed minimal gliosis or macrophage permeation. The middle segment of the cerebral peduncle showed similar changes to the pyramidal tract, reflecting degeneration of the descending corticospinal pathway.

In the motor cortex the giant cells of Betz were readily identified in all the four levels examined and subjective assessment could not confirm depletion. There was no significant excess of astrocytic (Fig. 2 e) or microglial gliosis in the motor cortex which showed similar appearances to the rest of the neocortex. No neocortical ubiquitinated inclusions were found in any neocortical region.

Ubiquitin ICC revealed very infrequent inclusion bodies within LMN of the spinal cord and facial motor nucleus (Fig. 3 a). Although typical skeins were identified many inclusions showed a granular and floccular morphology. These inclusions were not revealed by staining for SMI31, SMI32, or an antibody against SOD1 (Binding Site, 1 : 1500,) which differs from previous reports of sporadic ALS [22]. There was no evidence of hyaline or Lewy body-type inclusions.

Other brain regions

No ubiquitinated inclusions were detected in any of the other neuronal populations surveyed throughout the brain and cord with the exception of the dentate gyrus. In this structure the dentate granule cells contained rounded inclusion bodies indistinguishable from those described in

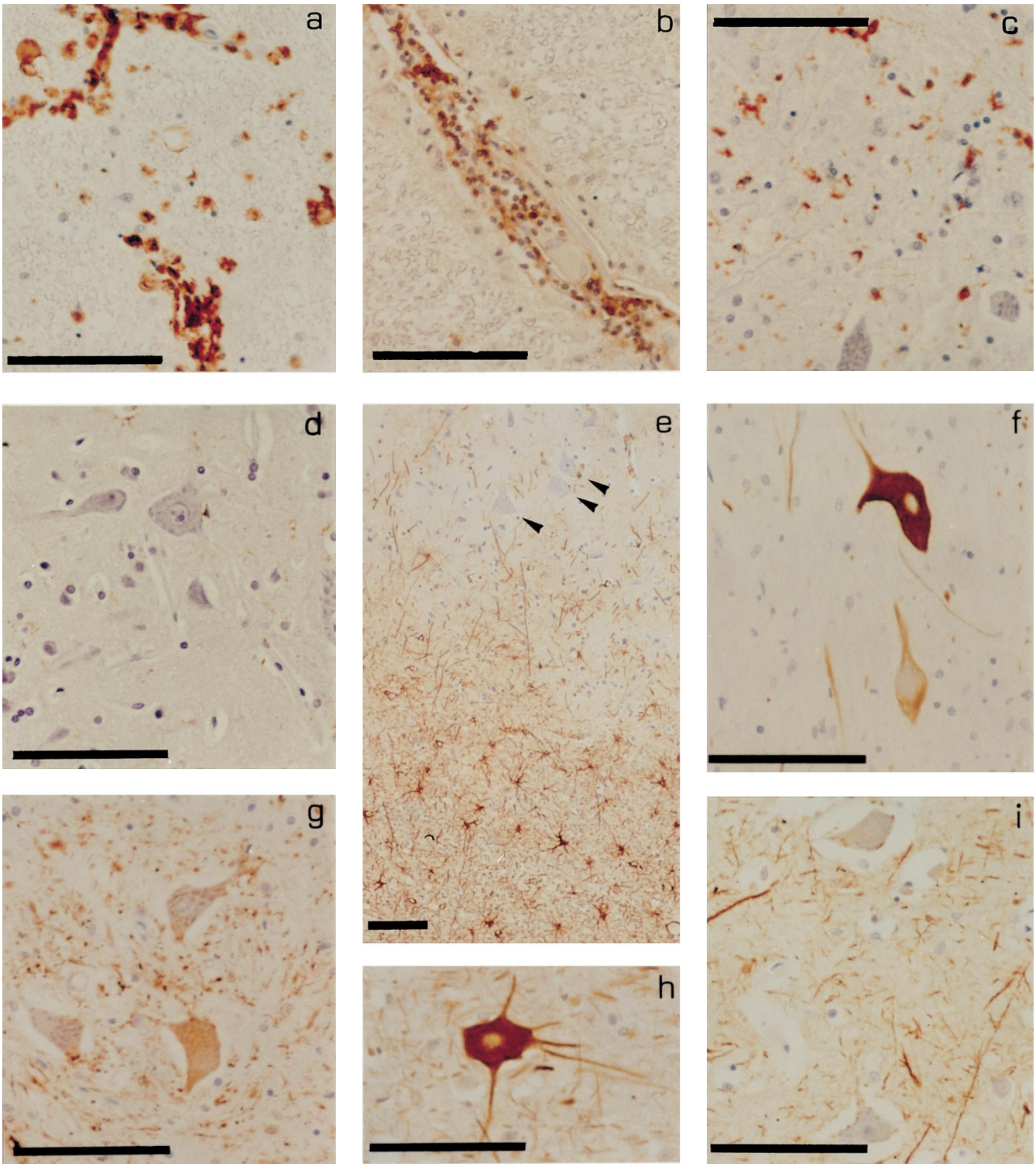

Fig. 2 **a** Macrophage permeation in the lateral corticospinal tract [immunoperoxidase (Ipx) for CD68; C6 segment]. **b** Perivascular lymphocytic infiltration of the corticospinal tract (Ipx for leucocyte common antigen; C6 segment). **c** Macrophage permeation of the seventh nerve nucleus is markedly more intense than **d** in the sixth nerve nucleus (Ipx for CD68; lower pons). **e** Motor cortex and subcortical white matter. The latter shows brisk reactive gliosis despite preserved motor neurons (*arrowheads*; Ipx for glial fibrillary acidic protein. **f**, **g** Lower motor neurons stained respectively for non-phosphorylated and phosphorylated neurofilament epitopes (Ipx for SMI32 and SMI31 respectively; C7 segment). **h**, **i** Upper motor neurons stained respectively as in **f** and **g** (Ipx for SMI32 and SMI31). *Scale bars* = 0.1 mm

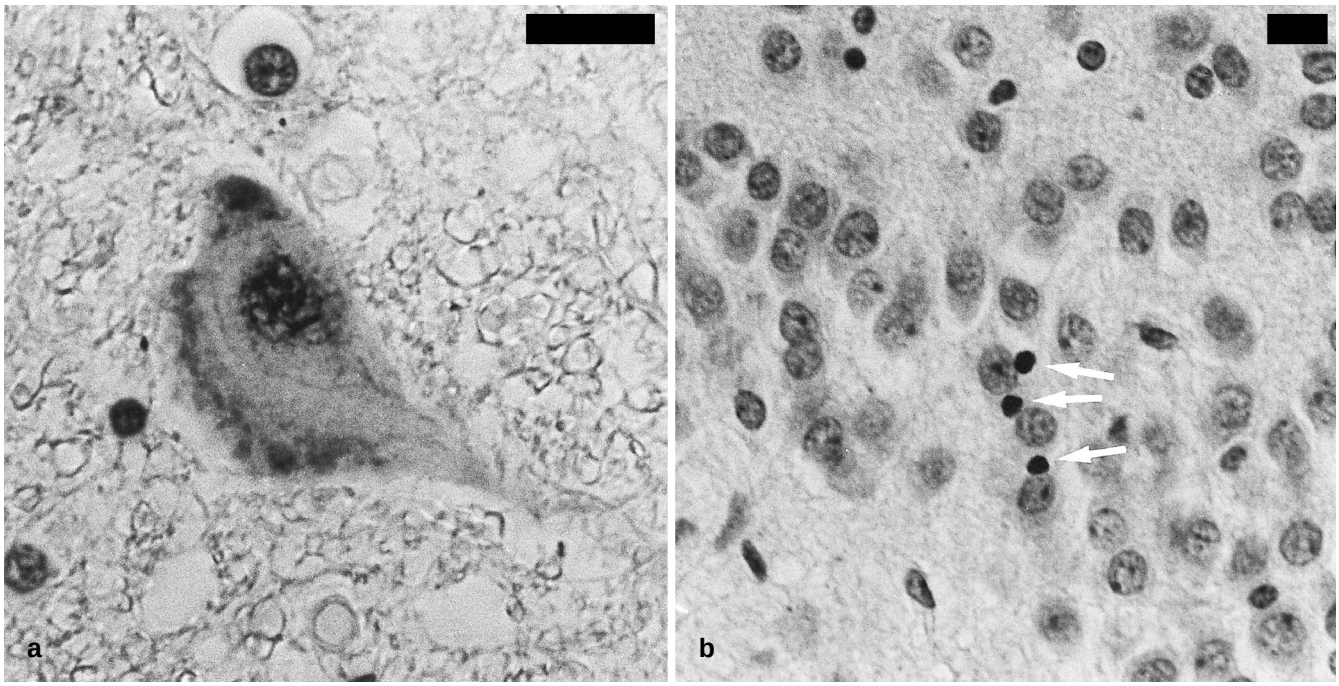

Fig. 3 **a** Skein-like inclusion body in facial motor nucleus neuron. **b** Round inclusion bodies in neurons of the dentate granule cell layer (both Ipx for ubiquitin). *Scale bars* = 0.02 mm

ALS-dementia (Fig. 3b) and in a subset of patients with frontal lobe dementia in the absence of motor system pathology [14, 27].

In the brain stem significant neuronal loss was present in the inferior olivary nucleus. This was associated with reactive gliosis and increased numbers of marcophages in the white matter of the olivary hilus and the inferior cerebellar peduncle. There was a diffuse increase, in reactive astrocytes and macrophages in the medullary reticular formation and medial lemniscus, but the dorsal efferent vagal nucleus and solitary tract were only minimally affected. The nucleus cuneatus showed marked gliosis and macrophage permeation with some neurons showing central chromatolysis. In the ventral pons there was increased macrophage permeation among the pontine nucleus in addition to the descending tracts but no other significant evidence of involvement. The locus coeruleus was uninvolved. In the midbrain the substantia nigra showed minimal incontinence of pigment and was not significantly depleted. The red nucleus in contrast showed astrocytic and macrophage permeation. The raphé nucleus, superior colliculus and periaqueductal grey matter were uninvolved. In the cerebral hemispheres there was a diffuse astrocytic gliosis of the white matter which was especially prominent in the frontal lobe. Severe gliosis with increased prominence of CD68-immunoreactive microglia was also noted in the thalamus and amygdala complexes. The remaining deep grey nuclei were only minimally gliotic.

Neurofilament epitopes

Spinal cord levels were examined using the monoclonal antibodies SMI31 and SMI32. Both antibodies gave variable immunoreactivity to the various neuronal populations present. In the limb enlargements the remaining LMN were variably reactive to SMI32, the intensity of immunoreactivity ranging among individual neurons from absent to intense (Fig. 2f). Large neurons in the dorsal horn showed similarly variable immunoreactivity. SMI31 immunoreactvity was present in axons throughout the spinal white matter. The density of immunoreactive axons mirrored the areas of myelin loss. In the ventral horn a proportion of surviving neurons showed several patterns of immunoreactivity with SMI31. Most surviving LMN were completely negative; others showed weak diffuse somatodendritic immunoreactivity (Fig. 2g). Only very few neurons showed strong focal somatic immunoreactivity and a subgroup showed focal immunoreactivity in dendrites only. Strongly immunoreactive somata of large somatic afferent neurons were readily demonstrable in the dorsal horn. There was no evidence that immunoreactivity to either antibody was associated with the ubiquitinated inclusions present in spinal LMN in this case and these findings appear to represent an abnormality of neurofilament phosphorylation in a subgroup of neurons which is more marked in the sensory system. ICC for SMI31 and SMI32 was performed in control cases dying from non-neurological illness. The pattern of SMI32 immunoreactivity was not distinguishable from the present case. SMI31 immunoreactivity significantly differed only in the extent and intensity of labelling of large dorsal horn sensory neurons.

The pattern of immunoreactivity in the motor cortex was indistinguishalbe from controls – i.e. strong SMI32 staining of Betz cells and other large pyramidal neurons with no reactivity to SMI31 (Fig. 2h, i).

Table 1 Comparison of pathological findings in four cases of SOD1-linked familial amyotrophic lateral sclerosis (*n* nerve, *NS* not stated in published paper, *NFT* tau-immunoreactive neurofibrillary tangle)

		Proband	Ref 16	Ref 21	Ref 25
Clinical					
Mutation		E100G	I113T	I113T	A4T
Age at presentation (years)		36	48	63	40
Duration (months)		39	252	24	9
Pathology					
Lower motor neuron	Spinal ventral horn	Depleted, inclusions-ubiquitin +ve	Depleted, no inclusions	Depleted, inclusions neurofilament and Bielschowsky +ve	Depleted, inclusions-Lewy body-like, ubiquitin, SMI31 +ve
	n. XII	Depleted, CD68 +ve	NS	NS	Depleted, inclusions
	n. VII	Depleted, inclusions ubiquitin +ve	Depleted	NS	Depleted inclusions
	n. VI	Normal	NS	NS	NS
	n. V	Depleted, CD68 +ve	NS	NS	NS
	n. III	Normal	Infrequent NFT	NS	NS
	Onuf. n.	Normal	Normal	NS	NS
Upper motor neuron	Betz cells	Normal, CD68, GFAP –ve	NS	Normal	Normal
	Corticospinal tract	Myelin loss below cerebral peduncle	Myelin loss in spinal cord	Normal	Mild myelin loss in spinal cord
Spinocerebellar and cerebellar	Clarke's n.	Depleted	Depleted	NS	Depleted, inclusions
	Spinocerebellar tract	Myelin loss	Myelin loss	NS	Myelin loss
	Inferior Olivary n.	Depleted, CD68 +ve	Depleted, gliosis, infrequent NFT	NS	NS
	Dentate n.	Normal	Infrequent NFT	NS	Normal
	Red n.	CD68 +ve	NS	NS	NS
Somatosensory	Dorsal columns	Central myelin loss	Normal	NS	Central myelin loss
	Cuneate nucleus	Depleted CD68 +ve	NS	NS	Depleted, inclusions
	Thalamus	CD68 +ve	NS	NS	Normal
Basal ganglia	Caudate	Normal	Normal	NS	Normal
	Putamen	Normal	Normal	NS	Normal
	Globus pallidus	Normal	Frequent NFT	NS	Normal
Brain stem/ midbrain	Substantia nigra	Mild depletion	Mild depletion, infrequent NFT	NS	NS
	Locus ceruleus	Normal	Mild depletion, infrequent NFT	NS	NS
	Raphe n.	Normal	NS	NS	NS
	Reticular formation	Normal	NS	NS	Infrequent inclusions
	Dorsal Vagus n.	Normal	NS	NS	NS
Cerebral cortex	Hippocampus	Dentate granule cell inclusions, ubiquitin +ve	NS	NS	Normal
	Amygdaloid	CD68 +ve	NS	NS	Normal
Cerebral white matter		Diffuse gliosis (GFAP)	NS	NS	Normal

Discussion

The substantial proportions of FALS cases (~70%) have pathological involvement of the posterior columns [19, 28]. These case reports now need to be reappraised following the identification of the genetic basis of a subgroup of autosomal dominant cases of ALS. Only 20–25% of familial autosomal dominant ALS is linked to mutations in the SOD1 gene and therefore the previous diversity of pathological reports of FALS must include an unspecified heterogeneity of genetic mutations [28]. Even

among the SOD1-linked cases there may be heterogeneity since approximately 39 mutations in several exons have been described [2]. The pathogenesis of these cases has not been established but the evidence supports a toxic gain of function for the mutant protein [4]. Clinicopathological correlations are urgently required for the different mutations since there are only three published accounts of the pathology of FALS where a SOD1 gene mutation has been characterised [16, 21, 25].

Comparison with previous SOD1-linked cases

A comparison of the pathological findings in the present case and three others reported is given in Table 1. Takahashi et al. [25] reported a case with an exon 1 mutation (A4T) in the SOD1 gene. This case also fell into the broad category of "ALS with posterior column involvement" [7] with additional features. Lewy-body like inclusions were visible in H&E sections which were immunoreactive for ubiquitin and phosphorylated neurofilaments. In the present case (E100G) the inclusions were only infrequently demonstrable by ubiquitin ICC in ventral horn motor neurons, showed variable skein and floccular morphology, and were not demonstrable by SMI31 ICC. The degeneration reported by Takahashi et al. [25] in the brain stem motor nuclei and the preservation of Betz cells in the motor cortex are essentially similar to the present case. Their case also showed variable staining of non-motor neurons in the cord and brain stem with phosphorylated neurofilament antibodies, a finding which we confirm in our case.

Two contrasting cases with Ile113→Thr mutations (I113T) in exon 4 [16, 21] showed marked differences. In the case reported by Orrell et al. [16] the posterior columns were uninvolved and ubiquitinated inclusions were not found in LMN. Increased immunoreactivity to phosphorylated medium weight neurofilaments (antibody BF10) was reported in spinal lower motor neurons. This finding was also prominent in the case of Rouleau et al. [21] where the LMN inclusions were immunoreactive to an unspecified anti-neurofilament antibody. In the brain the most striking difference both to the present case and the cases of Takahashi et al. [25] and Rouleau et al. [21] was the presence of tau-immunoreactive neurofibrillary tangles in multiple midbrain, brain stem and cerebral regions. The distribution and morphology of these lesions is reminiscent of progressive supranuclear palsy. These differences highlight the phenotypical heterogeneity of FALS cases even with the same mutation. The rapidly progressive form of FALS associated with the A4V mutation (exon 1) is also not associated with long tract degeneration (R. H. Brown, personal communication).

Pathobiology of FALS

Multisystem pathology

The present case, and those reviewed in Table 1, emphasise the extent to which FALS is a multisystem disorder [16, 21, 25, 28]. The prominent pathological evidence of involvement of the ascending proprioceptive pathway, afferent and efferent cerebellar pathways and the cerebrum is, however, not reflected in the clinical picture [11]. This discrepancy may arise because of a masking effect due to the severity of motor impairment. This explanation readily accounts for the absence of cerebellar signs in affected regions since their clinical demonstration is not feasible in the presence of severe weakness. However, loss of proprioception should be demonstrable even in a paralysed limb. The lack of corresponding clinical data may reflect the focus of clinical interest on the progression of motor weakness, especially at later stages of the disease. Electrophysiological testing has demonstrated impaired sensory pathways in sporadic ALS [10] and it is clearly appropriate to pay particular attention to sensation and cerebellar function in familial cases.

Ubiquitinated inclusions and the cytoskeleton

The presence of ubiquitinated inclusions in the motor neurons of the ventral horn resembles the findings described in sporadic ALS. Diffuse cerebral white matter astrocytosis and dentate granule cell inclusions are also found in cases of ALS-dementia [9, 14, 27]. These observations are of particular importance concerning the relationship between the sporadic and familial forms of the disease. The presence of such inclusions in familial cases with defined genetic abnormalities argues strongly for common pathogenetic processes. However, it does not exclude the possibility that the formation of such inclusions may be a late stage "common pathway" resulting from neurodegeneration arising from different mechanisms. Ubiquitinated lesions were not demonstrated in other neuronal groups which are markedly affected (dorsal sensory neurons, Clarke's nucleus) in the present case but were reported by Takahashi et al. [25]. Given the phenotypic diversity between LMN, Clarke's nucleus, and the granule cells of the dentate gyrus, the factors which predispose to or protect from such inclusion body formation are not obvious, although these neuronal populations have major efferent and afferent glutamatergic connectivity. A similar problem exists in elucidating the factors determining vulnerability to excessive neurofilament phosphorylation, which varies markedly between these differing SOD1-linked cases.

Inflammatory cell infiltration

In the spinal cord there was an excess both of macrophages and of lymphocytes in the areas of myelin degeneration and, less extensively, the grey matter. These infiltrates are of equivalent prominence to those found in the majority of sporadic cases of ALS [26]. Since the present case has arisen on the basis of a defined genetic mutation, it seems unlikely that a primary autoimmune mechanism is involved in the pathogenesis. If therefore seems likely

that the extensive long-tract degeneration present in this case is associated with secondary reactive inflammatory cell infiltration. This observation is potentially of importance because the presence of such infiltrates is frequently cited in support of the autoimmune hypothesis for ALS [23].

Mechanism of neurodegeneration

Myelin degeneration and axonal loss were prominent in the pyramidal tract up to the level of the cerebral peduncles, associated with a macrophage/lymphocyte reaction. However, in the motor cortex and adjacent premotor area there was no evidence of axonal or myelin degeneration, Betz cells were preserved, ubiquitinated inclusions were absent, and the pattern of neurofilament phosphorylation was normal. In the subcortical white matter there was reactive gliosis (similar to the other frontal areas examined) and only a minimal excess of macrophages. Similar findings were reported by Takahshi et al. [25] and argue strongly for distal axonal degeneration as the basis for the long-tract degeneration observed. Given that the most prominently affected regions of the central nervous system comprised those with long projecting axons, these observations support the hypothesis that a major determinant of the vulnerability of motor neurons in ALS may be the size of their axonal compartment. If the neurodegenerative process in FALS leads to a failure to maintain normal axonal function, possibly through cytoskeletal injury [3, 6], then the motor system would be expected to be especially vulnerable.

Further systematic examination of cases of ALS with defined genetic mutations should be made to clarify the spectrum of clinical pathological and molecular changes.

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