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Coexistence of Huntington's disease and amyotrophic lateral sclerosis: a clinicopathologic study

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Abstract

We report a retrospective case series of four patients with genetically confirmed Huntington's disease (HD) and sporadic amyotrophic lateral sclerosis (ALS), examining the brain and spinal cord in two cases. Neuropathological assessment included a polyglutamine recruitment method to detect sites of active polyglutamine aggregation, and biochemical and immunohistochemical assessment of TDP-43 pathology. The clinical sequence of HD and ALS varied, with the onset of ALS occurring after the mid-fifties in all cases. Neuropathologic features of HD and ALS coexisted in both cases examined pathologically: neuronal loss and gliosis in the neostriatum and upper and lower motor neurons, with Bunina bodies and ubiquitin-immunoreactive skein-like inclusions in remaining lower motor neurons. One case showed relatively early HD pathology while the other was advanced. Expanded polyglutamine-immunoreactive inclusions and TDP-43-immunoreactive inclusions were widespread in many regions of the CNS, including the motor cortex and spinal anterior horn. Although these two different inclusions coexist in a small number of neurons, the two proteins did not co-localize within inclusions. The regional distribution of TDP-43-immunoreactive inclusions in the cerebral cortex was somewhat similar to that of expanded polyglutamine-immunoreactive inclusions. In the one case examined by TDP-43 immunoblotting, similar TDP-43 isoforms were observed as in ALS. Our findings suggest the possibility that a rare subset of older HD patients is prone to develop features of ALS with an atypical TDP-43 distribution that resembles that of aggregated mutant huntingtin. Age-dependent

neuronal dysfunction induced by mutant polyglutamine protein expression may contribute to later-life development of TDP-43 associated motor neuron disease in a small subset of patients with HD.

Keywords

Huntington's disease; Amyotrophic lateral sclerosis; polyglutamine; TDP-43

Introduction

Huntington's disease (HD) is an inherited neurodegenerative disorder caused by an expanded CAG repeat that encodes an abnormally long polyglutamine stretch in the disease protein huntingtin [39]. Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder that occurs sporadically or as a familial disorder. Both diseases are associated with abnormal protein accumulation: HD represents one of at least nine polyglutaminopathies [6], whereas ALS is characterized by the accumulation of the 43 kDa TAR-DNA-binding protein (TDP-43) [21]. Mutations in TDP-43 have been identified in sporadic and familial ALS, and thus ALS is now recognized as one of several TDP-43 proteinopathies [2, 13, 37, 44].

Despite the fact that HD and ALS are relatively rare and clinically distinct, more than ten patients with HD have been reported to show ALS-like clinical features [14, 25, 27, 31, 32]; the coincidental occurrence of both diseases, however, is predicted to be only 2–6 cases per billion [9, 15]. Notwithstanding the recent exclusion of the HD disease gene as a risk factor for ALS [17, 29], these cases raise the question: does mutant huntingtin have a deleterious effect on motor neurons that predisposes HD mutation carriers to motor neuron degeneration? To address this question, we first must define the pathologic features of such cases. Although the pathology of a single case with concurrent familial ALS and HD has been reported [31], there is no pathologic report of sporadic ALS occurring in genetically confirmed HD.

Here we report three individuals with confirmed HD and one HD mutation carrier who developed ALS. We performed pathologic and biochemical studies in two cases to assess whether dual pathological findings of ALS and HD are indeed present, and whether they show characteristic features that suggest effects of mutant huntingtin on ALS pathology.

Patients and Methods

Patients

Patients were referred to the Departments of Neurology at the University of Iowa (Case 1), National Hospital of Saigata (Case 2), University of Michigan (Case 3), and University of Alberta (Case 4). Written informed consent for autopsy including use of tissue for research purposes was obtained from next of kin. The present study was approved by the institutional review boards of the Iowa University School of Medicine, IA and Niigata University School of Medicine and Saigata National Hospital, Niigata, Japan.

Neuropathologic techniques and immunohistochemistry

Neuropathologic examination was performed in cases 1 and 2. Formalin-fixed, paraffin-embedded sections were prepared from brain and spinal cord and stained with hematoxylin and eosin and Klüver-Barrera (K.B.) staining methods. Sections were also immunostained using polyclonal antibodies against phosphoserines 409 and 410 of TDP-43 (pS409/410-2; Cosmo Bio Co., Ltd., Tokyo, Japan; 1:4,000), TDP-43 (10782-2-AP; Protein Tec Group Inc., Chicago, IL; 1:4,000) and ubiquitin (Dako, Glostrup, Denmark; 1:1,000), and

monoclonal antibodies against expanded polyglutamine (1C2) (Chemicon, Temecula, CA; 1:10,000), huntingtin (EM48) (MAB5374; Chemicon, Temecula, CA; 1:100) and p62 (3/p62 LCK LIGAND; BD biosciences, San Jose, CA; 1:500). Four- μ m-thick sections were pre-treated with formic acid for 1C2 immunostaining, or in a microwave oven in a 10-mM citrate buffer (pH 6.0) for other antibodies. Immunolabeling was detected using the avidin–biotin-peroxidase complex method with Vectastain ABC kit (Vector, Burlingame, CA), and visualized with diaminobenzidine/H₂O₂ solution. Counterstaining was carried out with hematoxylin. Double-label immunofluorescence was performed on sections of spinal cord (case 1 and 2), motor cortex (case 1) and frontal cortex (case 2), using antibodies against pS409/410 of TDP-43 (1:2,000) or TDP-43 (1:2,000) together with 1C2 (1:2,000) or EM48 (1:50). Secondary antibodies were Alexa Fluor 488 goat anti-rabbit IgG and Alexa Fluor 568 or 555 goat anti-mouse IgG (Molecular Probes; 1:1,000). Nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI). Sections were analyzed by confocal laser-scanning microscope (LSM510-V4.0 ; Carl Zeiss Co., Ltd.). To detect active sites of polyglutamine aggregation, 40- μ m-thick sections were cut from spinal cord and motor cortex of case 1 and control case with a vibrating microtome and immunostained with the polyglutamine peptide recruitment method as described previously [10, 24]. Briefly, sections were immunostained using a biotinylated polyglutamine peptide containing approximately 30 glutamine residues. Immunolabeling was detected using the avidin–biotin-peroxidase complex method with Vectastain ABC kit and visualized with diaminobenzidine/H₂O₂ solution.

Fractionation of frozen tissues and TDP-43 immunoblotting

Protein lysates were generated as described [8, 38] from frontal, motor and temporal cortices of case 2, one ALS, two HD and two control brains. Briefly, frozen tissues were homogenized in buffer A [10 mM Tris-HCl (pH 7.5), 1 mM ethylene glycol-bis[β -amino-ethylether]-tetra-acetic acid, 1 mM dithiothreitol, 10% sucrose] and centrifuged at 25,000 $\times$ g for 30 min at 4°C. The resulting pellets were extracted in buffer A containing 1% Triton X-100 and centrifuged at 180,000 $\times$ g for 30 min at 4°C. These pellets were subsequently homogenized in buffer A containing 1% sarcosyl, incubated for 1 h, and centrifuged at 180,000 $\times$ g for 30 min at 22°C. The sarcosyl-insoluble pellets were solubilized in 8 M urea buffer. After centrifugation at 25,000 $\times$ g for 30 min at 22°C, the supernatants were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and analyzed by immunoblotting with anti-TDP-43 polyclonal antibody (1:2,000) and anti-pS409/410 of TDP-43 monoclonal antibody (Cosmo Bio Co., Ltd; 1:2,000). Detailed case information is in Online Resource 1.

Gene analysis of TARDBP, progranulin and C9ORF72 genes

High-molecular-weight genomic DNA was extracted. We amplified all the exons of TARDBP (NM_007375.3) and progranulin (NM_002087.2) genes with use of a series of primers, followed by sequence reaction. For screening of GGGGCC repeat expansion in C9ORF72 (NM_018325.2), fluorescence fragment length analysis of PCR fragments was performed on an ABI 3130xl genetic analyzer (Applied Biosystems, Foster City, CA, USA) and Peak Scanner software v1.0 (Applied Biosystems) according to previously described methods [3].

Results

Case 1

In her mid-fifties the patient, who had a family history suggestive of HD, developed chorea in all limbs, mild irregularities in speech, emotional lability and difficulty concentrating. Neurological examination at age 58 revealed subtle dysarthria, choreiform movements of the face and limbs, slowed and irregular alternating hand movements, and lower limb

hyperreflexia without spasticity. No muscle weakness or atrophy was noted. Mild caudate atrophy was seen on magnetic resonance imaging (MRI), and HD genetic testing revealed a pathogenic CAG repeat of 46 in the *HTT* gene, establishing the diagnosis of HD. One year later she developed right hand weakness. A brachial plexus lesion was suspected, prompting surgery to remove a cervical rib, but her weakness progressed and soon involved all limbs and neck. At age 60, she was noted to have distal atrophy of upper limbs, fasciculations in all limbs, diffuse hyperreflexia and extensor plantar responses. Electromyography (EMG) revealed extensive denervation in both upper extremities. Except for having HD, she fulfilled a diagnosis of probable ALS according to El Escorial criteria [1], and she was placed on riluzole. She died of respiratory weakness at age 61.

Case 2

The patient developed chorea and cognitive changes in her mid-thirties. Her father, sister and numerous other relatives had shown similar symptoms. She was referred to the neurology clinic at age 44 where an examination revealed choreiform movements of the face, neck and limbs without weakness or muscle atrophy. Caudate atrophy was seen on the brain computed tomography consistent with HD, and the diagnosis was confirmed with a CAG repeat of 47. Her cognitive impairment progressed and at age 48 she was bed-ridden. Examination revealed bilateral extensor plantar responses, rigidity and chorea. At age 57, extensor plantar responses were still evident but she was hypotonic with no involuntary or volitional limb movements. MRI showed brain atrophy with areas of signal hyperintensity in the cerebral white matter on T2-weighted images. She developed progressive respiratory decline and died at age 58.

Case 3

The patient was referred for neurological evaluation of HD at age 62. Numerous relatives, including three siblings, her mother and maternal grandmother, were affected with HD but there was no family history of ALS. She had developed chorea in her mid-fifties, which slowly worsened and was later accompanied by incoordination as well as cognitive and personality changes. Examination revealed choreiform movements in all limbs, trunk and face with hand clumsiness, postural instability and lower limb hyperreflexia. No speech or swallowing impairment, weakness or muscle atrophy was noted at this point. Genetic analysis confirmed the diagnosis of HD with a CAG repeat of 42. At age 66 she developed dysarthria and dysphagia, and within several months was anarthric and unable to swallow. EMG revealed extensive denervation in upper and lower extremities and thoracic paraspinal muscles. Further laboratory tests were negative including anti-GM1 antibodies. Except for having HD, she fulfilled a diagnosis of clinically probable, laboratory-supported ALS according to El Escorial criteria [1].

Case 4

The patient was referred at age 48 for neurological evaluation following predictive testing for HD that revealed an expanded CAG repeat of 39. His father had developed symptoms of HD in his mid-fifties and died at age 66. An examination revealed no neurological deficits at the time of predictive testing. At age 56 he developed dysarthria, which progressed over the following year such that his speech became nearly incomprehensible and was accompanied by drooling and nasal regurgitation. An examination revealed pseudobulbar affect with emotional incontinence, hyperactive jaw jerk and tongue atrophy. Mild weakness was evident in all limbs along with fasciculations in the arms, spasticity, diffuse hyperreflexia and extensor plantar responses. MRI revealed signal changes in the corticospinal pathways bilaterally from motor cortex to brainstem. EMG revealed acute and chronic denervation in multiple muscle groups including paraspinal muscles. A diagnosis of definite ALS was made according to El Escorial criteria [1]. At age 58, he required a keyboard to facilitate

communication. His gait became wide-based with a steppage and spastic quality, requiring a walker.

Neuropathologic findings, case 1

The brain weighed 1,346 g before fixation and had selective atrophy of the precentral gyrus. Coronal brain sections revealed atrophy of the neostriatum without gross atrophy of the cerebral cortices (Fig. 1a). Marked myelin pallor was seen in the corticospinal tracts in the brainstem and spinal cord (Fig. 1b). Neuronal loss and gliosis were evident in the spinal anterior horn, most marked in the cervical cord (Fig. 1c). Bunina bodies were occasionally found in the remaining anterior horn cells (Fig. 1d) and in neurons of the hypoglossal nucleus. The motor cortex showed severe loss of pyramidal neurons, including Betz cells, and gliosis was evident (Fig. 1e). The remaining cerebral cortex was well preserved (Fig. 1f). Moderate neuronal loss and gliosis were evident in the caudate nucleus (Fig. 1g) and dorsal putamen (Vonsattel grading; grade 2) [42].

Immunohistochemistry revealed 1C2-immunoreactive (ir) neuronal intranuclear inclusions (NIIs) and neuronal cytoplasmic inclusions (NCIs) as well as TDP-43-ir NCIs in diverse areas of the CNS (Table 1). NIIs and NCIs were observed with anti-huntingtin (EM48) and anti-polyglutamine (1C2) antibodies in spinal anterior horn cells (Fig. 1h, i) and neurons of the entire cerebral cortex including motor cortex (Fig. 1j, k), predominantly in layer V-VI with occasional round or ellipsoid inclusions in the neuropil (Fig. 1l). Polyglutamine recruitment staining in the spinal cord and motor cortex revealed aggregation foci distributed in the cytoplasm and proximal dendrites of anterior horn cells (Fig. 1m) and pyramidal neurons (Fig. 1n), whereas no aggregation was observed in a normal control (Fig. 1o).

Using TDP-43 antibody, only a few NCIs were observed in the temporal cortex and none in dentate granular cells despite widespread NCIs in the motor and parietal cortex. TDP-43-ir NCIs varied in shape, adopting filamentous skein-like (Fig. 1p, r), dash-like (Fig. 1q), circumferential (Fig. 1s), and round (Fig. 1t) patterns. TDP-43-ir cytoplasmic inclusions were also seen in glial cells in motor cortex, adjacent white matter, and corticospinal tract (Fig. 1u). In the spinal cord and motor cortex, anti-p62 and anti-ubiquitin antibodies labeled NCIs (Fig. 1C-v, w) and GCIs (Fig. 1C-x, y) that were similarly shaped but less frequent than the TDP-43-ir inclusions.

Neuropathologic findings, case 2

The brain weighed 708 g before fixation, showing severe global cerebral atrophy. Coronal brain sections revealed severe cortical and white matter atrophy with marked myelin pallor in the entire cerebrum and marked atrophy of the neostriatum (Fig. 2a). Myelin pallor was also seen in the corticospinal tract in the brainstem and spinal cord. Neuronal loss and gliosis were evident in the hypoglossal nucleus and spinal anterior horn, especially in cervical and thoracic segments (Fig. 2b). Bunina bodies (Fig. 2c) and round inclusions (Fig. 2d) were occasionally found in the remaining anterior horn cells and in neurons of brainstem motor nuclei. Neuronal loss and gliosis were severe throughout the cerebral cortex especially in layers V and VI (Fig. 2e). Myelinated fibers were sparse and gliosis was evident in the adjacent cerebral white matter (Fig. 2f). Neuronal loss and gliosis were severe in the caudate nucleus (Fig. 2g), and moderate in the putamen and globus pallidus (Vonsattel grading; grade 4) [42].

Immunohistochemistry revealed 1C2-ir NIIs and NCIs as well as TDP-43-ir NCIs in diverse areas of the CNS (Table 1). Numerous NIIs and neuropil inclusions were observed with 1C2 and EM48 antibodies throughout the cerebral cortex, accentuated in frontal and motor cortex

and more frequent in layers II and III (Fig. 2h, i). Scattered glial intranuclear inclusions were also present in the cerebral white matter (Fig. 2j). 1C2-ir NIIs and NCIs were also seen in spinal anterior horn cells (Fig. 2k, l).

With TDP-43 antibody, many NCIs were detected in the cerebral cortex, particularly in the frontal cortex, mainly in layer II with numerous dot-like short neurites and scattered long neurites (Fig. 2m, n). Numerous NCIs were also seen in other regions including the dentate gyrus (Fig. 2o). Many round or skein-like TDP-43-ir NCIs were detected in the remaining lower motor neurons of the spinal anterior horn (Fig. 2p) and the trigeminal motor (Fig. 2q), hypoglossal and ambiguus nuclei. No TDP-43-ir NIIs were detected in the CNS. Based on the classification system for FTLTDP, the TDP-43 cortical pathology of case 2 could be considered type A [18, 19, 33] (i.e. numerous short dystrophic neurites and NCIs concentrated primarily in neocortical layer II), although the presence of long neurites and dot-like neuropil staining is atypical.

Double-label immunofluorescence, cases 1 and 2

TDP-43 did not co-localize with expanded polyglutamine or mutant huntingtin in NCIs, NIIs, GCIs or neuropil inclusions (Fig. 3a–l). TDP-43-ir NCIs and 1C2- or EM48-ir NIIs or NCIs coexisted in a small number of neurons, but they located separately in individual neurons of the spinal cord and motor and frontal cortex (Fig. 3j–l). In these areas, approximately half of the TDP-43-ir NCIs and several GCIs were immunoreactive for p62. Less frequently, NCIs and GCIs were also immunoreactive for ubiquitin (not shown).

TDP-43 immunoblot analysis, case 2

In lysates examined from case 2, ALS, HD and control cases, anti-TDP-43 antibody showed a ~43 kDa band consistent with non-phosphorylated TDP-43, with an additional ~45-kDa band being observed only in case 2 and the ALS case. Anti-phosphorylated TDP-43 antibody detected a ~45-kDa band and ~26-kDa fragment, as well as an indistinct ladder-like smear in case 2 and the ALS case, but not in the HD or control cases (Fig. 4). In addition, the pattern of ~26-kDa bands corresponding to TDP-43 fragments was similar in case 2 and the ALS case, consistent with what has been described in ALS [8] (data not shown).

Gene analysis of TARDBP, progranulin and C9ORF72, case 2

Case 2 showed no mutation in the TARDBP or progranulin gene, and no expansion of the C9ORF72 hexanucleotide repeat.

Discussion

Here we reported clinical and neuropathologic features in a series of patients with genetically confirmed symptomatic HD (three cases) or possessing the HD mutation (one case) who also manifested clinical and/or pathologic features of ALS. Neuropathological and biochemical evaluation of the spinal cord and brain from two patients confirmed the coexistence of pathological features of HD and ALS. Our findings suggest that the HD pathogenic process can involve motor neurons while also indicating that, in these two HD cases, TDP-43-associated processes contribute to motor neuron degeneration, as in typical ALS.

Pathologic findings of HD and ALS coexisted in both examined cases. Both showed obvious upper and lower motor neuron loss with Bunina bodies and ubiquitin-ir skein-like inclusions in remaining lower motor neurons, which are characteristic of ALS [15, 28], as well as neuronal loss and gliosis in the neostriatum together with polyglutamine inclusion-

containing neurons, which are hallmarks of HD [9, 11, 43]. A single reported autopsy case with HD and familial ALS likewise had these characteristics of ALS and HD, but also showed degeneration of posterior columns and dorsal spinocerebellar tracts, which was not present in our cases [31].

As shown in Table 1, TDP-43-ir NCIs were found in many regions beyond the affected lower motor nuclei and motor cortex. This pattern represents a broader distribution than seen in classical sporadic ALS, more reminiscent of a “type 2 ALS” pattern of TDP-43 pathology [22]. The pattern, however, also displayed features atypical of type 2 ALS. For example, TDP-43-ir NCIs were absent from the dentate gyrus in case 1 and Ammon’s horn in both cases. In these regions, TDP-43-ir NCIs were seen in over 85 % of patients with type 2 ALS [22]. In case 2, only a small number of TDP-43-ir NCIs were seen in the amygdaloid nucleus, where numerous NCIs appear in typical type 2 ALS [7]. In both cases, 1C2-ir neurons were also few in these regions. Unlike type 2 ALS [22] our cases lacked prominent TDP-43 pathology in temporal cortex. Instead, TDP-43-ir NCIs were abundant in the parietal cortex in case 1 and frontal cortex in case 2, where more frequent 1C2-ir neurons were also observed than in the temporal cortex. Thus, the distribution of TDP-43 in our HD/ALS cases resembles a classical ALS distribution (type 1 [22]) with a superimposed, atypical cortical distribution that may somewhat mirror the distribution of 1C2-ir pathology. However, the frequencies of the two different inclusions were not always consistent: abundant 1C2-ir neurons but rare or a few TDP-43-ir NCIs in some regions and vice-versa. Therefore, we could not draw a firm conclusion from the present data that the TDP-43 pathology would be directly affected by mutant huntingtin.

In neurons of the motor cortex and lower motor neurons, we also detected expanded polyglutamine inclusions and aggregation foci which suggest ongoing huntingtin aggregation [10, 24]. Although 1C2-ir NIIs in anterior horn cells have been described in HD [11, 43] we confirmed their presence and, using the polyglutamine recruitment technique, found evidence for active polyglutamine aggregation in motor neurons. Thus, we conclude that TDP-43 dependent and huntingtin-dependent pathogenic processes likely occur simultaneously in motor neurons in such cases.

We found neurons coexisting TDP-43-ir NCIs and 1C2/EM48-ir NIIs or NCIs, but they localized separately in all individual neurons. Thus, we failed to observe co-localization of mutant huntingtin protein and TDP-43 in NCIs, NIIs or neuropil inclusions in two cases in this study. We also immunohistochemically examined the frontal cortex and neostriatum of other 11 patients with HD who did not show clinicopathological features associated with ALS (10 male, 1 female, age; 57.82 [27–82] years, CAG repeat; [43–47] in available 7 patients) and the spinal cord of 7 of the patients with an anti-pTDP-43 antibody. We observed only a small number of TDP-43-ir neuropil inclusions similar to mutant huntingtin neuropil inclusions in the frontal deeper cortical layers of 8 patients and much fewer in the neostriatum but no inclusion in the spinal cord. In contrast, others reported frequent colocalization of these two proteins in neuropil inclusions and dystrophic neurites in the cortex and neostriatum of HD cases [34]. This discrepancy may reflect differences in the cases examined or in methods of tissue preparation.

Motor neuron degeneration is recognized in other polyglutamine diseases: for example, spinobulbar muscular atrophy is a form of motor neuron degeneration [36], and three patients with spinocerebellar ataxia type 2 (SCA2) and one with SCA6 have been described with concomitant ALS [5, 12, 20, 23]. Once TDP-43 was discovered to be a key component in ALS, TDP-43-ir NCIs were detected in neurons from various areas of SCA2 disease brain [4, 40] and in lower motor neurons and axons in patients with Machado-Joseph disease (also known as SCA3) [35, 38]. Interestingly, in the reported cases, co-localization of TDP-43 and

mutant polyglutamine proteins has never been observed, and coexistence of these proteins in the same neurons is not frequent [38, 40]. Thus, the features appear to be similar to those of our two cases. In addition, expanded CAG repeats in the SCA2 disease gene are associated with increased risk for ALS [4, 30, 41]. Although the pathophysiological mechanism remains unclear, intermediate expansion in the SCA2 disease protein ATXN2 has been suggested to perturb neuronal proteostasis thereby favoring TDP-43 mislocalization [4, 30]. Since two studies examining CAG length in the HD disease gene in sporadic ALS and controls have concluded that, unlike the SCA2 gene, the HD disease gene is not a risk factor for ALS [17, 29], we can not exclude the possibility that our patients suffer from both HD and ALS by chance alone. It is certain that abundant TDP-43-ir NCIs in our two cases is not a common feature of HD cases. Yet in a rare subset of HD patients, mutant huntingtin may predispose motor neurons to TDP-43 associated pathomechanisms and modify TDP-43 pathologic features.

Five patients with genetically confirmed HD who developed ALS-like features have previously been reported [14, 25, 27, 31, 32]. A review of the clinical features in these patients and our patients (Table 2) suggests several common features. First, although the sequence and interval between the onset of HD and ALS varied, the CAG repeat expansions in the HD gene were relatively short in all patients, typically resulting in mid- to later-life symptom onset. Consistent with this, HD onset occurred after at least the mid-thirties in all cases, and one patient had not yet developed overt symptoms of HD when ALS symptoms began at age 56. Second, the onset of ALS occurred after the mid-fifties in all but one patient. These observations suggest that age-dependent changes may promote the deleterious effect of mutant huntingtin on motor neurons. Similarly, in Machado-Joseph disease, affected individuals who are older and have the smallest disease-causing expansions are most prone to develop motor neuron degeneration [26].

In summary, we suggest the possibility that a rare subset of older HD patients is prone to develop features of ALS with an atypical TDP-43 distribution that resemble to that of aggregated mutant huntingtin. Age-dependent neuronal dysfunction induced by mutant polyglutamine protein expression rarely may contribute to TDP-43 associated pathomechanisms, and a small proportion of the patients would develop phenotypic appearance of motor neuron diseases in their later-life.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Brooks BR, Miller RG, Swash M, Munsat TL. World Federation of Neurology Research Group on Motor Neuron Diseases. El Escorial revisited: revised criteria for the diagnosis of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Other Motor Neuron Disord. 2000; 1:293–299. [PubMed: 11464847]

2. Da Cruz S, Cleveland DW. Understanding the role of TDP-43 and FUS/TLS in ALS and beyond. *Curr Opin Neurobiol.* 2011; 21:904–919. [PubMed: 21813273]
3. DeJesus-Hernandez M, Mackenzie IR, Boeve BF, et al. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron.* 2011; 72:245–256. [PubMed: 21944778]
4. Elden AC, Kim HJ, Hart MP, et al. Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. *Nature.* 2010; 466:1069–1075. [PubMed: 20740007]
5. Furtado S, Payami H, Lockhart PJ, et al. Profile of families with parkinsonism-predominant spinocerebellar ataxia type 2 (SCA2). *Mov Disord.* 2004; 19:622–629. [PubMed: 15197699]
6. Gatchel JR, Zoghbi HY. Diseases of Unstable Repeat Expansion: Mechanisms and Common Principles. *Nat Rev Genet.* 2005; 6:743–755. [PubMed: 16205714]
7. Geser F, Martinez-Lage M, Robinson J, et al. Clinical and pathological continuum of multisystem TDP-43 proteinopathies. *Arch Neurol.* 2009; 66:180–189. [PubMed: 19204154]
8. Hasegawa M, Arai T, Nonaka T, et al. Phosphorylated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Ann Neurol.* 2008; 64:60–70. [PubMed: 18546284]
9. Hedreen, JC.; Roos, RAC. Huntington's disease. In: Dickson, DW., editor. *Neurodegeneration: The Molecular Pathology of Dementia and Movement Disorders.* ISN Neuropath Press; Basel: 2003. p. 229–241.
10. Heng MY, Duong DK, Albin RL, et al. Early autophagic response in a novel knock-in model of Huntington disease. *Hum Mol Genet.* 2010; 19:3702–3720. [PubMed: 20616151]
11. Herndon ES, Hladik CL, Shang P, Burns DK, Raisanen J, White CL 3rd. Neuroanatomic profile of polyglutamine immunoreactivity in Huntington disease brains. *J Neuropathol Exp Neurol.* 2009; 68:250–261. [PubMed: 19225411]
12. Infante J, Berciano J, Volpini V, et al. Spinocerebellar ataxia type 2 with Levodopa-responsive parkinsonism culminating in motor neuron disease. *Mov Disord.* 2004; 19:848–852. [PubMed: 15254952]
13. Kabashi E, Valdmanis PN, Dion P, et al. TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. *Nat Genet.* 2008; 40:572–574. [PubMed: 18372902]
14. Kanai K, Kuwabara S, Sawai S, et al. Genetically confirmed Huntington's disease masquerading as motor neuron disease. *Mov Disord.* 2008; 23:748–751. [PubMed: 18186118]
15. Kato, S.; Shaw, P.; Wood-Allum, C.; Leigh, PN. Amyotrophic lateral sclerosis. In: Dickson, DW., editor. *Neurodegeneration: The Molecular Pathology of Dementia and Movement Disorders.* ISN Neuropath Press; Basel: 2003. p. 350–368.
16. Kiernan MC, Vucic S, Cheah BC, et al. Amyotrophic lateral sclerosis. *Lancet.* 2011; 377:942–955. [PubMed: 21296405]
17. Lee T, Li YR, Chesi A, et al. Evaluating the prevalence of polyglutamine repeat expansions in amyotrophic lateral sclerosis. *Neurology.* 2011; 76:2062–2065. [PubMed: 21562248]
18. Mackenzie IR, Baborie A, Pickering-Brown S, et al. Heterogeneity of ubiquitin pathology in frontotemporal lobar degeneration: classification and relation to clinical phenotype. *Acta Neuropathol.* 2006; 112:539–549. [PubMed: 17021754]
19. Mackenzie IR, Neumann M, Baborie A, et al. A harmonized classification system for FTLD-TDP pathology. *Acta Neuropathol.* 2011; 122:111–113. [PubMed: 21644037]
20. Nanetti L, Fancellu R, Tomasello C, Gellera C, Pareyson D, Mariotti C. Rare association of motor neuron disease and spinocerebellar ataxia type 2 (SCA2): a new case and review of the literature. *J Neurol.* 2009; 256:1926–1928. [PubMed: 19644730]
21. Neumann M, Sampathu DM, Kwong LK, et al. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science.* 2006; 314:130–133. [PubMed: 17023659]
22. Nishihira Y, Tan CF, Onodera O, et al. Sporadic amyotrophic lateral sclerosis: two pathological patterns shown by analysis of distribution of TDP-43-immunoreactive neuronal and glial cytoplasmic inclusions. *Acta Neuropathol.* 2008; 116:169–182. [PubMed: 18481073]
23. Ohara S, Iwahashi T, Oide T, et al. Spinocerebellar ataxia type 6 with motor neuron loss: a follow-up autopsy report. *J Neurol.* 2002; 249:633–635. [PubMed: 12021959]

24. Osmand AP, Berthelie V, Wetzel R. Imaging polyglutamine deposits in brain tissue. *Methods Enzymol.* 2006; 412:106–122. [PubMed: 17046655]
25. Papageorgiou SG, Antelli A, Bonakis A, et al. Association of genetically proven Huntington's disease and sporadic amyotrophic lateral sclerosis in a 72-year-old woman. *J Neurol.* 2006; 253:1649–1650. [PubMed: 17219039]
26. Paulson HL. Dominantly inherited ataxias: lessons learned from Machado-Joseph disease/spinocerebellar ataxia type 3. *Semin Neurol.* 2007; 27:133–142. [PubMed: 17390258]
27. Phukan J, Ali E, Pender NP, et al. Huntington's disease presenting as amyotrophic lateral sclerosis. *Amyotroph Lateral Scler.* 2010; 11:405–407. [PubMed: 19530012]
28. Piao YS, Wakabayashi K, Kakita A, et al. Neuropathology with clinical correlations of sporadic amyotrophic lateral sclerosis: 102 autopsy cases examined between 1962 and 2000. *Brain Pathol.* 2003; 13:10–22. [PubMed: 12580541]
29. Ramos EM, Keagle P, Gillis T, et al. Prevalence of Huntington's disease gene CAG repeat alleles in sporadic amyotrophic lateral sclerosis patients. *Amyotroph Lateral Scler.* 2012 [Published online Mar 13, 2012].
30. Ross OA, Rutherford NJ, Baker M, et al. Ataxin-2 repeat-length variation and neurodegeneration. *Hum Mol Genet.* 2011; 20:3207–3212. [PubMed: 21610160]
31. Rubio A, Steinberg K, Figlewicz DA, et al. Coexistence of Huntington's disease and familial amyotrophic lateral sclerosis: case presentation. *Acta Neuropathol.* 1996; 92:421–427. [PubMed: 8891076]
32. Sadeghian H, O'Suilleabhain PE, Battiste J, Elliott JL, Trivedi JR. Huntington chorea presenting with motor neuron disease. *Arch Neurol.* 2011; 68:650–652. [PubMed: 21555641]
33. Sampathu DM, Neumann M, Kwong LK, et al. Pathological heterogeneity of frontotemporal lobar degeneration with ubiquitin-positive inclusions delineated by ubiquitin immunohistochemistry and novel monoclonal antibodies. *Am J Pathol.* 2006; 169:1343–1352. [PubMed: 17003490]
34. Schwab C, Arai T, Hasegawa M, Yu S, McGeer PL. Colocalization of transactivation-responsive DNA-binding protein 43 and huntingtin in inclusions of Huntington disease. *J Neuropathol Exp Neurol.* 2008; 67:1159–1165. [PubMed: 19018245]
35. Seidel K, den Dunnen WF, Schultz C, et al. Axonal inclusions in spinocerebellar ataxia type 3. *Acta Neuropathol.* 2010; 120:449–460. [PubMed: 20635090]
36. Sobue, G.; Adachi, H.; Katsuno, M. Spinal and bulbar muscular atrophy. In: Dickson, DW., editor. *Neurodegeneration: The Molecular Pathology of Dementia and Movement Disorders.* ISN Neuropath Press; Basel: 2003. p. 275-279.
37. Sreedharan J, Blair IP, Tripathi VB, et al. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science.* 2008; 319:1668–1672. [PubMed: 18309045]
38. Tan CF, Yamada M, Toyoshima Y, et al. Selective occurrence of TDP-43-immunoreactive inclusions in the lower motor neurons in Machado-Joseph disease. *Acta Neuropathol.* 2009; 118:553–560. [PubMed: 19526244]
39. The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell.* 1993; 72:971–983. [PubMed: 8458085]
40. Toyoshima Y, Tanaka H, Shimohata M, et al. Spinocerebellar ataxia type 2 (SCA2) is associated with TDP-43 pathology. *Acta Neuropathol.* 2011; 122:375–378. [PubMed: 21830155]
41. Van Damme P, Veldink JH, van Blitterswijk M, et al. Expanded ATXN2 CAG repeat size in ALS identifies genetic overlap between ALS and SCA2. *Neurology.* 2011; 76:2066–2072. [PubMed: 21562247]
42. Vonsattel JP, Myers RH, Stevens TJ, Ferrante RJ, Bird ED, Richardson EP Jr. Neuropathological classification of Huntington's disease. *J Neuropathol Exp Neurol.* 1985; 44:559–577. [PubMed: 2932539]
43. Yamada M, Tsuji S, Takahashi H. Pathology of CAG repeat diseases. *Neuropathology.* 2000; 20:319–325. [PubMed: 11211058]
44. Yokoseki A, Shiga A, Tan CF, et al. TDP-43 mutation in familial amyotrophic lateral sclerosis. *Ann Neurol.* 2008; 63:538–542. [PubMed: 18438952]

Figure 1. Neuropathologic findings, case 1

Coronal section at the level of the lenticular nucleus (a). Transverse sections of the ventral medulla and spinal cord at the C5 level. Degeneration of corticospinal tracts (arrowheads) (b). Low-power view of the spinal anterior horn at the C6 level (c). Bunina body (arrowhead) in a cervical anterior horn cell (d). Betz cell (arrowhead) and a Betz cell-sized hole containing lipofuscin-laden macrophages (arrow) indicating that Betz cells had presumably disappeared (e). The deeper layers of middle frontal cortex (f). Caudate nucleus (g). Klüver-Barrera staining (b). Hematoxylin and eosin (c–g). Bar = 2 mm (b), 100 μ m (c, f, g), 10 μ m (d), 50 μ m (e).

Expanded polyglutamine (1C2)- and huntingtin (EM48)-immunoreactive (ir) inclusions (h–l). Neuronal intranuclear (h) and cytoplasmic (i) inclusions in cervical anterior horn cells. Granular inclusions in Betz cell (j). An intranuclear inclusion in a pyramidal neuron in

motor cortex (k). Neuropil inclusions in layer V of motor cortex (l). Polyglutamine recruitment methods (m–o). Aggregation foci in an anterior horn cell (m) and a pyramidal neuron in motor cortex (n). Anterior horn cell from a control case (o). Bar = 10 μ m (h–k, m–o), 20 μ m (l).

TDP-43-ir neuronal and glial inclusions (p–u). Skein-like inclusions in a cervical anterior horn cell (p). Dash-like inclusions in Betz cell (q) and a pyramidal neuron (r, arrowhead). Glial cytoplasmic inclusions (GCIs) in motor cortex (r, asterisks). Circumferential inclusion in a putaminal neuron (s). Round inclusion in substantia nigra (t). GCIs in the white matter adjacent to motor cortex (u). p62-ir or ubiquitin-ir skein-like inclusions in spinal anterior horn cells (v, w) and GCIs (x, y). Bar = 10 μ m (p–y).

Figure 2. Neuropathologic findings, case 2

Coronal section at the level of the lenticular nucleus (a). Low-power view of the spinal anterior horn at the C7 level (b). Bunina bodies (c, arrowheads) and a round inclusion (d, arrowhead) in cervical anterior horn cells. Low-power view of motor cortex with atrophic Betz cells (arrowhead) and a Betz cell-sized hole containing lipofuscin-laden macrophages (arrow)(e). High-power view of the white matter adjacent to motor cortex (f). Low-power view of caudate head (g). Klüver-Barrera staining (a). Hematoxylin and eosin (b–g). Bar = 5 mm (a), 100 μ m (b), 10 μ m (c, d), 50 μ m (e, g), 20 μ m (f).

Expanded polyglutamine (1C2)- or huntingtin (EM48)-immunoreactive (ir) inclusions (h–l). Intranuclear and neuropil inclusions in layers II of motor cortex (h). Intranuclear inclusion in a pyramidal neuron in motor cortex, layer V (i). Intranuclear inclusion in a glial cell in the white matter adjacent to motor cortex (j). Intranuclear (k) and cytoplasmic granular (l) inclusions in lumbar anterior horn cells. Bar = 10 μ m (h–l).

TDP-43-ir inclusions (m–q). Neuronal cytoplasmic inclusions (NCIs) with fine dot-like neuropil staining and a long neurite (arrowhead) in frontal cortex, layer II (m). Nuclei of neurons with NCIs were unstained with phosphorylation-independent TDP-43 antibody. Frontal cortex layer II (n). Multiple NCIs in the granular cells of the dentate gyrus (o). Round inclusion in a cervical anterior horn cell (p). Skein-like inclusions in trigeminal motor nucleus (q). Bar = 20 μ m (m, o), 10 μ m (n, p, q).

Figure 3. Association of TDP-43 and expanded polyglutamine or huntingtin

Co-immunofluorescence staining for TDP-43 (green; p409/410 TDP-43 [a–f, j–l], TDP-43 [g–i]) and expanded polyglutamine (red; 1C2 [a–c, g–i]) or mutant huntingtin (red; EM48 [d–f]). TDP-43-positive, 1C2 or EM48-negative neuronal cytoplasmic inclusions (arrow heads) and TDP-43-negative, 1C2 or EM48-positive neuronal intranuclear inclusions (arrow) and neuropil inclusions (double arrows) in layer V of motor cortex (a–c) and layer II of frontal cortex (d–f). TDP-43-positive, 1C2-negative skein-like inclusions (arrow heads) in the lumbar anterior horn cell (g–i). TDP-43-positive, 1C2-negative wisp-like cytoplasmic inclusions (arrow heads) and a TDP-43-negative, 1C2-positive neuronal intranuclear inclusion (arrow) in the same cervical anterior horn cell (j–l). The diffuse and irregular structure seen in all green, red and blue fluorescent channels represents autofluorescence (asterisk; i, l). Nuclei were stained with DAPI; blue. Bar = 10 μ m (a, d, g, j). a–c; case 1, d–l; case 2.

Figure 4. TDP-43 immunoblot analysis, case 2

Immunoblot analysis of the sarkosyl-insoluble, urea-soluble fraction from case 2 and one ALS, two HD and two control brains (frontal, motor and temporal cortices). Blots were probed with antibodies to TDP-43 (upper panels) and phosphorylated TDP-43 (pS409/410) (lower panels). Arrows and an arrowhead indicate ~45 and 43-kDa full-length isoforms of TDP-43, respectively, and an asterisk indicates a fragment of TDP-43.

Table 1

Distribution and severity of neuronal loss and pTDP-43 and 1C2 lesions in Case 1 and 2

	Case 1					Case 2				
	Neuronal loss	pTDP-43 NCIs	1C2 NI staining*	1C2 NC staining**	Neuronal loss	pTDP-43 NCIs	1C2 NI staining*	1C2 NC staining**		
<i>Cerebral cortex</i> (II–III/IV–VI)										
Frontal cortex	–/–	±/±	+/++	+/++	+/+/+	+/+/+	+/+/+	+/+/+	+/+/+	+/+/+
Motor cortex white matter [#]	+/+/+	+/+/+/+/+	+/+	+/+	+/+/+/+	+/+/+	+/+/+/+	+/+/+	+/+/+	+/+/+
Parietal cortex	–/–	+/+/+	+/+/+	+/+	+/+/+	+/±	+/+/+	+/+	+/+	+/+
Temporal cortex	–/–	–/±	+/+	+/+	+/+/+	+/+/+	+/+/+	+/+/+	+/+/+	+/+/+
Occipital cortex	–/–	–/–	+/+	+/+	+/+/+	+/±	+/+/+	+/+/+	+/+/+	+/+
<i>Subcortical area</i>										
Ammon	–	–	±	–	–	–	+	±	±	±
Dentate gyrus	–	–	±	–	–	++	±	–	–	–
Amygdaloid nucleus	na	na	na	na	–	+	+	+	+	+
Putamen	++	+	+/+	++	+/+	++	++	++	++	++
Caudate nucleus	++	–	++	+	+/+	±	++	+	+	+
Globus pallidus	+	–	±	±	+	±	±	±	±	±
Internal capsule [#]		+				+				
Thalamic nuclei	–	+/+/+	+	+	–	++	++	++	++	++
<i>Brain stem</i>										
Periaqueductal gray	–	±	±	–	–	–	+	+	+	+
Oculomotor nucleus	–	–	–	–	–	±	–	–	–	–
Red nucleus	–	±	–	–	na	na	na	na	na	na
Substantia nigra	–	±	±	+	+	+	±	±	±	±
Pyramidal tract of midbrain [#]		++				±				
Locus ceruleus	–	–	–	±	–	–	±	±	±	±
Trigeminal motor nucleus	–	±	–	–	–	+/+/+	–	–	–	–
Pontine nuclei	–	+	+	++	–	+	++	++	++	++
Pyramidal tract of pons [#]		+				±				
Hypoglossal nucleus	–	+/+/+/+	–	–	+	+/+/+	–	–	–	–

Case 1							Case 2		
	Neuronal loss	pTDP-43 NCIs	1C2 NI staining*	1C2 NC staining**	Neuronal loss	pTDP-43 NCIs	1C2 NI staining*	1C2 NC staining*	1C2 NC staining**
Ambiguous nucleus	na	na	na	na	±	+++	—	—	—
Dorsal nucleus of vagus	—	—	—	—	—	—	—	±	±
Reticular formation	—	±	±	±	—	±	±	+	+
Inferior olivary nucleus	—	+	±	++	—	±	—	—	—
Pyramis [#]		++				—			
<i>Cerebellum</i>									
Cortex	—	—	—	—	+	—	±	+	+
Dentate nucleus	—	—	±	++	+	—	++	+++	+++
Cerebellar white matter [#]		+				±			
<i>Spinal cord</i>									
Anterior horn	+++++	+++++	±	±	++	+++++	±	+++	+++
Corticospinal tract [#]		±—+				±			
Intermediate lateral nucleus	—	±	±	—	—	—	—	—	—
Clarke's column	—	—	±	±	+	—	±	±	±
Posterior horn	—	±	±	±	—	—	+	+	+

Percentage of neurons expressing pTDP-43 or expanded polyglutamine (1C2) immunoreactivity; —, 0%; ±, ≤3 cells/section; +, <1%; ++, 1–10%; +++, 10–30%; ++++, 30–60%; +++++, >60%

* Neuronal intranuclear,

** Neuronal cytoplasmic diffuse and aggregated staining

[#] pTDP-43 immunoreactive glial pathology severity; —, none; ±, ≤3 cells/section; +, rare; ++, occasional; +++, many

NCIs = neuronal cytoplasmic inclusions; na = not available

Table 2

Case reports of genetically confirmed HD and ALS

Case or [Reference]	Age, Gender	Family history	CAG repeats	Illness sequence (Age at onset)	Initial symptoms of HD	ALS type [16]
[Rubio 1996] [31]	81 y.o. ^a , M	ALS (mother) Neuropsychiatric disorder (mat. uncle)	45	HD (57) only. Pathologically diagnosed with ALS.	Personality change	NA
[Papageorgiou 2006] [25]	73 y.o. ^b , F	Involuntary movements (brother)	40	HD (72) followed by ALS 18 months later.	Involuntary head and limb movements	Limb-onset
[Kanat 2008] [14]	48 y.o. ^b , M	Involuntary movements (father, siblings)	46	ALS (41) followed by HD one year later.	Involuntary limb and trunk movements	Limb-onset
[Phukan 2009][27]	56 y.o. ^b , M	HD (pat. first cousin)	≤40	ALS (56) followed by HD seven months later.	Involuntary face and limb movements	Limb-onset
[Sadeghian 2011][32]	69 y.o. ^b , M	None	40	HD (67) followed by ALS (69).	Memory problems	Limb -onset
Case 1	61 y.o. ^a , F	Chorea (pat. grandmother)	46	HD (early 50's) followed by ALS (59).	Involuntary facial and limb movements	Limb-onset
Case 2	58 y.o. ^a , F	HD (father, pat. grandfather and relatives, sister)	47	HD (mid-30's). Pathologically diagnosed with ALS.	Involuntary face, neck and limb movements, Cognitive impairment	NA
Case 3	67 y.o. ^b , F	HD (numerous mat. relatives)	42	HD (50's) followed by ALS (66).	Involuntary limb and trunk movements	Bulbar-onset
Case 4	58 y.o. ^b , M	HD (father)	39	ALS (56) only. Confirmed HD mutation carrier.	NA	Bulbar-onset

^aAge at death.^bSupposed age at when the case was reported. HD = Huntington's disease; ALS = Amyotrophic lateral sclerosis; NA = non-applicable