Published in final edited form as:

Acta Neuropathol. 2015 July ; 130(1): 145–157. doi:10.1007/s00401-015-1431-2.

A truncating SOD1 mutation, p.Gly141X, is associated with clinical and pathologic heterogeneity, including frontotemporal lobar degeneration

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Abstract

Amyotrophic lateral sclerosis (ALS) is a degenerative disorder affecting upper and lower motor neurons, but it is increasingly recognized to affect other systems, with cognitive impairment resembling frontotemporal dementia (FTD) in some patients. We report clinical and pathologic findings of a family with ALS due to a truncating mutation, p.Gly141X, in copper/zinc superoxide dismutase (SOD1). The proband presented clinically with FTD and later showed progressive motor neuron disease, while all other family members had early-onset and rapidly progressive ALS without significant cognitive deficits. Pathologic examination of both the proband and her daughter revealed degeneration of corticospinal tracts and motor neurons in brain and spinal cord compatible with ALS. On the other hand, the proband also had neocortical and limbic system degeneration with pleomorphic neuronal cytoplasmic inclusions. Extramotor pathology in her daughter was relatively restricted to the hypothalamus and extrapyramidal system, but not the neocortex. The inclusions in the proband and her daughter were immunoreactive for SOD1, but negative for TAR DNA binding protein of 43 kDa (TDP-43). In the proband, a number of the neocortical inclusions were immunopositive for α -internexin, initially suggesting a diagnosis of atypical FTLT, but there was no evidence of fused in sarcoma (FUS) immunoreactivity, which is often detected in atypical FTLT. Analogous to atypical FTLT, neuronal inclusions had variable co-localization of SOD1 and α -internexin. The current classification of FTLT is based on the major constituent protein: FTLT-tau, FTLT-TDP-43, and FTLT-FUS. The proband in this family indicates that SOD1, while rare, can also be the substrate of FTLT, in addition to the more common presentation of ALS. The explanation for clinical and pathologic heterogeneity of SOD1 mutations, including the p.Gly141X mutation, remains unresolved.

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Keywords

amyotrophic lateral sclerosis; electron microscopy; frontotemporal lobar degeneration; immunohistochemistry; internexin-alpha; neurofilament; superoxide dismutase 1

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease with progressive loss of upper and lower motor neurons. Although the majority of ALS is seemingly sporadic, about 10–15% is familial and can occur as an autosomal dominant, autosomal recessive, or X-linked disorder [1]. Mutations in the gene encoding the antioxidant enzyme copper/zinc superoxide dismutase (*SOD1*) [25] account for about 20% of familial ALS [<http://alsod.iop.kcl.ac.uk>]. Since the first report of linkage of *SOD1* to ALS in 1991 [27], at least 166 *SOD1* mutations have been reported [for updated list see: <http://alsod.iop.kcl.ac.uk>]. Clinical phenotypes relating to the duration, age of onset, and penetrance are variable among *SOD1* mutation carriers both between and within families with the same mutations as well as for the various mutations [1].

ALS is traditionally considered a pure motor neuron disease, but is now recognized to be a multisystem disorder. Many patients with ALS exhibit mild cognitive impairment and a subset have severe impairment with features of frontotemporal dementia (FTD) (reviewed in [8]). It has been recently reported, however, that *SOD1*-related ALS is less likely to be associated with significant cognitive impairment compared to non-*SOD1*-related ALS [34]. Neuropathologically, *SOD1*-related ALS is heterogeneous, with some patients showing pathology relatively restricted to upper and especially lower motor neurons, while other patients show degeneration of the posterior and anterolateral funiculi in the spinal cord with and Lewy body like hyaline inclusions or conglomerate inclusions in anterior horn cells [12]. A less common phenotype is a slowly progressive disorder with long disease duration [2]. Bunina bodies, cystatin C containing inclusions that are characteristic of sporadic ALS [13], are not found in *SOD1*-related ALS.

In this report we describe the clinical and pathological features of a family with a nonsense mutation in exon 5 (p.Gly141X) of the *SOD1* gene, predicted to generate a truncated SOD1 protein at 140 amino acids. This mutation has been reported previously in a brief report of a 34-year-old man with a four-year disease course, whose mother also had early-onset ALS [2]. There was no description of neuropathology in either the patient or his mother. The proband in our family presented with clinical features suggestive of FTD and later developed progressive motor neuron disease, but her affected relatives only had features of ALS. Postmortem examination of the proband and her daughter revealed pathologic heterogeneity in the distribution of SOD1 immunoreactive inclusions as well as in severity of motor and extramotor neurodegenerative changes. The results suggest that a truncating p.Gly141X mutation in *SOD1* may cause not only familial ALS, but rarely also FTLD.

Materials and Methods

Clinical findings

Genealogy—The family pedigree is shown in Fig. 1, which illustrates clinically probable ALS affecting males and females in four generations without skipping generations and affecting approximately half of each at-risk generation consistent with an autosomal dominant, highly penetrant mutation. Only the proband (III-2) had significant cognitive impairment. The grandmother of the proband (I-2) and multiple other maternal relatives all died of ALS. The mother of the proband (II-2) died at 36 years of age. The son of the proband (IV-1) died at 44 years of age from ventilatory failure only 7-months after first clinically apparent weakness. He had no signs of cognitive impairment. He had rapidly progressive quadriparesis with bulbar features and early respiratory muscle involvement. There were widespread lower motor neuron signs (i.e. atrophy and fasciculations), but no definite upper motor neuron signs.

Clinical features of proband (III-2)—The proband was a 48-year-old, right-handed woman with 12 years of education. She was cognitively normal until 17-months before she died, when her ability to carry on a conversation became impaired. She also had unexplained weight loss, in the absence of dysarthria or dysphagia. She gradually developed behavioral and language abnormalities marked by childlike and preservative behaviors, neglect of personal hygiene, and uncharacteristic food preferences, as well as tangential and repetitive speech. She had a tendency to become lost when driving, and she had difficulty recalling the locations of personal items. Swallowing difficulties and a pseudobulbar affect developed within one year of onset of her language impairment. Her medical history was significant for polio at age five years, initially requiring respiratory support, but with complete recovery and no lasting motor deficits. Neurological examination at our center 12 months after symptomatic onset revealed diffuse muscle fasciculations, but no significant weakness or atrophy in her face or limbs. There were no upper motor neuron signs. The cranial nerves were intact. There were no sensory, extrapyramidal, or cerebellar abnormalities. Her gait was normal.

Routine blood and cerebrospinal fluid tests were normal. Her electroencephalogram was unremarkable. Brain CT and MRI studies showed a probable lacunar infarct in the left basal ganglia. Electromyography at an outside institution showed motor denervation, but details were not available. She was given a clinical diagnosis of FTD, potentially associated with ALS. Cognitive impairment worsened, and she developed gait impairment compatible with lower limb weakness two-to-three months after her initial evaluation at our center. She died at 49 years of age due to respiratory failure associated with possible aspiration pneumonia.

Clinical features of the daughter of the proband (IV-3)—The daughter of the proband (IV-3) developed progressive lower limb weakness at 40 years of age and was diagnosed with El Escorial clinically probable ALS four months after the onset of weakness. Motor findings remained confined to thoracic and lumbosacral segments 12 months after onset. Dysarthria and dysphagia appeared 15 months after onset. Her respiratory function deteriorated and elective tracheostomy with mechanical ventilation was initiated 17 months

after onset. A cognitive screen 2 months prior to death using the ALS Cognitive Behavioral Screen (ALS-CBS) was normal [36]. She died of an acute cardiopulmonary event at 42 years of age, 20 months after symptom onset.

Neuropathologic methods

The autopsies on the proband (III-2) and her daughter (IV-3) were performed at Mayo Clinic Florida with postmortem intervals of 3 hours and 3 days, respectively. Segments of spinal cord from IV-3 and right hemibrains from both III-2 and IV-3 were frozen. The left hemibrains and spinal cords were fixed in 10% formalin. Tissue blocks were taken from multiple cortical and subcortical structures, as well as the spinal cord at multiple levels. The blocks were embedded in paraffin and processed for histologic studies, including thioflavin S fluorescent microscopy.

Immunohistochemical methods—Paraffin-embedded 5- μ m thick sections were deparaffinized and stained with hematoxylin and eosin (H&E). To assess demyelination, Luxol fast blue (LFB) stain was also used. Selected sections were immunostained with a DAKO Autostainer (DAKO, Carpinteria, CA, USA) using 3, 3'-diaminobenzidine (DAB) as the chromogen. The primary antibodies used were rabbit polyclonal antibody against SOD1 (1:5000; gift from Zhuoshang Xu, Johns Hopkins University School of Medicine, Maryland [22]), mouse monoclonal antibody against phosphorylated neurofilament (pNF) (SMI 31, 1:10 000, Sternberger-Meyer, Inc., Lutherville, MD, USA), mouse monoclonal antibody against internexin-alpha (INA) (MAB 2E3, 1:50, gift from Gerry Shaw, University of Florida, Gainesville), mouse monoclonal antibody against ubiquitin (Ubq) (Ubi-1; MAB1510, 1:60 000; Chemicon, Burlingame, CA, USA), guinea pig polyclonal antibody against p62 (p62-N, 1:2000; Progen, Heidelberg, Germany), rabbit polyclonal antibody against cystatin C (A0451, 1:5000; Dako, Denmark), mouse monoclonal against phospho-TDP-43 (pS409/410, 1:5000; Cosmo Bio Co., LTD. Tokyo, Japan), rabbit polyclonal antibody against FUS (HPA008784; 1:500, SIGMA, St. Louis, MO, USA), mouse monoclonal against phospho-tau (CP13; 1:1000, gift from Peter Davies, Feinstein Institute for Medical Research, North Shore/Long Island Jewish Health Care System), mouse monoclonal antibody against pan amyloid-beta (A β) (33.1.1; 1:1000, gift from Pritam Das, Mayo Clinic) and rabbit polyclonal antibody against SOD1-exposed-dimer-interface (SEDI antibody; 1:100 [24]).

Double labeling immunohistochemical methods—Double-labeling immunohistochemistry was performed using SOD1 antibodies and either INA, pNF or GFAP antibody. The first immunohistochemical cycle used SOD1 antibodies. After the DAB reaction of the first cycle, the sections were treated with double stain blocking reagent (DAKO) for 3 min, followed by incubation with the second primary antibody (INA, pNF or GFAP) for 45 min at room temperature. The second antibody was detected with alkaline phosphatase labeled polymer (DAKO) applied for 30 min followed by chromogen solution containing 0.15 mg/mL of 5-bromo-4-chloro-3-indolyl-phosphate/nitro blue tetrazolium (SIGMA).

Semiquantitative analytical methods—The frequency of the SOD1-, pNF- and INA-positive inclusions was assessed with a semiquantitative design using a 5-point grading scale: 0 = no inclusions; 1 = a small number of inclusions; 2 = moderate number of inclusions; 3 = many inclusions; 4 = very many inclusions, analogous to the scale used for α -synuclein pathology in assessing pathology of dementia with Lewy bodies [19].

Ultrastructural methods—Small pieces of tissues from the superior frontal cortex and spinal cord anterior horn of formalin-fixed brain were processed for transmission electron microscopy, and post-embedding immunogold electron microscopy as previously described [16]. Antibodies used for immunogold electron microscopy were rabbit polyclonal against SOD1 [22], INA (ab7259, Abcam, Cambridge, MA) and GFAP (BioGenex, San Ramon, CA). Goat anti-rabbit IgG conjugated to 18 nm gold particles were from Jackson ImmunoResearch Laboratories (West Grove, PA). Thin sections were stained with uranyl acetate and lead citrate and examined with a Philips 208S electron microscope (FEI, Hillsboro, OR) fitted with a Gatan 831 Orius CCD digital camera (Gatan, Pleasanton, CA). Digital images were processed with Photoshop software.

Mutation analysis—*SOD1* gene analysis was performed in patients IV-1 and IV-3 (Fig. 1) using DNA extracted from whole blood samples using standard procedures. The 5 exons of *SOD1* were amplified by polymerase chain reaction (PCR) using Qiagen PCR products. PCR products were purified using the Agencourt Ampure method, and sequenced using Big dye terminator V3.1 products. Sequencing products were purified using the Agencourt CleanSEQ method and analyzed on an ABI 3730 DNA analyzer.

Results

Mutation analyses of SOD1

Sequencing analyses of the 5 exons of *SOD1* revealed a coding variant within exon 5 in patients IV-1 and IV-3 (Fig. 2). The heterozygous guanine-to-thymine change at position c. 424 (c.424 G>T) is predicted to result in a truncated protein at codon 141 (p.Gly141X).

Neuropathologic findings of proband (III-2)

The fixed brain weighed 1270 grams and had macroscopic atrophy of the frontal lobe, most marked in the posterior convexity with relative sparing of the medial temporal lobe. There was mild atrophy of the basal ganglia (Sup. Fig. 1). Histologic studies showed neuronal loss and gliosis in the frontal cortex, basal ganglia, thalamus, subthalamic nucleus, substantia nigra, and locus coeruleus. There was also degeneration in the corticospinal tract and lower motor neurons in brainstem and spinal cord. Scattered hypereosinophilic neurons in the neocortex and basal ganglia were consistent with agonal anoxic-ischemic encephalopathy. The pathologic findings were consistent with FTLN and ALS.

Microscopically, the lateral corticospinal tract had mild myelin pallor, but there was no myelin loss in the posterior or anterolateral funiculi. Neuronal loss and gliosis were moderate-to-severe in the anterior horns of the spinal cord. The neurons of Clarke's nucleus were also slightly decreased. Conglomerate inclusions were recognized as homogeneous and

faintly eosinophilic inclusions on H&E stained sections. Immunohistochemical studies demonstrated that conglomerate inclusions were strongly immunopositive for pNF and partially immunopositive for p62 and weakly or focally positive for SOD1. They were negative for Ubq and INA. p62- and SOD1-immunopositive skein-like inclusions were also seen in motor neurons (Fig. 3a, b), but no other neuronal cytoplasmic inclusions could be found in the spinal cord. In the hypoglossal nucleus, neuronal loss and gliosis were moderate-to-severe, respectively. Analysis of hypoglossal serial sections revealed that these inclusions were strongly immunostained by pNF, but showed less staining by SOD1 (Fig. 3c–e).

In the motor cortex, moderate gliosis and loss of pyramidal neurons, including Betz cells, was evident. The frontal cortex had moderate neuronal loss, as well as marked superficial spongiosis and gliosis. The temporal cortex and cingulate gyrus had only mild neuronal loss, spongiosis, and gliosis. Several different types of inclusions were visible on H&E and immunohistochemical stains. The most common type of neuronal inclusion was round or oval in structure, and ranged from eosinophilic to basophilic (Fig. 3f). Round neuronal inclusions were consistently SOD1-positive. Many were also positive for Ubq and p62. The eosinophilic round inclusions were often immunopositive for INA (Fig. 3g). Double-labeling for SOD1 and INA revealed co-localization of INA within SOD1 inclusions (Fig. 3h). Occasionally, small eosinophilic granular inclusions (similar to “cherry spots” [10] in neuronal intermediate filament disease [4]) were present within the round inclusions (Fig. 3i). These granular inclusions were immuno-negative for SOD1, pNF, INA, and cystatin-C. Neuronal inclusions with other morphologies, including crescent-shaped, annular rings, and tangle-like inclusions were identified using pNF, SOD1, or INA immunostaining (Fig. 3k–o). These inclusions could not be appreciated with H&E staining. Conglomerate inclusions were also present in the cortex; and they were immunopositive for INA as well as pNF. SOD1-positive Lewy body-like inclusions were present in frontal cortex (Fig. 3j). No significant pathology was revealed with immunohistochemistry for phospho-TDP-43 or FUS.

The dentate gyrus and pyramidal layer of the hippocampus showed no gliosis or cell loss, with the exception of focal gliosis in the subiculum. There were no neurofibrillary tangles or senile plaques in the hippocampus with thioflavin S fluorescent microscopy and tau immunohistochemistry. The inclusions in subiculum and entorhinal cortex were mainly crescent-shaped, annular rings, and tangle-like in morphology. The inclusions had similar immunohistochemical staining characteristics to those in the cortex. Ubiquitin- and SOD1-positive intracytoplasmic inclusions were observed in the hippocampal dentate fascia granular cells. The amygdala had moderate neuronal loss and severe gliosis with frequent SOD1-, pNF-, and INA-positive inclusions.

Some of the large neurons in the basal ganglia had conglomerate inclusions that were immunoreactive for SOD1 and pNF, but not for Ubq or p62. Some were immunopositive for INA. There were numerous faintly eosinophilic and round inclusions in the mammillary body. They showed intense immunoreactivity for SOD1, INA, and p62; however, Ubq immunoreactivity was faint or absent. A small proportion of these inclusions were

immunoreactive for pNF. Many SOD1-positive grain-like structures were detected in the nucleus accumbens.

Conglomerate inclusions were present throughout the hindbrain in varying densities. The substantia nigra and the locus coeruleus had moderate neuronal loss with extraneuronal neuromelanin and gliosis. The pontine nucleus was unremarkable, but there were scattered conglomerate inclusions or round inclusions. The inferior olive nucleus showed mild neuronal loss and gliosis, as well as numerous conglomerate inclusions. Cerebellar Purkinje cells and granule cells were well preserved. The dentate nucleus had mild neuronal loss and gliosis with a few conglomerate inclusions.

A few astrocytic hyaline inclusions were detected only in the neocortex, and they had an eosinophilic core with a surrounding pale halo (Fig. 4a, b). The immunoreactivity for SOD1 was mainly restricted to the halo of astrocytic hyaline inclusions (Fig. 4b). Additionally, many oligodendroglial cytoplasmic inclusions and threads were evident with SOD1 and p62 immunohistochemistry, confined mainly to the corticospinal tract (Fig. 4c, d).

The frequency of SOD1-, pNF-, and INA-positive inclusions is shown in Table 1. In almost all anatomical regions, more pathology was demonstrated with SOD1 immunohistochemistry than with pNF or INA immunohistochemistry. SOD1-positive inclusions were most numerous in the frontal cortex, cingulate cortex, amygdala, and mammillary body. pNF-positive inclusions were widely distributed, but they were most common in brainstem. INA-positive inclusions were also numerous and widely distributed, but they were most common in neocortex. The frequency of double-labeled inclusions varied within cortical, subcortical, and hindbrain regions (Table 1). Average SOD1/pNF double-labeled inclusions was highest in the hindbrain, most notably in the dorsal motor nucleus of vagus, the medullary tegmentum, and the cerebellar dentate nucleus, where all inclusions were double labeled. In contrast, the highest SOD1/pNF double-labeling in the forebrain was in temporal cortex (28–44%).

Characterization of inclusions with SOD1 exposed dimer interface (SEDI) antibody

The sections from frontal, temporal, and motor cortices, as well as the medulla, hippocampus, and spinal cord were investigated using SEDI antibody [24], which recognizes an epitope in the SOD1 domain that is lost due to truncation by the p.Gly141X mutation. The presence of SEDI immunoreactivity would imply that the inclusions recruited wild type SOD1 that formed pathologic dimers. This did not seem to be the case. Despite the presence SOD1 immunoreactive lesions in all of areas studied with SEDI immunohistochemistry, no inclusions were detected (Fig. 5a–c). In another SOD1 mutation carrier (an ALS patient with p.Ala4Val SOD1 mutation), numerous large hyaline conglomerate inclusions were labeled with the SEDI antibody (Fig. 5d). The inclusions in the daughter of the proband were also negative with SEDI immunohistochemistry.

Ultrastructural features of inclusions

The ultrastructural features of skein-like and Lewy body-like inclusion in neocortex and conglomerate inclusions in the spinal cord motor neurons were similar to those reported previously in ALS [13]. Previous reports have shown these structures mostly in the spinal

cord. It is noteworthy that, despite the small areas sampled for electron microscopy, they were more readily observed in the frontal cortex than spinal cord. Immunoelectron microscopy confirmed that some inclusions were composed exclusively of INA-positive filaments (Fig. 6), while other inclusions had co-localization of SOD1 and INA in neurons (Fig. 7) or SOD1 and GFAP in astrocytes (Fig. 8). The INA-only inclusions contained loose filaments, about 10 nm in diameter. SOD1-labeled filaments had slightly larger diameter filaments (10–15 nm) that formed densely packed or loose aggregates, which were also strongly labeled for Ubq (data not show). Neurons bearing inclusions with mixed immunoreactivity showed either intermingling of INA- and SOD1-immunolabeled filaments or separate filamentous aggregates, findings that are analogous to those seen in neuronal intermediate filament inclusion disease [32].

Neuropathologic findings in the daughter of the proband (IV-3)

Macroscopically, there were no abnormalities in the brain, which weighed 1420 grams, well within normal limits. Coronal sections of the supratentorial compartment showed no ventricular enlargement. Transverse sections of brainstem had visible neuromelanin pigmentation in the substantia nigra and locus ceruleus appropriate for age. In contrast to the brain, the spinal cord had macroscopic atrophy, with poor demarcation between gray and white matter on transverse sections.

Microscopically, the neocortex was unremarkable except for motor cortex, which had mild neuronal loss (Betz cells, in particular) and gliosis. The substantia nigra had mild focal neuronal loss with extraneuronal neuromelanin and focal gliosis. In the medulla oblongata, the hypoglossal nucleus had severe neuronal loss and gliosis. Myelin pallor and axonal degeneration were observed in the mid-third of the cerebral peduncle (i.e. corticospinal tract) and the medullary pyramids. Neuronal loss and gliosis were severe in the motor neurons of the spinal cord. The neurons of Clarke's nucleus were preserved. The corticospinal tracts showed moderate to severe degeneration, but the posterior columns and anterolateral funiculi were preserved.

Eosinophilic hyaline inclusions were detected in motor neurons of the spinal cord, hypoglossal nucleus, and neurons in the subthalamic nucleus with H&E stains. No Bunina bodies were detected. SOD1 immunohistochemistry showed neuronal cytoplasmic inclusions, glial cytoplasmic inclusions, and dystrophic neurites in the brain and spinal cord, with a predilection for the hypothalamus and central gray matter as well as the extrapyramidal system (Table 1). The neuronal cytoplasmic inclusions were immunopositive for p62, but mostly negative for Ubq. A few of the inclusions were immunoreactive for INA. The pathology was consistent with ALS, with limited extramotor pathology in a unique distribution favoring central gray and extrapyramidal structures.

Discussion

In the present study, we report striking clinical and neuropathological heterogeneity in a family with FTD and ALS clinical phenotypes due to a nonsense mutation in exon 5 (p.Gly141X) of *SOD1*. The proband showed progressive cognitive and behavioral decline and later progressive motor degeneration. Cognitive impairment included compulsive

behaviors, dietary change, and language problems with limited lower motor neuron signs and no upper motor neuron signs. These features were consistent with behavioral variant FTD [28] with subtle evidence of concomitant motor neuron disease initially, and more significant motor impairment later, with notable gait difficulties. The family history is compatible with autosomal dominant ALS with the proband (III-2) as an obligate gene carrier, given confirmation of the *SOD1* mutation in her affected son and daughter. The SOD phenotype in this family is characterized by relatively early onset, mixed upper and lower or predominantly lower motor neuron signs, and rapid disease progression. Variable clinical phenotype associated with the p.Gly141X mutation in our family is noteworthy, particularly with respect to FTD, which overshadowed motor features, as an early clinical presentation in the proband, while other affected family members had rapidly progressive motor impairment without overt cognitive impairment. In large series of *SOD1*-related ALS [<http://alsod.iop.kcl.ac.uk>], there is wide phenotypic variability between mutations, but also between individuals with the same mutation. Features of FTD are rarely mentioned in *SOD1*-related ALS. A patient with fulminant FTD and ALS has been reported in a patient with an p.Ile113Thr *SOD1* mutation, but postmortem studies were not available to determine the nature of the neuropathologic substrate of the neurobehavioral syndrome [14]. In contrast, neuropathologic studies of our proband revealed not only degeneration of corticospinal tract and loss of upper and lower motor neurons compatible with ALS, but also neuronal loss and gliosis in frontal lobe and widespread pleomorphic neuronal and glial inclusions. The predilection of neocortical degeneration to the frontal lobe correlates well with her cognitive and behavioral deficits.

In neuropathologic studies of ALS with p.Ile113Thr *SOD1* mutation [11,15,26], neuronal conglomerate inclusions, similar to those in our cases, are a consistent finding. Using the same *SOD1* antibodies, researchers have previously described strong immunostaining of hyaline inclusions in the anterior horn cells of familial (p.Ala4Val) and in sporadic ALS [33]. We found conglomerate inclusions in various regions, including the anterior horn motor neurons, brainstem motor neurons, dorsal motor nucleus of the vagus, pontine nucleus, inferior olivary nucleus, dentate nucleus, substantia nigra, locus coeruleus, thalamus, basal ganglia, and neocortex. Furthermore, we demonstrated that INA was co-localized with *SOD1* within many conglomerate inclusions in the neocortex. In contrast, conglomerate inclusions in the spinal cord were negative for INA, even though INA is expressed in spinal cord [7], albeit less in the adult than during development.

There is a wide range of disease duration among *SOD1*-related ALS, which can be divided into rapidly progressive forms with survival <2 years, classic forms with survival 2–5 years, and relatively benign forms with survival >5 years [23]. The most common phenotype in our family was rapidly progressive ALS. Neuropathologically, *SOD1*-related ALS often has mild corticospinal tract involvement, in contrast to severe degeneration of lower motor neurons [12]. The daughter of the proband had this pattern of ALS. The pattern of motor neuron degeneration was similar in the proband, but neurodegeneration extended to non-motor frontal association cortices. The proband had extensive *SOD1* immunoreactive inclusions in neocortex and the limbic system, while her daughter had more restricted pathology affecting hypothalamus and central gray as well as extrapyramidal neurons, but almost no pathology in the neocortex. This difference suggests that phenotypic heterogeneity

is not determined exclusively by the particular *SOD1* mutation, but also undefined environmental and genetic modifiers.

Both the proband and her daughter had pleomorphic neuronal and glial inclusions, including Lewy body like inclusions and conglomerate inclusions in motor and extramotor neurons. These inclusions were immunoreactive for SOD1, but not for tau, TDP-43, or FUS. Some of the inclusions were also immunopositive for neuronal intermediate filaments (i.e. INA and pNF). Immunoreactivity to pNF and INA was first reported in spheroids of sporadic ALS in 1992 and has subsequently been characterized to a greater extent [6,35]. While pNF pathology has been described in *SOD1* mutation carriers [9], to our knowledge, there has not been a report of INA immunoreactive neuronal inclusions in patients with *SOD1* mutation. However, mice expressing transgenes to multiple different SOD1 mutations develop the ALS-characteristic spheroids composed of both pNF and INA [31]. It has been reported that Lewy body like inclusions or conglomerate inclusions can be observed in extramotor motor neurons in some *SOD1* mutation cases, but these tend to be exceptional patients in terms of long disease duration due to aggressive ventilatory support [29,30,11]. In contrast, the proband and her daughter had disease of relatively short duration, implying that disease duration is unlikely to be a driving factor for their extensive extramotor pathology and more likely related to specific nature of the truncating SOD1 mutation itself. One might speculate that the truncation produces a protein fragment with enhanced aggregation properties, analogous to truncations in other protein aggregation disorders, including TDP-43 [38].

SOD1 is a 32-kDa homodimeric protein located mainly in the cytoplasm. Each monomer contains eight-stranded beta barrels as well as binding sites for one copper iron and one zinc ion per monomer. The SEDI sequence is located over residues 145–151, and is buried within the natively folded SOD1 protein [24]. When the SOD1 dimer is disrupted or becomes monomeric, the sequence is subsequently exposed to the exterior and can be detected with the stereospecific SEDI antibody. The p.Gly141X mutation is predicted to produce an SOD1 protein truncated after amino acid residue 141, eliminating the SEDI sequence from the mutated protein. We confirmed the lack of SEDI immunoreactivity in SOD1-positive inclusions in various brain regions and the spinal cord. Although SEDI antibody does not necessarily label all misfolded forms of SOD1, the absence of SEDI immunoreactivity suggests that the truncated protein is not able to induce templated misfolding and recruitment of the wild-type SOD1 protein into the inclusions. This phenomenon has been noted in other neurodegenerative protein aggregation disorders, such as tauopathies [20].

Implications of SOD1 p.Gly141X to frontotemporal degeneration

The neuropathologic substrate of FTD is heterogeneous, with the common feature being relatively selective neocortical degeneration of the frontal and temporal lobes [3], as well as more heterogeneous subcortical involvement. The current classification of FTLN is based on the major constituent protein that accumulates within inclusions: tau (FTLD-tau), TDP-43 (FTLD-TDP), and FUS (FTLD-FUS) [17]. The patient reported here indicates that SOD1, while rare, can be a neuropathologic substrate for FTLN. While this case raises the possibility of a discrete nosologic entity, namely FTLN-SOD, further neuropathologic examples are needed to define this molecular class. A striking feature of the neocortical

pathology in the proband was presence of INA-positive neuronal inclusions that initially suggested the possibility of neuronal intermediate filament inclusion disease (NIFID) [4,10]; however, no FUS immunoreactivity was detected. Most, but not all cases of NIFID are associated with FUS [21], which has led to the suggestion that accumulation of intermediate filaments (*e.g.*, INA and NF) is a secondary disease process. The findings in this case would tend to support this hypothesis.

INA is classified as a type IV intermediate filament and is expressed by most, if not all, neurons [37]. In the adult brain, INA is expressed at relatively low levels, and there is selective anatomical expression with greater immunoreactivity being seen in the cerebellar granule cells, the source of thin-caliber parallel fibers, and in the neuronal cell bodies and processes of cortical layer II neurons [7]. Although many INA-positive inclusions were seen in superficial layers of cortex, the wide distribution of INA-positive inclusions throughout subcortical regions and brainstem does not coincide with the populations of neurons that generally have higher levels of expression of INA. It has been reported that INA immunoreactivity is increased after blockage of axonal transport and that overexpression of INA can lead to neuronal dysfunction and neurodegeneration [5,18]. Our data suggests that the aggregation of INA might be a secondary response to the disruption of the axonal transport system.

Final comments

Similar to other mutations in *SOD1*, the p.Gly141X mutation was associated with intrafamilial phenotypic heterogeneity including FTD-ALS in the proband and rapidly progressive ALS in other family members. Autopsies on two family members showed motor and extramotor SOD1 immunoreactive inclusions, with variable involvement of neuronal intermediate filament proteins in a subset of the inclusions. The extent and distribution of extramotor pathology in the two individuals was strikingly different despite similar age of onset and disease duration. These findings indicate that other genetic and environmental factors influence the phenotype of p.Gly141X SOD1 mutation. The unique characteristics of the truncated SOD1 protein may have contributed to the fulminant FTLD phenotype. Future studies investigating this hypothesis in cellular or animal models will be needed to support this hypothesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We are grateful to the patient and her family members who agreed to brain donation, without which this study would have been impossible. We would like to thank Mayo Clinic Professor Emeritus Dr. Haruo Okazaki for his assistance with the neuropathologic diagnosis of the proband (patient III-2). We also acknowledge expert technical assistance of Linda Rousseau and Virginia Phillips for histology, and Michael DeTure, Beth Marten, and Deanne Gibson for brain banking. This research was funded by NIH grants P50 NS072187, P50 AG16574, R01 NS065782, R01 AG026251, The ALS Therapy Alliance, Mayo Foundation (ALS Center donor funds) and the ALS Association. We thank Dr. David Borchelt, University of Florida Center for Translational Research in Neurodegenerative Disease, for providing antibody to SOD1.

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Fig. 1.

Pedigree of the family. An arrowhead indicates the proband. To protect confidentiality, some individuals are not shown, and sex is portrayed using a diamond. Black infill in the upper right quarter of a symbol in solid indicates the diagnosis of ALS and in the left lower quarter the diagnosis of frontotemporal dementia.

Fig. 2.
DNA sequence traces of SOD1 exon 5, showing the coding c.424 G>T mutation in patient IV-1 (top) and wild-type SOD1 sequence in an unaffected individual (bottom).

Fig. 3.

(a & b) Serial sections of the anterior horn cells of the spinal cord with skein like inclusions are immunoreactive for SOD1 and p62. (c & d) Serial sections of the hypoglossal nucleus with conglomerate inclusions are slightly eosinophilic and show weak immunoreactivity for SOD1. (e) In the same neuron, double immunostaining for SOD1 (brown) and pNF (blue) shows co-localization within inclusions. (f & g) Serial sections of the cortex reveal that most round inclusions are slightly eosinophilic and are the most common type of cortical inclusion. (g) The eosinophilic round inclusions are often immunopositive for alpha-internexin (INA). (h) Double immunostaining for SOD1 and INA shows co-localization within inclusions. (i) Occasionally, small hyper-eosinophilic granular inclusions are present within the round inclusions. (j) Lewy-body like hyaline inclusions are also seen. (k–o) Other morphological types of neuronal cytoplasmic inclusions are tangle like inclusions. (k, i, n) These inclusions are immunopositive for pNF, SOD1, and INA by single or (m, o) double immunostaining. (p) Cortical conglomerate inclusions can be detected on a hematoxylin and eosin stain (H&E). (q & r) Double immunostaining for SOD1 (brown) and pNF (blue) or for SOD1 (brown) and INA (blue) reveals that SOD1 is co-localized with pNF and INA in conglomerate inclusions of cortex. (a & b) Sections from the spinal cord, (c–e) hypoglossal nucleus, and (f–r) frontal cortex are shown. (a) Scale bar, 10 μ m

Fig. 4.

(a) Astrocytic hyaline inclusions are seen in frontal cortex. (b) Double immunostaining with SOD1 (brown) and GFAP (blue) demonstrates a SOD1-positive inclusion (arrow) is found in a GFAP-positive astrocyte. (c & d) Glial cytoplasmic inclusion immunoreactive for SOD1 and p62 can be seen in the internal capsule. (a) Scale bar, 10 μ m

Fig. 5.
(a & b) Serial sections of the hippocampus reveal that abundant SOD1 immunoreactive inclusions are negative with SEDI antibody. **(c)** Spinal cord section is also negative with the SEDI antibody. **(d)** In contrast, spinal cord section from another SOD1 mutation carrier (A4V) is labeled with SEDI antibody. **(a–d)** Scale bar, 50 μ m

Fig. 6. (a) Immunogold labeling of serial sections of the same cortical neuron showing INA-positive, but (b) SOD1-negative filaments in loose, random orientation. N, nucleus. * indicates enlarged areas. Arrows point to gold particles. Bars, 1 μ m; 0.2 μ m.

Fig. 7.
(a & b) Immunogold localization of INA and SOD1 to filaments in serial sections of the same cortical neuronal inclusion. Note the compact nature of the inclusion. * indicates enlarged areas. Arrows point to gold particles. Bars, 1 μ m; 0.2 μ m.

Fig. 8.
(a–c) Immunogold localization of INA, SOD1, and GFAP to filaments in serial sections of the same cortical astrocytic inclusion. SOD1 is localized throughout the main inclusion; INA is more peripheral; GFAP is mainly at the tightly packed glial fibrils surrounding the inclusion. * indicates enlarged areas. Arrows point to gold particles. Bars, 1 μ m; 0.2 μ m.

Table 1

Semiquantitative grading of immunoreactive pathology and the frequency of SOD1 co-labeled neuronal inclusions in different anatomical regions.

Anatomical region	SOD1		pNF	INA	SOD1/pNF	SOD1/INA
Patient:	IV-3	III-2				
Motor cortex	++	+	++	++	23%	39%
Frontal cortex	-	+++	++	+++	6%	33%
Cingulate cortex	-	+++	+	+++	9%	26%
Entorhinal cortex	-	+++	+	++	28%	41%
Temporal cortex	-	+	+	+	44%	55%
Hippocampus-dentate	-	++	-	-	0%	0%
Hippocampus-pyramidal	-	+	+	+	17%	38%
Subiculum	-	+++	++	+	39%	32%
Amygdala	+	+++	++	+++	4%	10%
Nucleus accumbens	+	+++	-	+	0%	2%
Substantia innominata	+++	++	-	-	0%	0%
Putamen	+	++	+	+	39%	53%
Globus pallidus	++	+++	+	+	30%	46%
Thalamus	+	++	+	+	56%	17%
Subthalamic nucleus	++	na	na	na	na	na
Hypothalamus	+++	+++	+	++	18%	19%
Substantia nigra	++	+	-	+	0%	43%
Pontine nuclei	-	++	++	++	83%	56%
Locus coeruleus	+	+++	++	+	55%	29%
Inferior olivary nucleus	-	++	++	++	83%	58%
Oculomotor nucleus	-	++	-	+	0%	40%
Dorsal motor nucleus of vagus	+	++	++	+	100%	80%
Medullary tegmentum	+++	++	++	+	100%	0%
Hypoglossal nucleus	+++	+++	+++	-	75%	0%
Cerebellar gray matter	-	-	-	-	0%	0%
Cerebellar dentate	++	+	+	-	100%	0%
Spinal cord anterior horn	+++	+++	+++	+	25%	0%

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Grading: –, none; +, sparse; ++, moderate; +++, frequent; +++++, very severe.

Abbreviations: na, not available. INA = internexin-alpha, pNF = phosphorylated neurofilament, SOD1 = superoxide dismutase 1