Acta Neuropathol (2006) 111: 300–311
DOI 10.1007/s00401-006-0046-z

ORIGINAL PAPER

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Hereditary diffuse leukoencephalopathy with spheroids: clinical, pathologic and genetic studies of a new kindred

Received: 9 November 2005 / Revised: 9 January 2006 / Accepted: 10 January 2006 / Published online: 8 March 2006

Abstract Hereditary diffuse leukoencephalopathy with spheroids (HDLS) is a rare autosomal dominant disorder characterized by cerebral white matter degeneration with axonal spheroids leading to progressive cognitive and motor dysfunction. We report clinical and pathological features, as well as molecular genetic analysis, of a family with HDLS. A pedigree consisting of 27 persons in 5 generations contained 6 affected individuals. Dementia and depression were common; two individuals presented with a syndrome resembling corticobasal degeneration (CBD). Postmortem neuropathologic evaluation of three affected individuals revealed enlargement of the lateral ventricles and marked attenuation of cerebral white matter, but preservation of white matter in brainstem and cerebellum, except for the corticospinal tract. Histopathologic studies showed a loss of myelinated fibers, lipid-laden macrophages and bizarre astrocytes, as well as abundant axonal spheroids that were immunoreactive for phosphorylated neurofilament protein and amyloid precursor protein (APP), but not αB-crystallin and variably with ubiquitin. By electron microscopy, axonal spheroids contained aggregates of intermediate filaments or of organelles that were predominantly vesicular and lamellar. The cerebral cortex had focal neuronal degeneration with αB-crystallin-immunoreactive ballooned neurons. In summary, the present report describes a previously unreported kindred with HDLS with individuals presenting as CBD. Immunohistochemistry for APP and αB-crystallin demonstrates distinctive neurodegeneration in cerebral axons and perikarya.

Keywords Hereditary diffuse leukoencephalopathy with spheroids (HDLS) · Parkinsonism · Corticobasal degeneration · Neurofilament · Amyloid precursor protein

Abbreviations HDLS: Hereditary diffuse leukoencephalopathy with spheroids · eIF2B: Eukaryotic translation initiation factor 2B · MMSE: Mini-Mental Status Examination · H&E: Hematoxylin and eosin · LFB–PAS: Luxol fast blue–periodic acid Schiff · APP: Amyloid precursor protein · GFAP: Glial fibrillary acidic protein · PCR: Polymerase chain reaction · MAPT: Microtubule associated protein tau · FTDP-17: Frontotemporal dementia and parkinsonism linked to chromosome · CBD: Corticobasal degeneration · MRI: Magnetic resonance imaging · EEG: Electroencephalogram · CT: Computed tomogram

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Introduction

Hereditary diffuse leukoencephalopathy with spheroids (HDLS) was first described by Axelsson et al. [2] in a

large Swedish kindred. The disorder is inherited in an autosomal dominant pattern. In that family, the disease had a variable neurological presentation, with the onset of symptoms occurring in the fourth decade of life, but was invariably progressive. Psychiatric symptoms were usually the first and predominant neurological manifestation of HDLS. Other frequently observed features included gait instability, incoordination and seizures [2]. Neuropathological studies of four affected individuals in this kindred showed widespread leukoencephalopathy characterized by the presence of neuroaxonal spheroids and by the loss of myelin sheaths and axons.

At present, only six families with HDLS have been well described clinically and pathologically [2, 8, 14, 22, 25, 29]. Variability of neurological symptoms is noted within each kindred. Sporadic cases of diffuse leukoencephalopathy with spheroids have also been reported; these individuals also have a heterogeneous clinical presentation [3, 6, 28]. The available evidence suggests clinical and genetic heterogeneity in diffuse leukoencephalopathy with spheroids.

Several other hereditary neurodegenerative disorders affecting white matter [25], including vanishing white matter disease (VWM) [19], share clinical or pathological similarities to HDLS. While the genetic basis for HDLS remains unknown, VWM has been discovered to be mutations in one of the five subunits of eukaryotic translation initiation factor eIF2B [24]. The present study is a description of clinical, pathological and genetic studies in a new kindred with HDLS, and the first report of screening for mutations in eIF2B in HDLS.

Materials and methods

We reviewed available genealogical and clinical records. Neuropathologic studies and molecular genetic analyses were carried out on three affected individuals. The ethical committees and/or institutional review boards of all involved institutions approved this research.

Clinical and genealogical studies

Historical material, family records and interviews with family members were evaluated to construct the pedigree. Medical and historical records were collected and reviewed. For the proband, who was followed at the Mayo Clinic, examination instruments included standardized medical history and neurological examination forms, Unified Parkinson's Disease Rating Scale, Hoehn and Yahr staging, Schwab and England activities of daily living scale and Mini-Mental Status Examination (MMSE). The clinical data of other family members or deceased patients were obtained from available clinical records.

Pathological studies

Neuropathologic studies were performed on the proband (III-6), as well as her uncle (II-1) and her aunt (II-4). Individual II-4 died in 1996; II-1 died in 2003; and III-6 died in 2004. Reexamination of stored tissue of II-4 while the proband was still alive lead to the initial diagnosis, which was confirmed on subsequent review of II-1 and the eventual autopsy of the proband.

In addition to standard histological stains (i.e., hematoxylin and eosin—H&E), special stains for myelin [Woelcke–Heidenhain or Luxol fast blue–periodic acid Schiff (LFB–PAS)] and axons (Bodian or Bielschowsky) were used to characterize white matter pathology in cerebrum, basal ganglia, diencephalon, brainstem and cerebellum. Spinal cord was not available on any of the cases. Alzheimer type pathology was also assessed with thioflavin-S fluorescence microscopy, in addition to silver stains and immunohistochemistry for tau and Aβ.

Immunohistochemical studies were also performed to characterize the lesions and tissue reaction. Axonal spheroids were studied with antibodies to phosphorylated neurofilament protein (SMI-31; 1:20,000; Sternberger Monoclonals, Lutherville, MD, USA), phospho-tau (CP13; 1:500; Peter Davies, Albert Einstein College of Medicine, Bronx, NY, USA), α-synuclein (polyclonal antibody; 1:3,000 [7]), αB-crystallin (polyclonal antibody; 1:2,500; Novacastra, Newcastle upon Tyne, UK), amyloid precursor protein (APP) (22C11; 1:250; Chemicon, Temucula, CA, USA) and ubiquitin (Ubi-1; 1:40,000; Gerry Shaw, University of Florida, Gainesville, FL, USA). Reactive glial changes were assessed with glial fibrillary acidic protein (GFAP; 1:5,000; Biogenex, San Ramon, CA, USA) and HLA-DR (LN3; 1:10; ICN Biomedicals, Aurora, OH, USA). Aβ was detected with immunohistochemistry (6F/3D; 1:100; DAKO, Santa Barbara, CA, USA). The deparaffinized and rehydrated sections were pretreated with 95% formic acid for 30 min for α-synuclein and Aβ, but not for other antibodies. Subsequently, all sections were steamed in distilled water at high power setting for 30 min prior to immunohistochemistry with a DAKO Autostainer using 3,3′-diaminobenzidine as the chromogen. After immunostaining, the sections were counterstained briefly with hematoxylin.

Electron microscopy

Tissue samples from fixed brain were dissected from cerebral white matter and placed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 overnight. After washing in cacodylate buffer, the tissue was post-fixed in 1% OsO_4, en bloc, stained with 1% uranyl acetate—50% ethanol, dehydrated in ethanols and propylene oxide, infiltrated and embedded in Epon 812 (Polysciences, Warrington, PA, USA). Ultrathin sections were counterstained with uranyl acetate and lead

citrate, and examined with a Philips 208S electron microscope.

Molecular genetic analysis

DNA samples were extracted from brain tissue and screened for mutations in the subunits of eIF2B. All exons for the five subunits of the eIF2B were amplified by polymerase chain reaction (PCR) using primers designed to flanking intronic sequence. Each PCR used 25 ng of genomic DNA in a 25 μl reaction mixture containing 20 pmol of each primer, 0.2 mM dNTPs, 1 unit of Taq polymerase (Qiagen; Valencia, CA, USA), 1× PCR buffer and 10% Q-solution (Qiagen; Valencia, CA, USA). Amplifications were performed oil-free in Hybaid Touchdown thermal cyclers (Hybaid; Middlesex, UK). Conditions were 35 cycles of 94°C for 30 s, 60–50°C touchdown annealing for 30 s, and 72°C for 45 s with a final extension of 72°C for 10 min. PCR products were purified using Multiscreen PCR (Millipore; Billerica, MA, USA) and then sequenced using Big Dye chemistry following manufacturers protocols on an ABI 3100 Genetic Analyzer (Applied Biosystems; Foster City, CA, USA).

In addition to screening for mutations in eIF2B, exons 1, 7 and 9–13 of the *MAPT* gene on chromosome 17q21 were sequenced and analyzed using methods similar to those reported previously in studies of frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) [9].

Results

Genealogical studies

The pedigree of this family is shown in Fig. 1. The pedigree contains 27 family members spanning 5 generations with 6 affected individuals (5 of them deceased). The mode of inheritance is consistent with an autosomal dominant pattern. The male-to-female ratio of affected individuals was 1:1.

Clinical studies

Clinical features of the affected individuals are summarized in Table 1. The mean age at onset of symptoms is 54.2 years (range 42–78 years). The mean disease duration is 16.2 years (range 2–34 years). The initial clinical symptoms were parkinsonism in two (II-4 and III-6), depression in two (I-1 and II-3) and memory loss in two (II-1 and II-2). During the clinical course, individuals developed additional symptoms: parkinsonism (II-1 and II-3), depression (II-1, II-2 and II-4), dementia (II-3, II-4 and III-6), apraxia (II-4 and III-6), seizures (II-1 and II-4) and pyramidal tract signs (III-6). The parkinsonian signs included rigidity, bradykinesia, postural instability and asymmetry and were poorly responsive to levodopa/carbidopa therapy. Resting tremor was either absent, mild and transient, or severe and persistent. The clinical presentation of individuals II-4 and III-6 resembled corticobasal degeneration (CBD).

Cases report and pathological findings

III-6 (proband): This 44-year-old woman had a 2-year history of atypical parkinsonism. She noted reduced dexterity in her right hand at age 42. Shortly thereafter that she became unsteady while walking, with a tendency to veer to the left. She then noted slurred speech and stumbling over words. Her symptoms progressed rapidly over the next 2 years. She also developed significant emotional lability, fluctuation of her mood and mild short-term memory deficits. Before the onset of her illness, she was healthy and had no history of any medical or neurological disorder. Neurological examination performed at age 44 revealed severe parkinsonism (Hoehn and Yahr stage V) characterized by left-sided rigidity and bradykinesia, but no tremor. There was moderately severe cerebellar ataxia in all four limbs (worse in the left extremities) and an ataxic dysarthria. Ideomotor apraxia of the left limb was evident and suggested the possibility of CBD. The deep tendon reflexes in the lower extremities were very brisk, with ankle clonus on the left side. Bilateral extensor plantar responses were elicited. Her gait was spastic. Her Schwab and England activities of daily living scale was 10. Her MMSE was 22/30. Routine blood and urine tests were normal. T2-weighted and fluid-attenuated inversion recovery magnetic resonance imaging (MRI) of the brain showed bilateral abnormal high intensity areas in deep cerebral white matter and asymmetrical cerebral atrophy that was worse on the right (Fig. 2). The [^{18}F]-2-fluorodeoxyglucose positron emission tomography showed generalized decreased uptake throughout both cerebral hemispheres, but most marked in the parietal lobes, especially on the right. The electroencephalogram (EEG) was normal. Cerebrospinal fluid examination was within normal limits, with no oligoclonal bands. Treatment with levodopa/carbidopa was of no benefit. She died at age 45, 3 years after the onset of the illness, from pneumonia (Table 2).

The brain weight was 1,120 g. The sulci and gyri revealed minimal frontal atrophy. The sagittal view showed marked hydrocephalus with thinning of the corpus callosum (Fig. 3). There was no atherosclerosis of the major vessels. Coronal section of the brain showed markedly dilated ventricular system, especially the lateral ventricles (Fig. 4). The cerebral white matter had marked atrophy with softening and gray discoloration. The basal ganglia showed mild atrophy, which was more severe in the caudate and putamen than the globus pallidus.

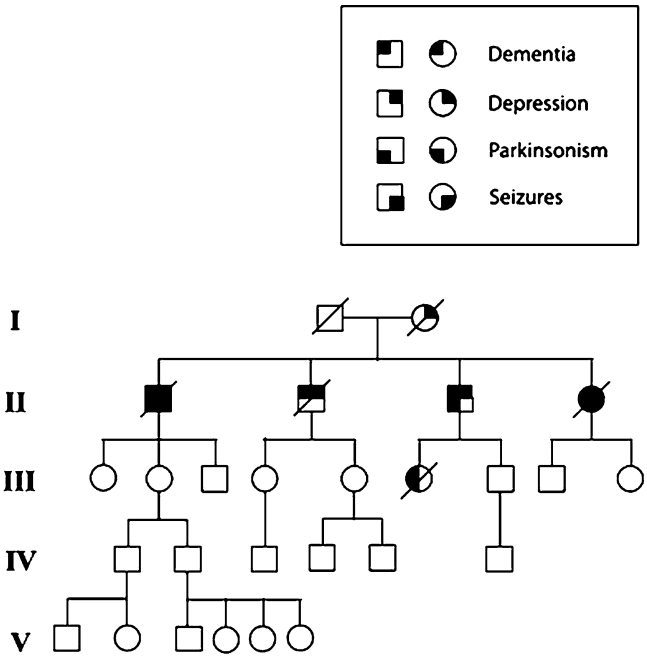

Fig. 1 Pedigree of the family with hereditary diffuse leukoencephalopathy with spheroids. The pedigree had 27 family members spanning 5 generations, with 6 affected individuals. *Circles* indicate females

Microscopically, the most significant pathology was in the cerebral white matter, affecting the centrum semiovale, periventricular white matter and corpus callosum. The white matter was rarefied and vacuolated, with marked myelinated fiber loss (Fig. 5), bizarre astrocytosis (Fig. 6) and many lipid-laden macrophages, as well as diffuse microglial activation (Fig. 5). In areas with myelin loss, axons were swollen and vacuolated and some had PAS-positive finely granular material. Spheroids were intensely immunoreactive for phosphorylated neurofilament protein and APP (Fig. 7); weakly reactive for ubiquitin; and negative for αB-crystallin, α-synuclein and tau. In some foci the overlying cortex also had numerous axonal spheroids (Fig. 8), as well as ballooned neurons that were immunoreactive for phosphorylated neurofilament and αB-crystallin (Fig. 9).

Electron microscopy of the axonal spheroids showed two major types of lesions. One was characterized by swollen axons containing dense bodies, lamellar bodies and vesicular profiles (Fig. 10a), while the other was characterized by swollen axons filled with densely packed and chaotically arranged intermediate filaments (Fig. 10b). Also present were hypertrophic astrocytes and lipid-laden macrophages. There were no ultrastructural features of lysosomal storage diseases (e.g., metachromatic leukodystrophy) or of long-chain fatty acid diseases (e.g., adrenoleukodystrophy).

Silver stains and thioflavin-S fluorescence microscopy revealed no Alzheimer type pathology in cortex or hippocampus, and the tau immunostains revealed no neuronal lesions or glial lesions typical of CBD. The basal ganglia were histologically unremarkable, except for a few axonal spheroids. The internal capsule and the corticospinal tract in the cerebral peduncle and brainstem had myelin pallor and axonal spheroids. The substantia nigra showed focal neuronal loss, but no neurofibrillary tangles. The raphe nucleus, locus ceruleus and reticular formation had preserved neuronal populations and no Lewy bodies. The cerebellum showed focal Purkinje cell loss and Bergmann gliosis in the vermis.

II-1: This 84-year-old man had a 6-year history of dementia, seizures, parkinsonism and psychiatric symptoms. The first symptom was a slowly progressive cognitive disturbance. When seen 4 years after the onset of his illness, his MMSE score was 14/30. He was diagnosed with probable Alzheimer's disease. He suffered from depression and had agitated behavior. Throughout the clinical course he had many syncopal episodes. At 83 years of age, he developed partial complex seizures with secondary generalization. EEG demonstrated left temporal slowing. At that time, the neurologic exam revealed persistent bilateral resting hand tremor that was worse on the left, cogwheel rigidity, bradykinesia and severe postural instability. For ambulation, he needed a wheeled walker. Frontal lobe release signs were elicited. Deep tendon reflexes were exaggerated throughout, but plantar responses were flexor. There was no evidence for hallucinations, sensory impairment or ataxia. His past history was negative for neurological disorders, includ-

Table 1 Clinical features of affected individuals in the hereditary diffuse leukoencephalopathy with spheroids kindred

Pedigree number	Sex	Current age	AAO	AAD	DD	Initial symptom	Clinical features					
							Dm	Dp	P	S	Ap	Others
I-1	F	–	42	76	34	Dp		+				
II-1	M	–	78	84	6	Ml	+	+	+	+		Agitation
II-2	M	–	59	79	20	Ml	+	+				Persistent vertigo
II-3	M	73	47	–	26	Dp	+	+	+			
II-4	F	–	54	62	8	P	+	+	+	+	+	
III-6	F	–	43	46	3	P	+		+		+	Ataxia, emotional lability, pyramidal signs

AAO age at symptomatic onset, *AAD* age at death, *DD* disease duration, *Dm* dementia, *Dp* depression, *P* parkinsonism, *S* seizures, *Ap* apraxia, *Ml* memory impairment, + present

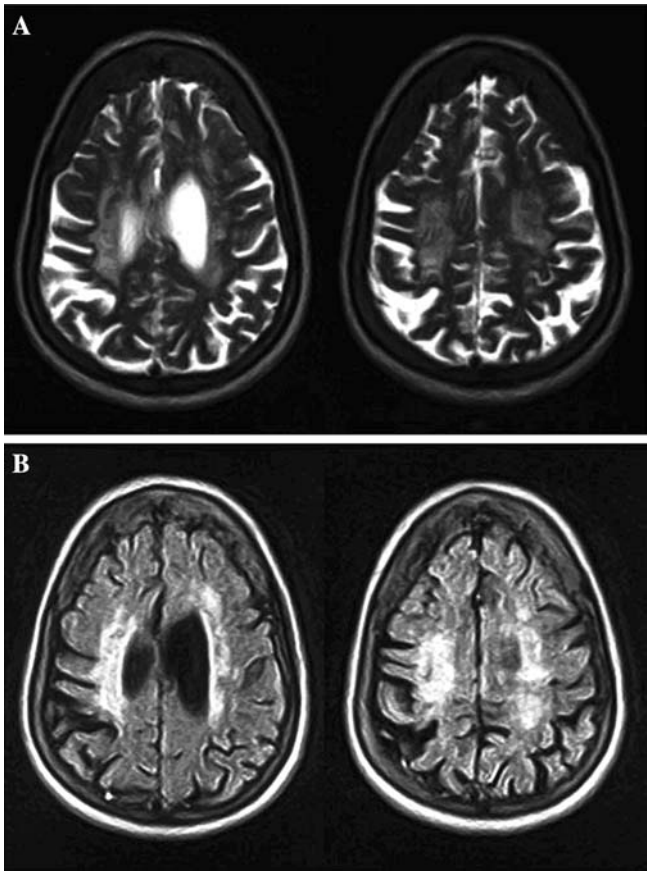

Fig. 2 T2-weighted (**a**) and fluid-attenuated inversion recovery magnetic resonance imaging (**b**) of the proband (III-6) shows bilateral abnormal high intensity areas in deep cerebral white matter and bilateral cerebral atrophy. Atrophy is worse on the *right*

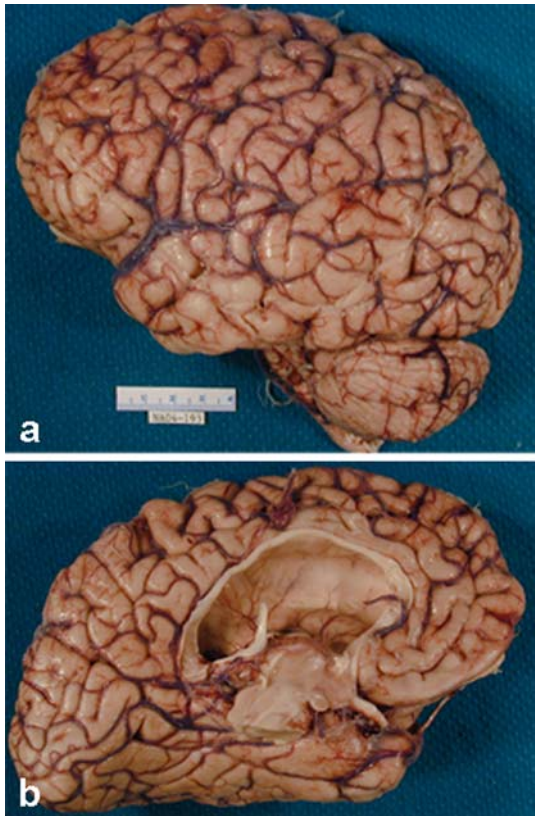

Fig. 3 Gross appearance of the brain of the proband (III-6). While the lateral convexity (**a**) is relatively unremarkable, the medial view (**b**) shows ventricular enlargement and marked thinning of the corpus callosum

ing head trauma. A head computed tomogram (CT) performed 3 years after the onset of his illness showed no structural abnormalities, except for diffuse cortical atrophy. He died 6 years after the onset of the illness of an unknown cause.

The brain weighed 1,250 g. There was frontal atrophy, and the lateral ventricles were enlarged. Microscopically, there was severe loss of cerebral myelin with sparing of subcortical U-fibers with moderate gliosis of the white matter. Many axonal spheroids were detected with Bodian stain, and immunohistochemistry for phosphorylated neurofilament protein in the cerebral cortex and white matter, but not in the basal ganglia,

Table 2 Neuropathology of three members of the hereditary diffuse leukoencephalopathy with spheroids kindred

Neuropathologic feature	II-1	II-4	III-6
Distribution of white matter pathology	Centrum semiovale Periventricular white matter Corpus callosum	Centrum semiovale Periventricular white matter Corpus callosum Internal capsule Cerebral peduncle White matter in the brain stem	Centrum semiovale Periventricular white matter Corpus callosum Internal capsule Cerebral peduncle
Distribution of axonal spheroids	Cerebral white matter and cortex	Cerebral white matter Basal ganglia Genu of internal capsule Frontopontine fibers in cerebral peduncle Cerebellar cortex	Cerebral white matter and cortex Basal ganglia Genu of internal capsule Frontopontine fibers in cerebral peduncle
Ballooned neurons	None	Cerebral cortex	Cerebral cortex
Senile plaques	Cortex, hippocampus and amygdala	Cortex	None
Amyloid angiopathy	Cortex and amygdala	Cortex	None
Braak NFT stage	Stage III-IV	Stage I	Stage 0

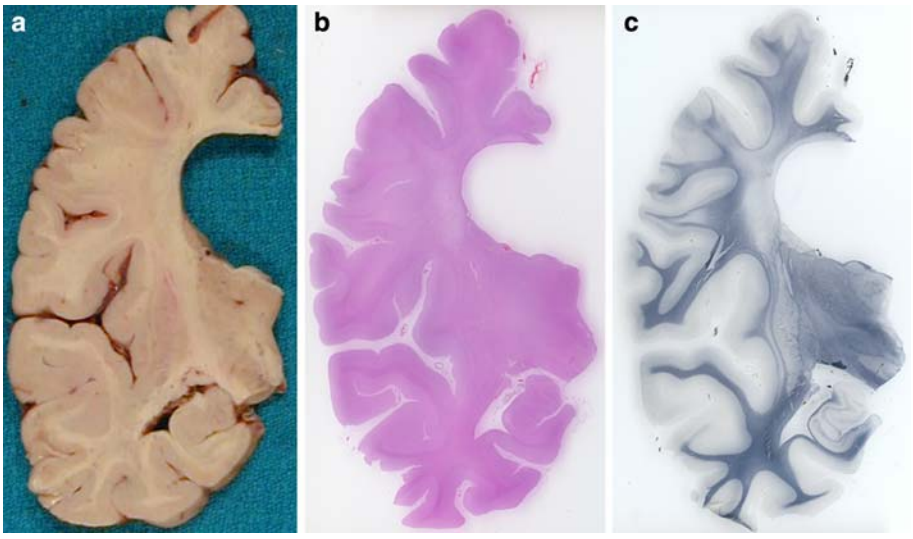

Fig. 4 A coronal section of the proband (**a**) at the level of the subthalamic nucleus shows ventricular enlargement, thinning of the corpus callosum and gray discoloration of the centrum semiovale and periventricular white matter. The internal capsule is grossly unaffected. Whole mount sections stained with hematoxylin and eosin (**b**) and the Bodian silver stain (**c**) show pallor of the centrum semiovale and periventricular white matter, with better preservation of the white matter in the temporal lobe

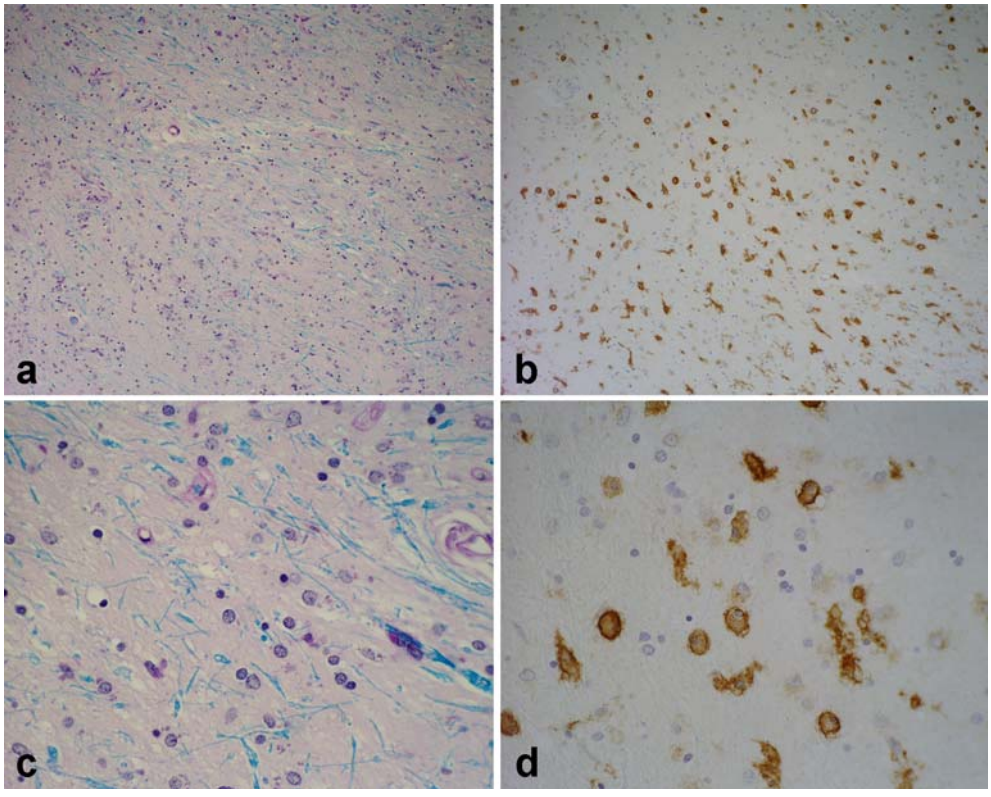

Fig. 5 Luxol fast blue–periodic acid Schiff (**a** and **c**) of the centrum semiovale of the proband shows marked loss of myelinated fibers and a gliotic neuropil. Adjacent sections stained for HLA-DR (**b** and **d**) to reveal activated microglia and macrophages shows many macrophages in the cerebral white matter, but no perivascular inflammatory cell infiltrates

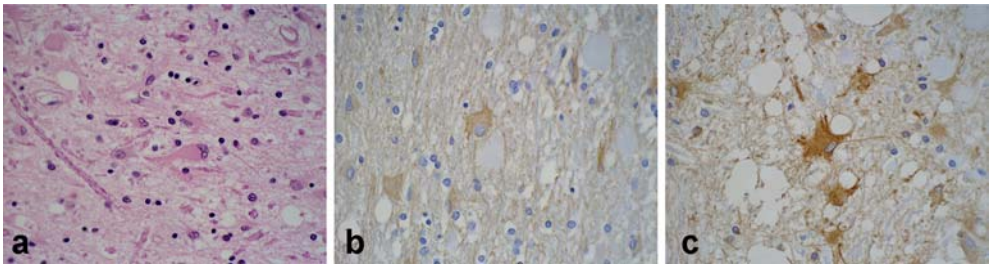

Fig. 6 Bizarre reactive astrocytes are visible in the white matter with hematoxylin and eosin (**a**). They are positive for glial fibrillary acidic protein (**b**) and also for αB-crystallin (**c**)

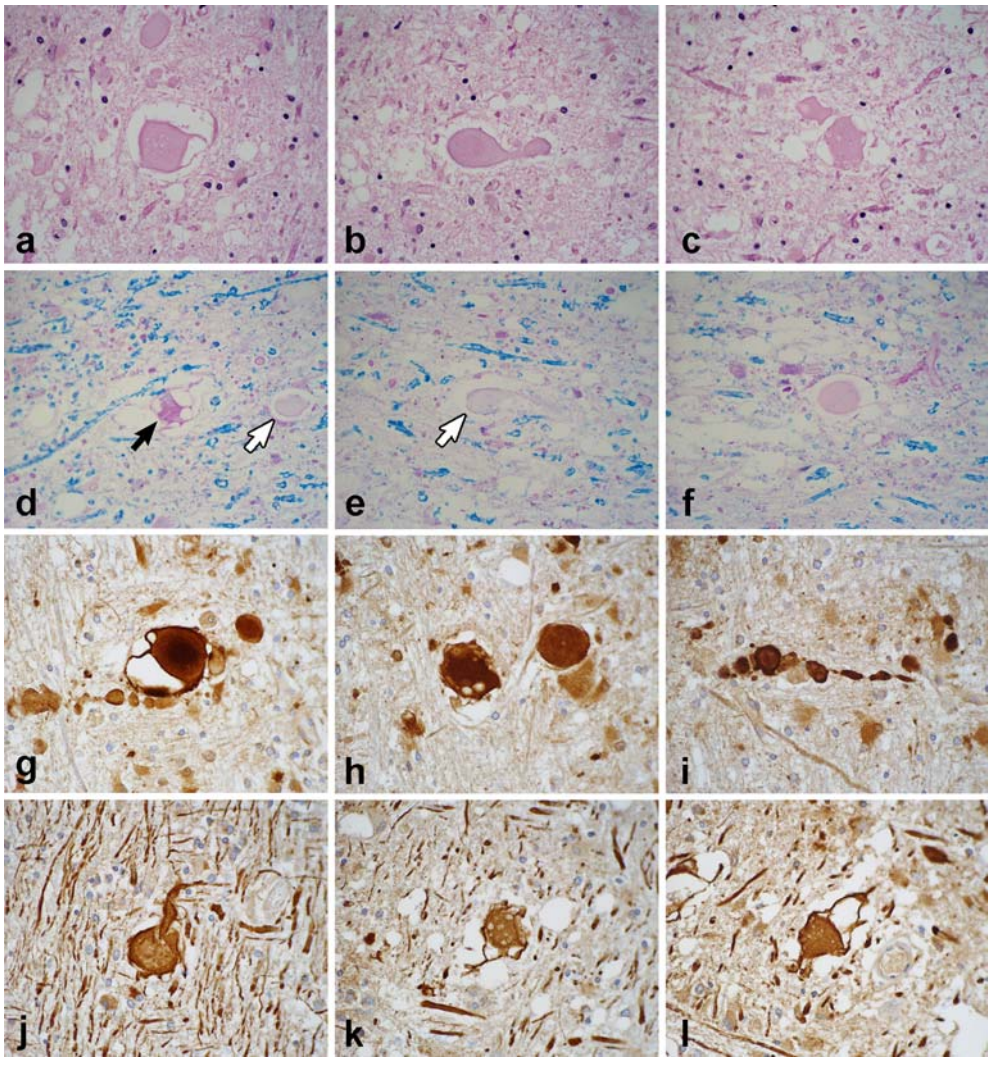

Fig. 7 Axonal spheroids in the white matter show a range of morphologies with hematoxylin and eosin (**a**, **b** and **c**), Luxol fast blue–periodic acid Schiff (LFB–PAS) (**d**, **e** and **f**), amyloid precursor protein (APP) (**g**, **h** and **i**) and phosphorylated neurofilament (**j**, **k** and **l**). Note the presence of vacuolation in many of the spheroids (**a**, **c**, **d**, **g**, **h**, **k** and **l**). Some spheroids contain PAS-positive granular material (**d**; *back arrow*) while others are pale on LFB–PAS (*white arrows* in **d** and **e**). In some spheroids neurofilament immunoreactivity is most dense at the *periphery* (**j**). In some spheroids APP immunoreactivity is most dense in the *center* (**g**)

brainstem or cerebellum. Mild neuronal loss and gliosis were noted in the basal ganglia.

Alzheimer type pathology was detected in cortex and hippocampus with Bodian stain and immunohistochemistry for Aβ, which also revealed focal amyloid angiopathy in leptomeninges and parenchyma. Both diffuse and neuritic plaques were present in the association cortices, amygdala and hippocampus, which also had granulovacuolar degeneration and Hirano bodies. Neurofibrillary tangles were present in the hippocampus and amygdala. The Braak neurofibrillary pathology stage was consistent with stage III-IV. Mild to moderate neuronal loss and gliosis with neuromelanin containing macrophages were present in the substantia nigra and locus ceruleus, and a few neurofibrillary tangles were also noted.

II-4: This 62-year-old woman had an 8-year history of progressive atypical parkinsonism. The parkinsonism was worse on the left side and was accompanied by apraxia. During the course of her illness, she suffered from depression and seizures. She was clinically considered to have CBD. An MRI of the brain obtained at 58 years of age showed bilateral high intensity lesions in the cerebral white matter, worse on the right. She died 8 years after the onset of her illness of an unknown cause.

The brain weighed 1,070 g and showed no macroscopic evidence of cortical atrophy. Coronal section of the brain showed loss of myelin, relatively sparing of U-fibers and marked ventricular enlargement. The microscopic examination showed rarefaction and vacuolation of the white matter with myelinated fiber loss, astrocytic gliosis and lipid-laden macrophages, as well as diffuse microglial activation. Axons within the demyelinated foci were swollen and intensely immunoreactive for phosphorylated neurofilament and APP, but negative for ubiquitin, α-synuclein, tau and αB-crystallin. The white matter tracts in the brainstem, including the cerebellar peduncles were unaffected; however, the fronto-pontine fibers on one side showed rarefaction, fiber depletion and axonal spheroids in longitudinal fibers. The medullary pyramids on both sides were unaffected.

The neocortex had a relatively unremarkable appearance. In the cortical areas overlying the regions

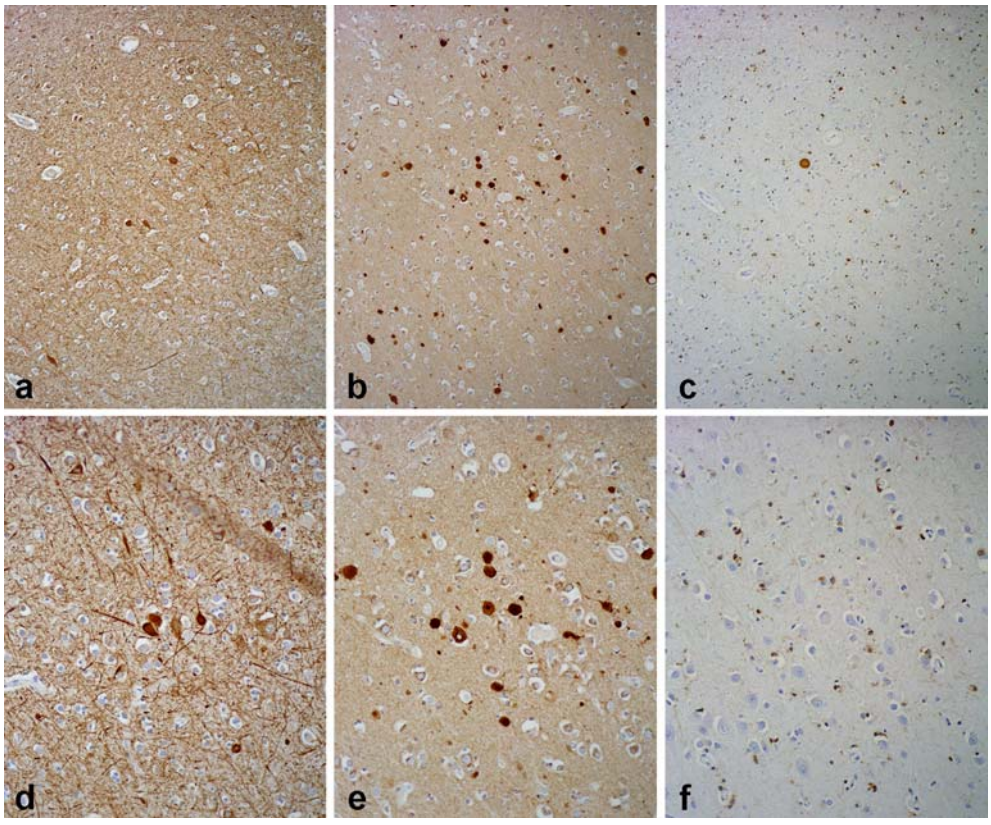

Fig. 8 Adjacent sections of cerebral cortex with immunohistochemistry for neurofilament (**a** and **d**), amyloid precursor protein (APP) (**b** and **e**) and αB-crystallin (**c** and **f**). There are scattered axonal spheroids that are more apparent with APP than neurofilament, while negative for αB-crystallin. In this region there was only a rare ballooned neuron (**c**)

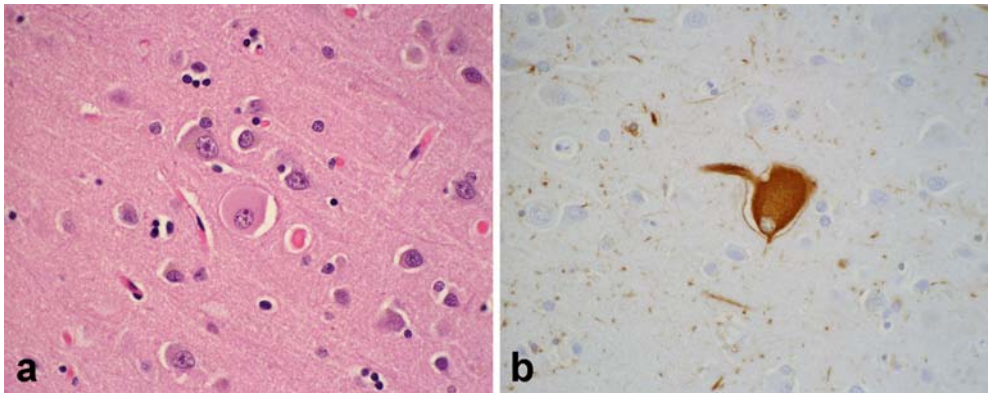

Fig. 9 Ballooned neurons in the cortex of II-4 are swollen and pale staining on hematoxylin and eosin (**a**) and immunoreactive for αB-crystallin (**b**)

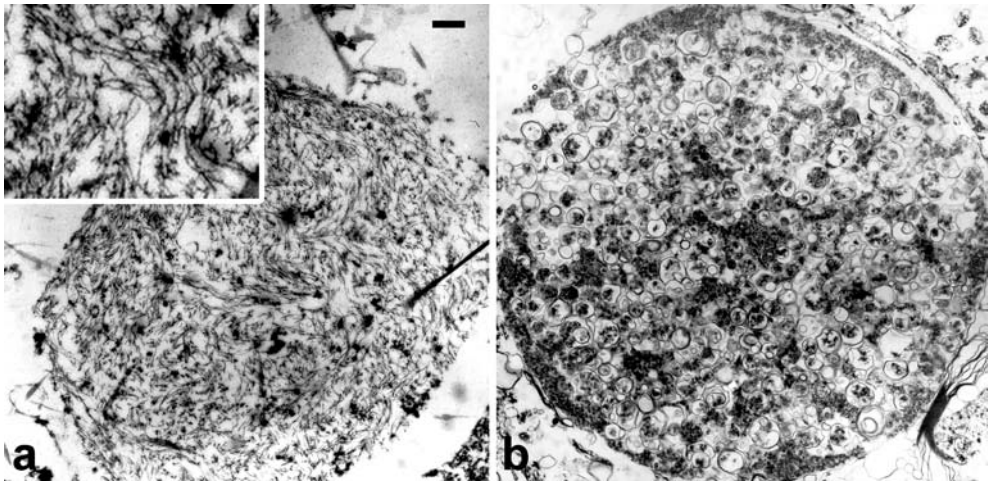

Fig. 10 Electron microscopy of axonal spheroids from white matter of the proband (III-6) reveals swollen axons filled with chaotically arranged neurofilaments (**a**) or vesicular and membranous material (**b**). *Inset* shows intermediate size filaments [*bar* = 1 μm for (**a**) and (**b**); 0.4 μm for inset]

Table 3 Genealogical and clinical characteristics of reported families with hereditary diffuse leukoencephalopathy with spheroids

	Axelsson et al. (1984) [2]	Yazawa et al. (1997) [29]	van der Knaap et al. (2000) [25]	Hancock et al. (2003) [8]	Marotti et al. (2004) [14]	Terada et al. (2004) [22]	Present report
Origin	Sweden	Japan	The Netherlands	Australia	USA	Japan	USA
Family member, *n*	71	19	NA	5	15	5	27
Affected individual, *n*	17	6	2	3	3	2	6
Male: female, *n*	10:7	1:5	1:1	1:2	1:2	0:2	3:3
Mean (and range) age at onset, year	36 (8–60)	21 (15–30)	33 (15–50)	43	40 (35–44)	49 (40–57)	54 (42–78)
Mean (and range) disease duration, year[a]	12 (0–32)	11 (2–26)	7	2	5 (4–7)	6 (4–8)	16 (2–34)
Cognitive and psychiatric features							
Memory disturbance	+	+	+	+	+	+	+
Depression	+			+	+	+	+
Behavioral change	+		+	+			
Disorientation	+					+	
Irritability	+	+			+		+
Anxiety	+				+		
Motor features							
Ataxia	+			+	+		+
Parkinsonism	+	+		+	+		+
Dysarthria/mutism	+	+	+	+	+		
Dyskinesia	+						
Dystonia	+			+			
Chorea			+				
Cortical signs[b]	+	+	+	+	+	+	+
Pyramidal signs	+	+	+	+	+	+	+
PIGD	+	+	+	+	+	+	+
Epilepsy	+	+			+	+	+
Urinary incontinence	+	+	+	+	+	+	
Autopsied cases, *n*	4	3	2	1	3	1	3
Diagnosis before death	Senile dementia, manic disorder, schizophrenia	Progressive dementia and limb paralysis	NA	Progressive cognitive decline with depressive features, AD	AD, leukodystrophy	AD	AD, CBD, FTDP-17

[a]Mean disease duration indicates from symptomatic onset to death

[b]Cortical signs include frontal release signs, apraxia, graphesthesia and tactile agnosia

+ present, *AD* Alzheimer's disease, *CBD* corticobasal degeneration, *FTDP-17* frontotemporal dementia and parkinsonism linked to chromosome 17, *NA* not available, *PIGD* postural instability and gait disturbance, *USA* United States of America

with white matter pathology, there were many ballooned neurons in the lower cortical layers that are immunoreactive for phosphorylated neurofilament and αB-crystallin. A few thioflavin-S fluorescent diffuse plaques were detected in the cortex and hippocampus, but there were no neurofibrillary tangles on silver stains or tau immunohistochemistry. The Braak neurofibrillary pathology stage was stage I. There was mild amyloid angiopathy that affected both parenchymal and leptomeningeal blood vessels and immunohistochemistry confirmed that amyloid in blood vessels and plaques was Aβ.

The substantia nigra, as well as the raphe nucleus, locus ceruleus and reticular formation had normal neuronal populations and neither Lewy bodies nor neurofibrillary tangles. Neurofilament immunohistochemistry revealed a few Purkinje cells with aberrant apical dendrites and axonal torpedoes.

Molecular genetic analysis

Molecular genetic analysis of the five subunits of eIF2B (*eIF2B1* gene on chromosome 12, *eIF2B2* on chromosome 14q24, *eIF2B3* on chromosome 1, *eIF2B4* on chromosome 2p23.3 and *eIF2B5* on chromosome 3q27) failed to identify any mutations or sequence variations. Furthermore, no mutations were detected in the *MAPT* gene on chromosome 17q21.

Discussion

Hereditary diffuse leukoencephalopathy with spheroids is a rare hereditary disorder, which is difficult to diagnose clinically, with the diagnosis often resting on postmortem neuropathology [2, 8, 14, 22, 25, 29]. Genealogical and clinical characteristics of previously reported families with HDLS are summarized in Table 3, with kindreds described worldwide, including Sweden, the Netherlands, Japan, Australia and the United States. Symptomatic onset is usually in the third to sixth decade of life. The principal clinical picture is characterized by dementia and psychiatric disturbances, including depression, irritability and anxiety (see Table 3). Cortical symptoms (e.g., frontal release signs, apraxia and graphesthesia), pyramidal signs, postural instability, gait disturbance and urinary incontinence are also frequent clinical features. There is significant intra- and inter-family variability of the clinical phenotype. While MRI may show striking abnormalities, there are no specific imaging characteristics of HDLS [25].

Phenotypic variability was noted in the present kindred. Atypical parkinsonism, dementia and psychiatric disturbances were seen alone or in combination in all affected family members. Individuals II-4 and III-6 presented with a syndrome resembling CBD, but individuals I-1 had only psychiatric disturbances and II-1 had Alzheimer type dementia with parkinsonism. Five HDLS patients with parkinsonism have been previously reported, and three of them (III-20 of Axelsson et al. [2]; the proband of Hancock et al. [8]; case 2 of Marotti et al. [14]) presented first with dementia and only later developed atypical parkinsonism, with CBD-like features. Two additional cases reported by Axelsson et al. (cases of II-8 and III-19) had symmetrical parkinsonism thought to be related to antipsychotic therapy [2]. Unlike previously reported HDLS cases with parkinsonism, two individuals in the present kindred (II-4 and III-6) had features of asymmetric parkinsonism and apraxia as early signs, with dementia developing later in the disease course.

Given the genealogical features and complex clinical phenotype of the proband, which included dementia, behavioral and personality changes and levodopa-unresponsive parkinsonism, the first diagnostic consideration was FTDP-17 [27]. This was ruled out by failure to detect mutations in the gene for tau protein, *MAPT*. The neuropathologic findings at autopsy also did not support this diagnosis.

The neuropathologic findings in three cases from the present kindred of HDLS were remarkably similar, including abundant axonal spheroids, a loss of axons and myelin in the cerebrum, bizarre astrocytes and ballooned neurons in the overlying cortex. The axonal spheroids were immunoreactive for phosphorylated neurofilament protein and APP, but only weakly and inconsistently immunoreactive for ubiquitin and negative for tau, α-synuclein and αB-crystallin. The axonal spheroids in HDLS are distributed in the internal capsule, cerebral peduncle as well as the corticospinal tract in the brainstem and spinal cord [2, 14, 29]. Autopsies of II-4 and III-6 revealed axonal spheroids in these structures, as well as a few in the basal ganglia. Some axonal spheroids were also detected in the cerebral cortex in two cases (II-1 and III-6), where they were clearly distinguished from ballooned neurons by their lack of αB-crystallin immunoreactivity. In contrast, ballooned neurons were positive for αB-crystallin.

The presence of ballooned neurons in the cerebral cortex is a histopathologic hallmark of CBD, but ballooned neurons are relatively nonspecific and can also be present in the limbic system of progressive supranuclear palsy, argyrophilic grain disease and in Pick's disease [4]. It is unclear whether the focal cortical degeneration with ballooned neurons may have contributed to some of the cortical clinical features, such as apraxia. While amyloid deposits have been reported in a sporadic case with HDLS [3], in the present kindred diffuse amyloid deposits and amyloid angiopathy were detected in a 62-year-old woman and an 84-year-old man, but not in a 44-year-old woman, which suggests that this feature may be merely coincidental age-related pathology. The possible contribution of apolipoprotein E to risk for Alzheimer type pathology was not investigated.

The differential diagnosis of progressive white matter disorders includes neuroaxonal dystrophies [10], cerebral

autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) [21], hereditary adult-onset leukodystrophy [5], late-onset metachromatic leukodystrophy [26], X-linked adrenoleukodystrophy [16] and VWM [19]. Clinical and neuropathologic features, including absence of arteriolar pathology of CADASIL, clearly excluded these diagnoses. Hallervorden–Spatz disease, which is now referred to as neurodegeneration with brain iron accumulation type I [10], is also included in the differential diagnosis and may present with extrapyramidal tract signs with histopathologic evidence of neuroaxonal spheroids [20]. While spheroids were detected in the basal ganglia in one case (II-4), they were sparse and the patient did not have other clinical manifestations of neurodegeneration with brain iron accumulation. Nasu–Hakola disease and adult-onset VWM also have leukoencephalopathy with axonal spheroids [11, 15, 17, 18]. Nasu–Hakola disease is an autosomal recessive disorder with onset in the second or third decades of life with pain and swelling of ankles and wrists after strain and spontaneous fractures [1]. Neurological symptoms are characterized by progressive dementia, disturbances of cortical function, upper motor neuron symptoms, involuntary movements, seizures and urinary incontinence [17]. Radiographic studies show cystic rarefactions of bone, and head CT shows brain atrophy with bilateral calcification in the basal ganglia [17]. These features were not detected in the present kindred.

Although initial reports of VWM suggested that it was an autosomal recessive disorder in children, several cases have been reported in adolescents and adults [19, 23]. The clinical features of VWM include chronic progressive encephalopathy, dementia, psychiatric symptoms and motor impairment [19, 23]. MRI studies have been reported on only three cases of HDLS [8, 25]. HDLS is characterized by patchy and inhomogeneous white matter abnormalities, which are often asymmetrical. They involve the frontal white matter and the white matter under the pre- and postcentral gyri most prominently, leaving the other areas relatively intact. The leukoencephalopathy in VWM is diffuse and homogenously involves almost all cerebral white matter.

Recently, VWM has been linked to mutations in each of the five subunits of the eukaryotic translation initiation factor 9 [24]. Mutation screening of genes for the eIF2B subunits, *EIF2B1*, *EIF2B2*, *EIF2B3*, *EIF2B4* and *EIF2B5*, failed to reveal any sequence variants or previously documented mutations in VWM [12, 24]. There are no known genes described in HDLS, but since our patient had clinical features suggestive of FTDP-17, we performed screening for mutations in *MAPT* with negative results.

In summary, the present report describes clinical and neuropathologic features of a previously unreported kindred of HDLS with variable clinical features, including those seen in CBD. HDLS can be differentiated from other disorders with familial parkinsonism or leukoencephalopathy with axonal spheroids by clinical and neuropathological findings. Eventual answers to the etiology and pathogenesis of HDLS will benefit from multicenter collaborative studies of multiplex kindreds in order to find a genetic linkage for this rare disorder.

Acknowledgments The authors are indebted to the members of this kindred for their interest and willingness to cooperate in this study. The study was partially supported by the Morris K. Udall Center for Excellence in Parkinson's Disease Research (P50 NS40256), VA Research Funds, the Indiana Alzheimer Disease Center (P30 AG10133) and the National Cell Repository for Alzheimer's Disease (U24 AG21886). YB is a recipient of Robert and Clarice Smith fellowship.

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