

# Familial amyotrophic lateral sclerosis with a point mutation of SOD-1: intrafamilial heterogeneity of disease duration associated with neurofibrillary tangles

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## Abstract

**Mutations of SOD-1 have recently been associated with autosomal dominant familial amyotrophic lateral sclerosis (ALS). A patient is described with a 20 year duration of motor neuron disease, with clinical features of ALS, who was heterozygous for a point mutation ATT to ACT leading to substitution of isoleucine for threonine at codon 113 in exon 4 of SOD-1. This mutation has previously been described in two families with ALS and three apparently sporadic cases of ALS. The patient described here had a family history suggestive of autosomal dominant inheritance of this genetic mutation; other members of the family having a more typical disease duration. Unusual pathological features included neurofibrillary tangles in neurons of the globus pallidus, substantia nigra, locus coeruleus, and inferior olivary nuclei, and absence of ubiquitin immunoreactive inclusions in motor neurons. This may reflect the slow progression of the neurodegeneration associated with the SOD-1 mutation in this patient. The prolonged survival, of over 20 years, with other family members having a more typical survival of two to three years, has important implications for genetic counselling in families with ALS in addition to the fundamental biological questions concerning the influence of these mutations on disease expression.**

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**Keywords:** amyotrophic lateral sclerosis; familial ALS; SOD-1; neurofibrillary tangles

Around 5-10% of patients with amyotrophic lateral sclerosis (ALS) (or motor neuron disease) have a family history of the disease, and in larger families show evidence of autosomal dominant inheritance. Genetic studies in such large families led to the identification of linkage to chromosome 21 in around 40% of families.<sup>1</sup> This linkage was recently attributed to mutations in the SOD-1 gene which encodes the enzyme copper-zinc superoxide dismutase (SOD).<sup>2</sup> Reduced SOD enzyme activity has been identified in erythrocytes and other tissues in these patients,<sup>3,4</sup> although it is possible that the disease expression is due to a

toxic effect of the mutant enzyme.<sup>5</sup> To date, around 25 different missense mutations of SOD-1 with single base pair substitutions,<sup>6</sup> and a single family with a two base pair deletion,<sup>7</sup> have been described. Such mutations are present in around 20% of all families with familial ALS in North America. The clinical implications of these mutations are of great importance. We describe a family with a SOD-1 mutation, in whom the considerable variability of disease expression illustrates the potential difficulties in prognosis and genetic counselling that may be encountered.

## Patients and methods

### CASE HISTORY

This woman first noticed tripping and difficulty climbing stairs at the age of 48. At the age of 49 she developed weakness of the right thumb, with difficulty grasping a car door or keys. At that time there was pronounced wasting of the right thenar eminence and interossei of the right hand, with severe weakness. There was also weakness of the right triceps. Reflexes were abnormally brisk in both arms, but examination was otherwise normal. An EMG showed features of active denervation of both hands and the right leg, with fasciculations in the right tibialis anterior and right thenar eminence, and normal motor and sensory conduction.

By the age of 60, she had progressive wasting and weakness, being only able to walk 100 yards, with elbow crutches. There was some weakness in both hands. She had slight difficulty with tongue movement. By the age of 63 the course of the disease had accelerated; she was confined to a wheelchair, with leg, trunk, and distal arm weakness. She still had proximal limb function and was able to feed herself. There was spasticity of the upper limbs. She had wasting and weakness of the face, mouth and tongue, with dysarthria. There were fasciculations in the tongue and a brisk jaw jerk, but no clinical signs of extrapyramidal disease or dementia. She died of pneumonia at the age of 69.

### PEDIGREE (FIG 1)

The proband is IV.13. One brother (IV.12) was killed in action in the 1914-18 war; the other brother (IV.10) is aged 80, and has no neurological disease. The proband's mother (III.8) died at the age of 50 with a diagnosis of "amyotrophic lateral sclerosis of the spine"

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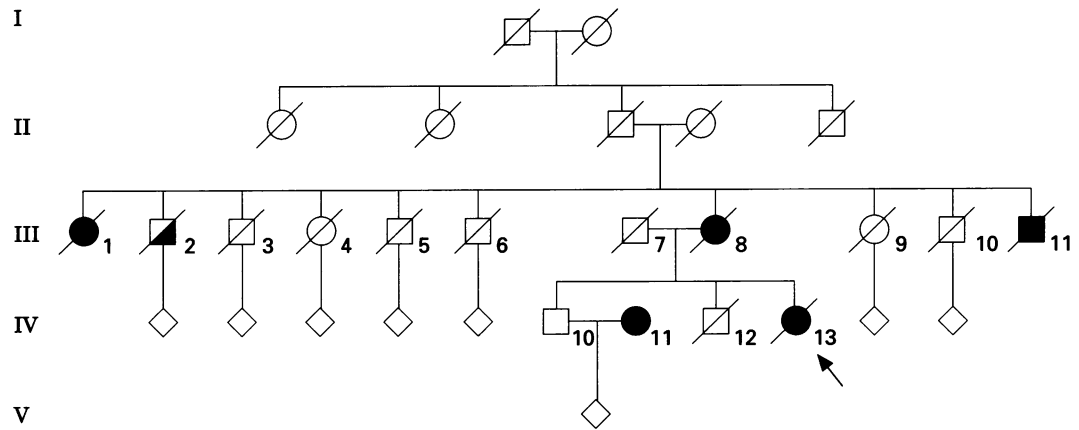
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Figure 1 Pedigree of family with ALS. Squares represent males, circles females, diamonds further generations of between one and three members. Oblique lines represent deceased members. Filled symbols represent those with ALS, the partially filled symbol possible ALS. The proband is indicated by an arrow.



and a disease duration of between two and three years. The family was large and contact has been lost among all the relatives, but the following descriptions are given.

III.1 This woman died at the age of about 50, in 1930, of ALS with a disease duration of between four and five years.

III.2 This man had a full life span, although there is a family rumour that he died of ALS, developing when aged 65.

III.3 This man died with no evidence of ALS.

III.4 This woman died of heart disease in her 70s.

III.5 This man was killed in action in the 1914–18 war.

III.6 This man died in his 70s of heart disease.

III.9 This woman died aged 89 of pneumonia.

III.10 This man died of old age.

III.11 This man developed mild leg weakness at the age of 70, presenting with weakness of the right arm followed by the left arm at the age of 72. This progressed to generalised weakness, wasting, and fasciculations of all limbs and tongue, with brisk reflexes and extensor plantar responses. He died of ALS, with a disease duration of around three years.

**Results**

**NEUROPATHOLOGICAL FINDINGS**

Before fixation the brain weighed 1360 g. The fixed brain appeared macroscopically unremarkable. The spinal cord showed obvious

atrophy of the anterior nerve roots, most pronounced in the mid-cervical region.

**Skeletal muscle**

Paraffin sections showed many atrophic angulated muscle fibres, some scattered singly within fascicles, others in relatively large groups. The appearances were typical of chronic partial denervation.

**Spinal cord**

Histological examination disclosed widespread loss of anterior horn cells and Clarke's column nuclei, with preservation of Onuf's nucleus. The anterior spinal nerve roots were depleted of fibres and contained excessive endoneurial collagen. Occasional axonal spheroids were present in the anterior horn regions of the lumbar cord. The posterior roots appeared normal. Pronounced fibre loss was noted in both anterior and posterior spinocerebellar tracts on myelin staining, with additional loss of myelin staining in the corticospinal tracts. The posterior columns appeared normal. No neurofibrillary tangles or other neuronal inclusions were seen in sections of spinal cord stained with haematoxylin and eosin or modified Bielschowsky silver impregnation.

**Brain**

Sections of the frontal and temporal lobes were unremarkable, and in particular there were no Alzheimer type changes. The globus pallidus showed diffuse gliosis, neuronal loss, and scattered neurofibrillary tangles (fig 2). These tangles were argyrophilic but did not fluoresce under ultraviolet light after thioflavin-S staining. No tangles were seen in the putamen or caudate nuclei, which appeared normal.

Mild neuronal loss, pigmentary incontinence, and neurofibrillary tangles were also present in the substantia nigra and locus coeruleus. Within the medulla there was neuronal loss, gliosis, and occasional neurofibrillary tangles in the inferior olivary nuclei. Neuronal loss was also seen in the seventh and 12th cranial nerve nuclei.

**Immunocytochemistry**

Sections of temporal lobe, basal ganglia, mid-brain, and pons were immunostained by a

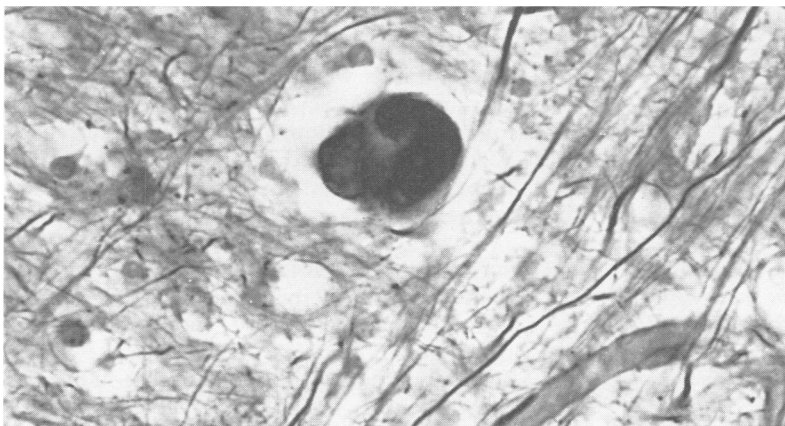


Figure 2 Neuron of globus pallidus, with intact nucleus, showing a neurofibrillary tangle. Palmgren's; × 380.

conventional avidin-biotin-horseradish peroxidase method. Controls included sections from cases of Alzheimer's disease and sections from which the primary antibodies were omitted during immunostaining. Occasional neurofibrillary tangles reacted with BF10 (a monoclonal antibody to phosphorylated medium weight polypeptide of neurofilament). Antibodies to ubiquitin (Dako) and RT-97 (a monoclonal antibody to phosphorylated heavy

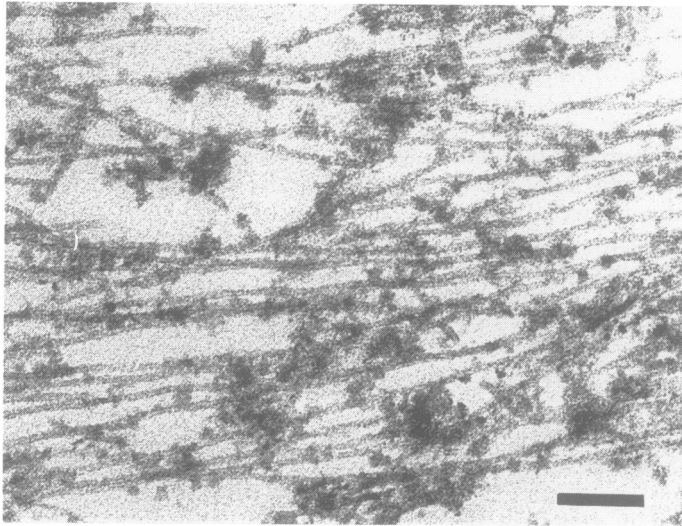


Figure 3 Electron micrograph showing an intraneuronal neurofibrillary tangle in the substantia nigra composed of straight 8 nm filaments; bar = 100 nm.

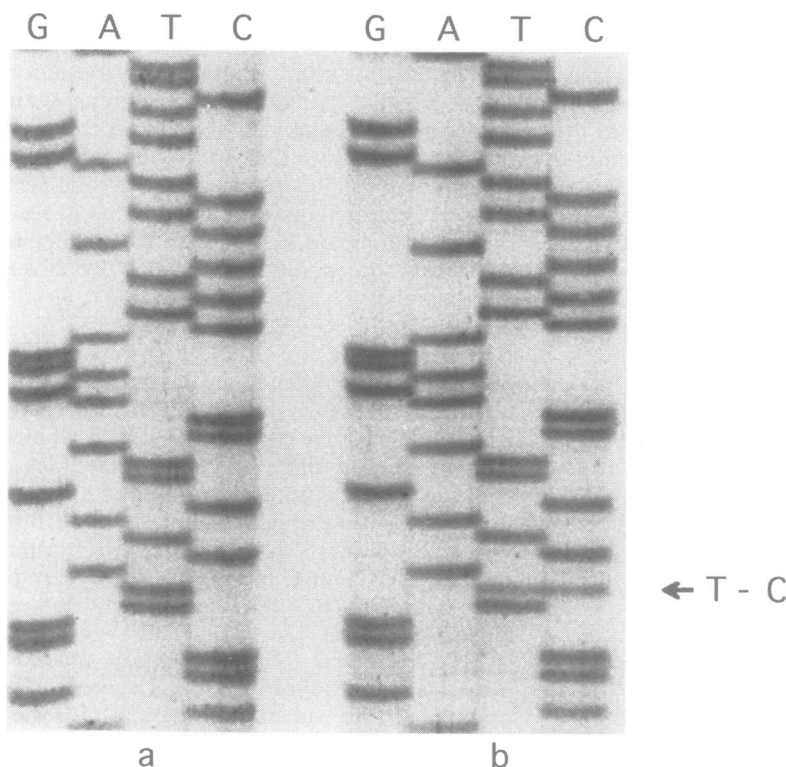


Figure 4 The sequence of exon 4 in a normal subject (a) and the proband (b). The four lanes for each individual represent the nucleotides guanine (G), adenine (A), thymine (T), and cytosine (C). These have been separated electrophoretically on a polyacrylamide gel, and the sequence of the nucleotides, which represents the "genetic code" of the DNA, is read sequentially from top to bottom. The nucleotides have separated according to their distance from the primer, giving the code T TCT GTG ATC etc. An additional C band (arrowed) is present in the proband (b) which is not present in the normal subject (a). In the proband (b), the mutant C band and its corresponding normal T band, are both of reduced intensity as this is a heterozygote point mutation T to C.

weight neurofilament) gave negative results. Occasional tangles showed weak tau protein (Sigma) immunoreactivity. Moderate numbers of tau positive neurites were present in the globus pallidus and dentate nuclei, but were only occasionally seen in the substantia nigra, oculomotor nuclei, and basis pontis. tau positive astrocytes were not noted.

Immunohistochemistry of multiple sections of spinal cord did not show any ubiquitinated inclusions, but showed diffuse immunoreactivity in the perikaryon of several anterior horn cells with BF10 neurofilament antibody.

#### Electron microscopy

Ultrastructural examination of sections of substantia nigra showed neurofibrillary tangles composed of bundles of straight filaments approximately 8 nm in diameter (fig 3).

#### SOD-1 SEQUENCE ANALYSIS

DNA was extracted from whole blood with Nucleon II (Scotlab). Each of the five exons of SOD-1 was amplified with appropriate primers. The primers for exon 4 were 5'CGCGACTAACAATCAAAGTGA and 5'CATCAGCCCTAATCCATCTGA. A polymerase chain reaction (PCR) was performed with 1 µg of genomic DNA in a 100 µl reaction volume with 5 µl DMSO, 1.5 mM MgCl<sub>2</sub>, 10 µl 10 × PCR buffer IV (Advanced Biotechnologies) (10 × buffer IV contains 200 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 750 mM Tris HCl (pH 9.0), 0.1% Tween), 200 µM dNTPs (Advanced Biotechnologies), 2.5 units Taq polymerase, 0.5 pM of biotinylated and non-biotinylated primers. The reaction mixture was overlaid with mineral oil before thermal cycling (Hybaid Omnigene thermal cycler), at 95°C for two minutes, 32 cycles of annealing at 55°C for two minutes, extension at 72°C for two minutes, strand separation at 95°C for one minute, and a final extension at 72°C for six minutes. The products were screened by electrophoresis in 2% agar stained with ethidium bromide, the product size being 232 base pairs. The amplified product was separated on Dynabeads M-280 Streptavidin (Dyna, Norway) by the standard protocol, and the single stranded product was directly sequenced using a Sequenase 7 Deaza dGTP sequencing kit with Sequenase version 2.0 T7 DNA polymerase (United States Biochemical Corporation) labelled with <sup>35</sup>SdATP (Amersham), according to the protocol. The DNA strands were electrophoresed on a 6% acrylamide gel (Severn Biotech Ltd) at 60 W for one to two hours, the gel transferred to Watman paper, dried, and exposed to Hyperfilm (Amersham) for up to seven days before developing and reading the sequence.

#### SOD-1 MUTATION IDENTIFICATION

A heterozygote point mutation was identified in the proband in exon 4, with the change of the codon ATT to ACT, causing the amino acid substitution Ile113Thr (fig 4). This was not present in the patient's clinically normal brother or in control specimens examined. There was no mutation in exons 1,2,3, and 5.

The mutation was confirmed by sequencing the complementary strand.

## Discussion

### SOD-1 MUTATION

Twenty five missense mutations of SOD-1 have now been described in familial ALS. The point mutation in exon 4, T to C, causing the amino acid substitution Ile113Thr, has been reported in two families with ALS<sup>2</sup> and in three patients with apparently sporadic ALS in Scotland.<sup>8</sup> The isoleucine at position 113 of SOD is highly conserved among species, including drosophila, swordfish, mouse, and human.<sup>3</sup> It is structurally located within the Greek key loop, and forms twofold symmetric dimer interactions. A substitution should significantly destabilise the mutant heterodimeric and homodimeric SOD. The mechanism by which these enzymic changes cause disease is yet to be defined, but may be by free radical damage due to reduced enzyme activity,<sup>9</sup> or other neurotoxic mechanisms due to a different function of the altered enzyme.<sup>5</sup> The clinical detail in these reports is sparse.

### PATHOLOGY

An unusual feature of this case is the finding of neurofibrillary tangles in neurons of the globus pallidus, substantia nigra, locus coeruleus, and inferior olivary nuclei. The ultrastructural and immunocytochemical findings suggest that the tangles are composed of neurofilaments. Neurofibrillary tangles are not usually seen in either sporadic or familial ALS,<sup>10</sup> although they are well recognised in Guamanian ALS<sup>11</sup> and have been described in cases of ALS occurring as a delayed complication of encephalitis lethargica.<sup>12,13</sup> The neurofibrillary tangles in Guamanian ALS have a more widespread distribution from those in the present case, also occurring in the cerebral cortex, dentate nucleus, and spinal cord,<sup>11</sup> having a paired helical structure<sup>14</sup> and exhibiting strong immunoreactivity with antibodies to tau protein.<sup>15</sup> Although Guamanian ALS may be related to environmental factors, recent segregation analyses support the involvement of a major gene in combination with environmental factors.<sup>16</sup> No mutation of SOD-1 was identified, however, in eight Guam patients with ALS and six patients with parkinsonism, some of whom had other affected family members.<sup>17</sup> Neurofibrillary tangles are a prominent feature of progressive supranuclear palsy, but the tangles in the patient we describe are much fewer than normally seen in progressive supranuclear palsy. The patient had clear clinical features of ALS, and not of progressive supranuclear palsy, although progressive supranuclear palsy may occasionally be familial.<sup>18</sup>

Although neurofibrillary tangles are not common in either sporadic or familial ALS, other cytoskeletal abnormalities often occur. These include various neuronal inclusions<sup>19</sup> and abnormal accumulations of neurofilaments within neuronal somata and axons,<sup>20-22</sup> with focal, rather than diffuse, neurofilamen-

tous accumulations being more common in familial cases.<sup>23</sup> Ubiquitin immunoreactive inclusions are a common finding in neurodegenerative diseases, including motor neurons of the anterior horn, brain stem, and, rarely, the cortex in ALS.<sup>24</sup> These inclusions are particularly associated with an aggressive and short duration of disease.<sup>25</sup> The absence of ubiquitinated inclusions in this patient may reflect the slow progression of the disease. This would support the hypotheses that ubiquitin immunoreactive inclusions result from the accumulation of altered cytoskeletal protein during relatively short duration, intense or sustained cell stress saturating ubiquitin mediated proteolytic clearance,<sup>26</sup> but not saturating the system in more slowly progressive disease. Alternatively, ubiquitination is not a primary cause of the neurodegeneration. The neuropathological findings in the current case may represent part of the range of neurofibrillary tangle formation described in cases of familial ALS.<sup>27-30</sup> Alterations in neurofilament density can cause slowing of axonal transport<sup>31</sup> and, conversely, toxic agents which interfere with axonal transport are associated with neurofilament accumulation.<sup>32-34</sup> The relation between mutations in the SOD-1 gene and abnormal neurofilament accumulation in ALS remains uncertain, although such mutations may lead to structural change in the SOD protein and interference with axoplasmic flow. Neurofilaments have been shown to accumulate in animal models of motor neuron disease<sup>35</sup> and, recently, transgenic animals have been described in which overexpression of neurofilament subunits by neurons<sup>36,37</sup> or a point mutation in the neurofilament subunit,<sup>38</sup> have resulted in disorders resembling motor neuron disease.

### CLINICAL GENETICS

The SOD-1 mutation segregates with the disease in an autosomal dominant manner, and can also be found in asymptomatic members of a family.<sup>39</sup> The age dependent and incomplete penetrance of the gene is acknowledged.<sup>5,39</sup> An additional complication in the counselling of people with the SOD-1 mutation is the variability of disease duration and severity.<sup>40</sup> If the proband presented now at age 48 years with a SOD-1 mutation, the advice given would have to be very general to anticipate her survival of over 20 years, living alone, and requiring a nursing home only after 17 years duration of disease at the age of 65. It would not be appropriate to take into account the rapid (two to three years) duration of disease of the other members of the family. Generally, a later onset of disease may be associated with a more rapid course,<sup>41</sup> but in itself cannot account for the variation in this family.

In Japan a mutation of SOD-1 was reported to be associated with a relatively benign disease.<sup>42,43</sup> In the members of these two families the mean survival after onset was 17 (SD 11) years. These statistics were based on only six patients, whereas a total of 23 were shown to be affected on the pedigrees.<sup>42</sup>

They suggested that this represents a new subtype, or mild form, of ALS associated with a unique mutation (His46Arg). We suggest that the prolonged survival data may be an artefact of the longest surviving being available for clinical and genetic analysis. The 95% confidence intervals of their data clearly include the more rapid (two to three years) survival, and counselling of a relatively benign disease for all those in such families may be inappropriate.

It is possible that this variable expression in a large family, including a relatively benign form of ALS, explains the appearance of apparently sporadic forms in smaller families with the same mutation. It is thus possible that a proportion of apparently sporadic forms, especially in smaller modern families, may be due to inheritance of SOD-1 mutations. It is important to determine the biological basis of the variable age of onset and identify other contributory factors to fully understand the causes and treatment of ALS.

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