RESEARCH ARTICLE

Hippocampal Sclerosis Dementia with Tauopathy

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In some elderly individuals with dementia, hippocampal sclerosis (HS) is the only remarkable autopsy finding. The cause of HS in this setting is puzzling, since known causes of HS such as seizures or global hypoxic-ischemic episodes are rarely present. We here describe a series of HS cases that have a widespread neuronal and/or glial tauopathy. Of 14 consecutive cases of HS, 12 had been clinically diagnosed with dementia and/or Alzheimer's disease (AD) while 2 were non-demented; 7 cases had also been clinically diagnosed with parkinsonism. In addition to HS, 6 cases also met pathologic diagnostic criteria for AD. Gallyas silver staining and immunohistochemistry with the AT8 antibody revealed a glial and/or neuronal tauopathy in 12 of 14 cases, with frequent positive neurons and/or glial cells in the neocortex, basal ganglia, thalamus and/or limbic regions; in addition, 8 of the 14 cases had argyrophilic grains. Screening for known tau mutations was negative in all cases. Western blots of sarkosyl-insoluble tau protein showed a mixture of 3- and 4-repeat forms. The results suggest that most cases of HS dementia are sporadic multisystem tauopathies; we suggest the term "hippocampal sclerosis dementia with tauopathy" (HSDT) for these.

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Introduction

In a subset of elderly individuals with dementia, hippocampal sclerosis (HS) is the only remarkable autopsy finding (1, 13, 15, 17, 31, 35, 41, 50, 52, 53, 55). The frequency of HS in autopsy series of elderly demented persons has been reported to be as low as 0.4% (53) and as high as 26% (17). Others have reported frequencies of 3.2% (13), 4.4% (41), 7.4% (50) and 12% (35). During life, these cases are usually diagnosed as Alzheimer's disease (AD) since they typically present with short-term memory impairment that progresses to dementia. The cause of HS associated with dementia (HSD) is unknown; there is rarely a history of seizures or of a global cerebral hypoxic-ischemic event, which are known causes of HS in other settings. It has been suggested that HSD is due to occult hypoxic/ischemic episodes (15) or is a sequela to limbic encephalitis (13).

The term "tauopathy" has been recently coined to describe brain diseases that are characterized by abnormal neuronal and glial accumulations of tau protein (2, 10, 14, 16, 19, 26, 38, 40, 42, 46). The primary tauopathies include progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), Pick's disease and a group of inherited conditions known as frontotemporal dementia and parkinsonism associated with mutations of the tau gene on chromosome 17 (FTDP-17). Alzheimer's disease may be considered a secondary tauopathy, as the primary pathology is amyloid deposition. The tauopathies have overlapping clinical syndromes whose features include varying degrees of memory impairment, behavioral changes, dementia and extrapyramidal motor abnormalities. There is also some overlap in their histopathologic features as all have glial and/or neuronal accumulations of hyperphosphorylated and aggregated tau protein; despite this, some, such as CBD, PSP and Pick's disease, may be distinguished by morphologic and topographic differences in the abnormal tau deposits.

We report that a majority of the HSD cases in our autopsied population have a widespread neuronal and/or glial tauopathy resembling that seen in FTDP-17.

Materials and Methods

Subjects. The subjects in this study are derived from the Brain Donation Program at Sun Health Research Institute, a non-profit organization devoted to the study of aging-related diseases. The Institute is located in Sun City, a suburb of Phoenix, Ariz. Mentally competent individuals enter the Program by voluntarily agreeing to brain removal after death; individuals with dementia are signed into the Program by their legal representative. The Brain Donation Program has been approved by the Sun Health Institutional Review Board. This study

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Case #	Presenting Features	Later Features	Clinical Diagnoses	Family History
1	Memory impairment, odd behavior, lack of judgement and insight	Gait abnormality, falling, syncopal episodes, rigidity, resting tremor, dysphagia, mutism, dementia	Probable AD, parkinsonism	none
2	Memory impairment, depression	Angry outbursts, paranoid ideation, impaired language function, falling, dementia	AD	none
3	Not available (sparse records)	Dementia (sparse records)	AD	none
4	Memory impairment, falling, cogwheeling rigidity, dysphagia	Paranoia, apathy, masked facies, bradykinesia, lip tremor, dysphagia, difficulty speaking, hallucinations, dementia	PD, dementia	none
5	Memory impairment	Parkinsonism (shuffling gait), falling, dementia	AD, PD	none
6	Not available (sparse records)	Dementia, HT, angina	Dementia	Yes, AD
7	Memory impairment, word-finding difficulty, difficulty reading, gait imbalance	Receptive and expressive aphasia, agnosia, difficulty with complex calculations, denial, dementia	AD	none
8	Parkinsonism (no details)	Verbally uncommunicative, falling, wheelchair-bound, dementia	PD, AD	Yes, AD
9	Memory impairment, bilateral action tremor of hands	Rigidity, bradykinesia	PD, non-demented	None
10	Not available (sparse records)	Aphasia, dementia	AD	AD, PD +/or FTD in four 2nd deg. relatives
11	Memory impairment, aggressive behavior, lack of insight, depression	Abnormal behavior, delusion, rigidity, resting tremor, gait abnormality, difficulty with speech, dementia	MID, AD, PD	Yes, AD
12	Essential tremor, falling	Action tremors of head, hands and voice, falling	Tremor disorder, non-demented	Yes, PD
13	Memory impairment,denial, alcoholism	Personality change, word-finding difficulty, stooped posture, dysphagia, postural instability, rigidity, bradykinesia, masked facies, gait abnormality, dementia	AD, DLB, parkinsonism	Yes, stroke
14	Memory impairment, personality change (withdrawal)	Aphasia, dyspraxia, loss of ability to walk, dementia	AD, FTD	Yes, AD

Table 1. *Clinical features of hippocampal sclerosis cases.* Information was obtained from review of clinical records and patient/proxy questionnaire. Family history is defined as yes only if there were one or more first-degree relatives with the stated condition except where noted. AD = Alzheimer's disease; PD = Parkinson's disease; DLB = dementia with Lewy bodies; MID = multi-infarct dementia; FTD = frontotemporal dementia.

includes all cases diagnosed with hippocampal sclerosis during the period between January 1, 1997 and July 30, 2001. Hippocampal sclerosis was defined as complete or near-complete neuronal loss (only rare or widely scattered neurons remaining) in all or part of area CA1 of the hippocampus. Cases with moderate or frequent neurofibrillary tangles or "ghost tangles" in CA1 were excluded. Clinical information was obtained by review of medical records obtained from the primary care physician, and from private neurologists, neuropsy-

chologists and psychiatrists, when available (the clinical research component of Sun Health Research Institute did not open until July 1, 2000 and therefore these cases had not received standardized clinical assessment).

Tissue processing. Brains were sliced coronally into 1 cm segments and then divided sagittally into left and right halves. Left-sided slices were immersion-fixed for 48 hours in 4% paraformaldehyde in 0.1 M phosphate buffer for 2 days at 4°C and then transferred through cryoprotectant solutions (2% DMSO/10% glycerol, then 2% DMSO/20% glycerol). Right-sided slices were rapidly frozen between slabs of dry ice. Following fixation and cryoprotection, diagnostic tissue blocks were taken from the fixed slices and embedded in paraffin wax; additionally, large (4 × 3 cm) tissue blocks were dissected for frozen sectioning. Sections from paraffin blocks were cut at 5 μm and stained with hematoxylin and eosin (H&E). Frozen sections (40 μm) from the large blocks were stained with H&E, Campbell-Switzer, Gallyas (7) and Thioflavine S methods. Immunohistochemical stains were performed on free-floating frozen sections as described previously (3). Antibodies used to detect abnormal tau deposits included AT8, which recognizes tau phosphorylated at Ser-202 and Thr-205, PHF-1, which recognizes tau phosphorylated at Ser-396 and Ser-404, and Alz-50, which recognizes a distinct tau conformation independent of phosphorylation state.

Apolipoprotein E genotyping. DNA for apolipoprotein E (apoE) genotyping was extracted from pieces of fixed cerebellar cortex. Tissue (100 mg) was digested to completion with proteinase K (1 mg/ml) at 55°C and extracted multiple times with phenol/chloroform. DNA was recovered by isopropanol precipitation. For each PCR reaction, approximately 500 ng of DNA from each sample was used. PCR primers and amplification conditions employed, and identification of apoE genotypes by Hha I digestion of amplified material, was carried out according to published protocols (24). Digested fragments were separated by electrophoresis through 8% acrylamide gels and identified by staining with ethidium bromide.

Tau mutation screening. All cases were screened for the presence of mutations in the tau gene using frozen frontal lobe neocortex. Tau exons 7, and 9 to 13 were amplified from genomic DNA with primers designed to include flanking intronic sequences. Cycling conditions were 35 cycles of 94°C for 30 seconds, 60 to 50°C "touchdown annealing" for 30 seconds and 72°C for 45 seconds with a final extension of 72°C for 10 minutes. All products were then purified, and for each exon, 100 ng of product was sequenced in both directions using Big Dye chemistry (Applied Biosystems) and relevant PCR primers. Sequencing was performed on an ABI377 automated sequencer and processed using Factura and Sequence Navigator software (Applied Biosystems). The presence or absence of tau mutations, as well as the tau haplotype, was determined from the sequence data.

Tau extraction and immunoblotting. Sarkosyl-soluble and insoluble tau protein fractions were extracted from fresh-frozen hippocampus and parahippocampal gyrus according to the method of Greenberg and colleagues (20). Samples were run on 10% SDS-polyacrylamide gels (SDS-PAGE), transferred to nitrocellulose membranes and probed with the WKS-44 antibody, which binds to the central region of tau.

Results

Clinical features of HSD cases. A summary of the clinical features is presented in Table 1. Fourteen (7.9%) cases met histologic criteria for HS, of the 177 consecutive cases in which the primary diagnosis was dementia. The cases included 10 males and 4 females, with a mean age at death of 81.7 years (range was 54-100 years). Twelve cases had been diagnosed during life with dementia; the medical history of the 2 others was negative for dementia. The mean duration of illness for the demented cases was 9.2 years (range was 5-17 years). Dementia cases were associated with a clinical diagnosis of Alzheimer's disease in 4 cases; with Alzheimer's disease and Parkinson's disease or parkinsonism in 4 cases; with Alzheimer's disease, Parkinson's disease and multi-infarct dementia in one case; and with Alzheimer's disease and/or frontotemporal dementia in one case. One of the non-demented cases was diagnosed with Parkinson's disease with memory impairment, while the other had no specific disease diagnosis but was noted to have postural instability, action tremors of the hands and head and a voice tremor.

Clinical features at presentation were available in 11 of the 14 cases. Memory impairment was the most common presenting feature, being present in 9 cases. Postural instability was a presenting sign in 3 cases. Behavioral changes were present at the outset in 4, depression in 2, parkinsonism in one and word-finding difficulty in one. At later stages of disease, dementia was present in 12, parkinsonism in 7, dysphasia in 8 and personality

Case #	Brain Weight	Cerebral Cortex and White Matter	Mesial Temporal Lobe	Basal Ganglia	SN neuron loss
1	1070	Atrophy: F++; T+++; P++	Atrophy: +++ in entire region; bilateral HS	Atrophy: Cd+++	None
2	780	Atrophy: F+++; T+++; P++; CWMR: ++	Atrophy: +++ in hip, PHG; unilateral HS;	No changes	+++
3	1055	Atrophy: + to ++ in all lobes; small old infarctions of CBL and SI	Atrophy: ++ in hip, PHG; unilateral HS	No changes	++
4	1360	No changes	Atrophy not apparent; bilateral HS	No changes	+
5	1150	Atrophy: F++; P++; T+++ ; CWMR: ++	Atrophy: ++ to +++ in entire region; bilateral HS	No changes	++
6	835	Atrophy: ++ to +++ in all lobes; frequent old cortical microinfarcts; large old PCA infarct of right temporal and occipital lobe; CWMR: +++	Atrophy: +++ in entire region; bilateral HS	No changes	None
7	1185	Atrophy: ++ to +++ in all lobes, knife-blade in left anterior temporal lobe	Atrophy: +++ in entire region; unilateral HS	Atrophy: lentiform nucleus ++	None
8	1170	CWMR: +	Atrophy: +++ hip, PHG, FG; unilateral HS	Sparse lacunar infarcts	+++
9	1130	Difficult to assess: acute subdural hematoma with cerebral edema	Could not be assessed; bilateral HS	No changes	++
10	800	Atrophy: +++ in all lobes, knife-blade in anterior frontal lobe; CWMR: +++	Atrophy: +++ in entire region; bilateral HS	No changes	++
11	1140	Atrophy: ++ generally, +++ in anterior frontal and anterior temporal lobe; CWMR: ++	Atrophy: +++ in entire region; bilateral HS	Old lacunar infarct	+
12	1160	Atrophy: F+, P+	Atrophy: +++ hip; unilateral HS	No changes	None
13	-	Atrophy: +++ generally, +++ in temporal lobe	Atrophy: +++ entire region; bilateral HS	No changes	+
14	980	Atrophy: +++ left F and T, + to ++ in other areas	Atrophy: +++ entire region; unilateral HS	Atrophy: Cd, Put +++	+++

Table 2. *Gross and microscopic pathologic features of hippocampal sclerosis cases.* The mesial temporal lobe is defined as the region encompassing the amygdala, uncus, hippocampal formation and parahippocampal gyrus. + = mild, ++ = moderate, +++ = severe; HS = hippocampal sclerosis; F = frontal lobe; T = temporal lobe; P = parietal lobe; PHG = parahippocampal gyrus; hip = hippocampus; FG = fusiform gyrus; Cd = caudate nucleus; Put = putamen; CBL = cerebellum; SI = substantia innominata; PCA = posterior cerebral artery; CWMR = cerebral white matter rarefaction.

and/or behavioral changes in 7. The latter included odd behavior, disinhibition, apathy, aggression, paranoia, and hallucinations.

The past medical histories were notable for cardiovascular disease in 11 of 14; this consisted of hypertension (3 cases), coronary artery disease (6 cases, with past myocardial infarctions in 3), cardiac arrhythmias (6 cases, 3 with pacemaker placement) and congestive heart failure (4 cases). A cerebrovascular disease history was present in 3 cases (transient ischemic attacks). None of the 14 cases had a history of cardiac arrest and/or stroke and/or seizures which preceded the onset of dementia. One case had a history of a syncopal episode that occurred after the onset of dementia.

The family histories were notable for 5 cases in which one or more first-degree relatives had dementia and/or parkinsonism, one case in which a son had

epilepsy and one case in which 3 second and third degree relatives had dementia and/or parkinsonism.

Neuropsychologic features of HSD cases. Detailed neuropsychologic assessments were available on 5 cases. Interpretation of these is complicated by the presence of conditions other than HS, including left frontoparietal craniotomy, coincident AD and progressive supranuclear palsy. Comments are based on report summaries, as the raw data was not available. However, the general trend across patients was for an initial presentation with memory and language deficits, with generative fluency more affected than receptive fluency. Depression and agitation was evident in most, but not all, of the tested subjects.

In one subject, with HSD as the only major pathologic diagnosis, testing done a year prior to death revealed that simple attention and working memory (serial 7s, "world" backwards, digit span forward and backward, Trails A) were well preserved. However, verbal learning for stories and word lists, as well as verbal fluency for letters and category (animals) was severely impaired. A sequencing task requiring mental set shifting (Trails B) was also impaired. Copying of a complex figure was intact, but memory for the figure after a delay was severely impaired.

Similarly, a second subject with HSD and histopathologically mild AD (Braak stage II) assessed 6 years prior to autopsy showed a pattern of impaired memory for verbal (stories) and visual (paired associates) material, impaired complex sequencing (Trails B), and conceptual impairment in understanding tasks. Performance on visuo-spatial construction (drawings) and simple mental and spatial sequencing tasks (WMS-R mental control, Trails A) were relatively spared. Some depression and agitation was noted, but the time of onset of the behavioral symptoms seemed to follow the cognitive deficits.

A subject with HSD, a past left frontoparietal craniotomy (for a subarachnoid cyst) and concurrent AD was evaluated 10 and 7 years prior to death. Initial testing showed above average intelligence (FSIQ = 123) with intact function, except for some problems with encoding complex material (stories). Testing 3 years later revealed a significant drop in overall IQ (FSIQ = 91), simple and complex visuo-spatial tracking (Trails A/B), verbal fluency (to both letter and category tasks), and memory (verbal worse than visual).

Another subject with progressive supranuclear palsy as well as HSD had neuropsychological testing done 6 years prior to death and showed significant depression but minimal cognitive deficits. Minor deficits were present in the area of memory, with poor learning, impaired delayed recall of word lists and poor delayed recall of visual designs.

The final subject had HSD as the only major pathologic diagnosis. Neuropsychological testing was done one to 2 years after symptom onset, 4 years prior to death. Features included a depressed and lethargic affect and a low average performance on the intelligence scale (FSIQ = 86). Memory was notably impaired for both learning and delayed recall of verbal material. Visual memory was in the low average range for immediate recall, but was significantly impaired after a delay. The participant was very concrete and performed poorly on frontal/executive tasks (Trails B, Stroop and Booklet Category tests). Fluency and confrontation naming were also impaired below that expected for his general level of intelligence.

Gross findings. The gross findings are summarized in Table 2. The brain weights varied from 780 to 1360 g, with a mean of 1063 g. All cases except one had noticeable gyral atrophy. In 9 cases this was focally severe in the frontal and/or temporal lobes; 3 of these were described as 'knife-edged." Two cases had old infarcts in the inferior temporal lobe; in one this was consistent with a posterior cerebral artery occlusion while the other was more consistent with a middle cerebral artery occlusion. All cases except one had severe atrophy of the hippocampus; in one additional case gross pathology could not be evaluated due to the effects of a large acute subdural hematoma. Of the 12 with hippocampal atrophy, 11 also had severe atrophy of the parahippocampal gyrus and in 4 the area of severe atrophy extended to the adjacent inferior temporal neocortical gyri. Five cases had severe atrophy of the amygdalae while 4 cases had marked atrophy of the mammillary bodies. All but 2 cases had moderate or marked dilatation of the lateral ventricles. In 3 cases there was moderate or severe atrophy of the caudate and/or putamen. The substantia nigra was markedly depigmented in 6 cases and moderately depigmented in 4. Four cases showed moderate atrophy of cerebellar folia; 2 of these affected the superior vermis, one involved the inferior vermis and one other affected the hemispheric cortex.

Microscopic appearance-classical stains. Staining with H&E and cresyl violet revealed hippocampal sclerosis in all 14 cases (Figure 1A). This consisted of complete or near-complete neuron loss in all or part of area CA1 (in some cases proximal portions of CA1 were

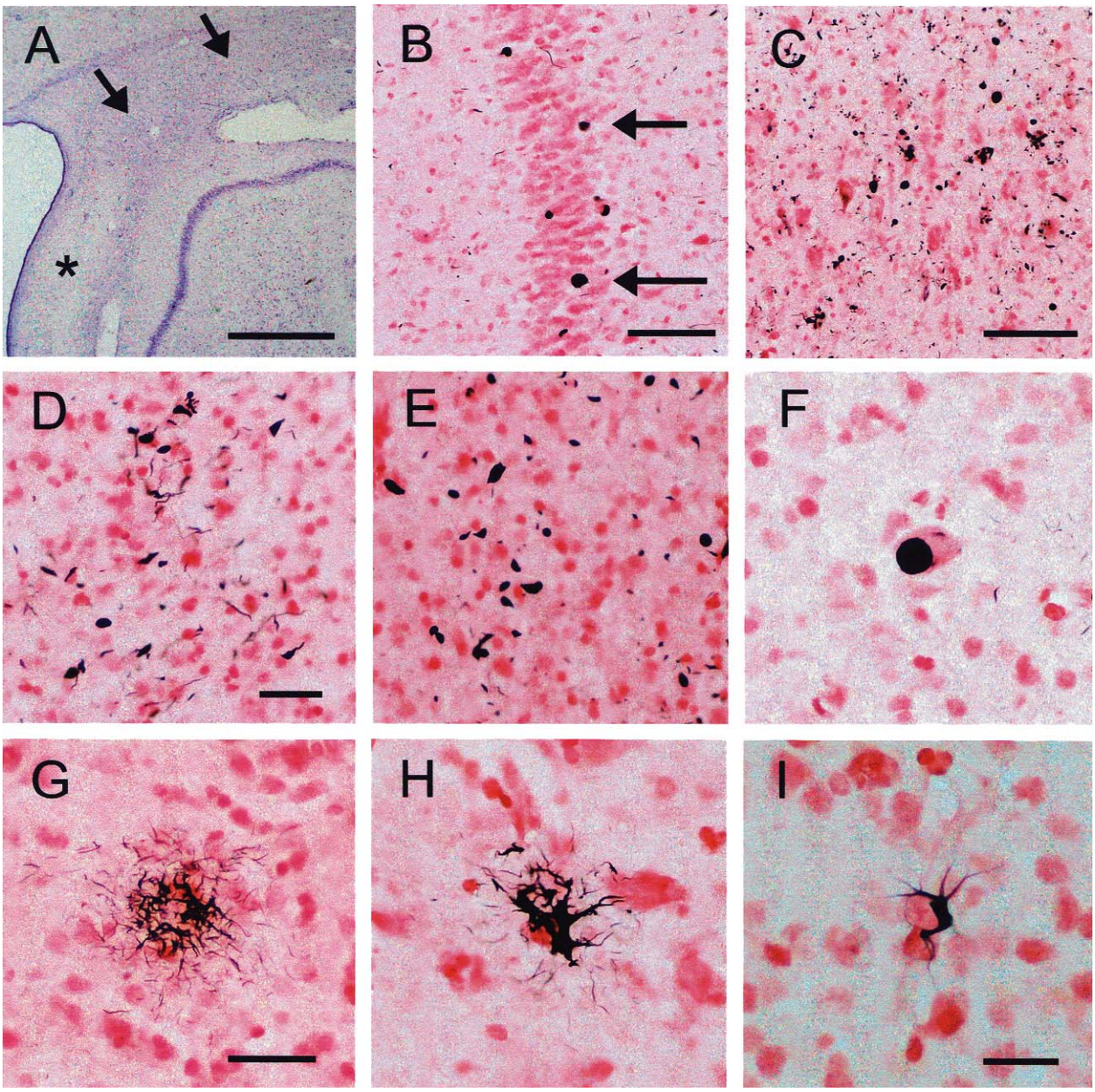


Figure 1. The section depicted in A has been stained with cresyl violet; while B through I are from sections stained with the Gallyas stain and counterstained with Neutral Red. All panels are taken from 40 μm fixed frozen sections. (**A**) Low-magnification photomicrograph of the hippocampus in an HSD case. A sclerotic portion of CA1 is marked by arrows. In comparison, a non-sclerotic, proximal part of CA1 and CA2 is also present (asterisk). Calibration bar equals 2 mm. (**B**) Gallyas-positive neurons in the dentate gyrus granule cell layer (arrows). Calibration bar equals 200 μm. (**C**) Argyrophilic grains, dots and short threads in the CA3 subfield of hippocampus. Calibration bar equals 100 μm. (**D**) Argyrophilic grains and threads in a sclerotic portion of CA1. Calibration bar equals 20 μm and serves for D-F. (**E**) Argyrophilic grains in the transentorhinal cortex. (**F**) Pick body-like neuronal inclusion in superior frontal gyrus. (**G**) Tufted astrocyte in superior frontal gyrus. Calibration bar equals 20 μm and serves for H also. (**H**) Thorned astrocyte in the globus pallidus. (**I**) Coiled body in frontal lobe white matter. Calibration bar equals 10 μm.

spared). Twelve cases had severe gliosis of the hippocampal formation and parahippocampal gyrus (the latter including subiculum, entorhinal cortex and transentorhinal cortex). In these cases there was marked neuron loss in the subiculum in addition to CA1 neuron loss. In 2 cases limbic gliosis and neuron loss was

Case #	Major Neuropathology Dx	CERAD Neuritic Plaque Density*	Braak Stage	CERAD NP AD Dx
1	HS, argyrophilic grains	Sparse	II	Not AD
2	AD, HS	Moderate	VI	Probable
3	AD, HS, small old infarctions CBL and SI	Moderate	V	Probable
4	PSP, PD, HS	Sparse	III	Possible
5	AD, HS, atrophy of superior cerebellar vermis	Zero	IV	Not AD
6	AD, VaD, HS, argyrophilic grains	Frequent	V	Definite
7	AD, HS, argyrophilic grains, acute purulent meningitis	Moderate	III	Probable
8	PD, AD, HS, argyrophilic grains	Frequent	III	Definite
9	PD, HS, argyrophilic grains, acute subdural hematoma	Frequent	III	Possible (not demented)
10	HS, argyrophilic grains	Sparse	III	Not AD
11	AD, HS, argyrophilic grains	Moderate	II	Probable
12	HS	Zero	II	Not AD
13	HS, argyrophilic grains	Sparse	III	Not AD
14	HS, atrophy of superior cerebellar vermis	Zero	I	Not AD

Table 3. *Neuropathologic scores and diagnoses of hippocampal sclerosis cases.* HS = hippocampal sclerosis; AD = Alzheimer's disease; PSP = progressive supranuclear palsy; PD = Parkinson's disease; VaD = Vascular dementia; CBL = cerebellum; SI = substantia innominata. * all cases are over age 75.

restricted to area CA1 of the hippocampus. In 9 of the 14 cases, HS was bilateral while in 5 it was unilateral; since examination of the contralateral hippocampus was restricted to a single anteroposterior plane, there is a possibility that it may have been present bilaterally in all cases, if more levels had been examined. Of the cases with unilateral HS, 4 of the 5 had noticeable neuronal loss and gliosis in the non-sclerotic side.

There was variable gliosis of neocortical areas. In 8 of the 14 cases this was judged as severe in at least one location, usually the frontal and/or temporal lobes; in one case the parietal lobe was also severely affected. The second cortical layer usually was the most gliotic and the gliosis here was often accompanied by considerable spongiosus. In 4 cases the full cortical thickness was gliosed. Frontal and/or temporal neocortical neuronal loss was readily apparent in 2 cases.

Of subcortical regions, severe gliosis of the amygdala was present in 8 of the 14 cases, with mild to moderate gliosis in 5 cases. The caudate nucleus and/or putamen showed variable gliosis in 8 cases. Of diencephalic areas, 3 cases showed gliosis of the hypothalamus, 4 cases had swollen neurons in the hypothalamus, 2 had gliotic mammillary bodies (but without evidence of Wernicke's encephalopathy) and 2 showed thalamic gliosis.

The substantia nigra showed pigmented neuron depletion (more than expected by age alone) in 7 cases, accompanied by increased amounts of extraneuronal pigment and gliosis; 3 cases were severe in terms of these changes while the others were judged to be moderate. Lewy bodies were present (cortical, subcortical and brainstem regions) in 3 cases.

The cerebellum showed patchy Purkinje cell loss in 4 cases and dentate nucleus changes in 4 cases (gliosis, swollen neurons, increased lipopigment in 3 cases, while one case, with the additional diagnosis of PSP, showed dentate nucleus grumose degeneration).

Staining with the Campbell-Switzer, Gallyas and Thioflavine S stains demonstrated the presence of neocortical senile plaques in 12 of the 14 cases. In most cases, diffuse plaques were more numerous than neuritic and/or cored plaques. The latter attained frequent densities in 3 cases, moderate densities in 4 cases and sparse densities in 4 cases; one case had diffuse plaques only. Neocortical neurofibrillary tangles were abundant and widespread in 3 cases (Braak Stage V or VI) while in 11 cases these were absent, rare or sparsely present (Braak Stages I-IV). In the hippocampus, sparse to moderate tangles were present in some cases where part of the proximal CA1 region was spared from HS, but otherwise tangles were rare or sparse in hippocampal subfields. Ghost tangles were not present in sclerotic regions of CA1. In the entorhinal cortex, tangles were present in all cases, attaining frequent densities in 7. The caudate and/or putamen showed rare or sparse tangles in 5 of the 14 cases while the globus pallidus had sparse to moderate densities in 2 cases. The subthalamic nucleus had rare tangles in one case and frequent tangles in 2 cases. Seven cases displayed tangles at rare to sparse densities in the substantia nigra. Argyrophilic

Case #	Gallyas	AT8	Blot	Genotype
1	AG	AG, PRT, AX, T	3R/4R	H1H1
2	Negative	CB, PRT, NPT, AX, T	3R/4R	H1H2
3	Negative	CB, PRT, PA, FA, NPT, AX, T	3R/4R	H1H2
4	NI, PRT, TA, CB	CB, PRT, PA, FA, AX, T	3R/4R	H1H1
5	Negative	PRT, PA, NPT, AX, T	3R/4R	H1H1
6	AG	AG, PRT, PA, NPT, AX, T	3R/4R	H2H2
7	AG	AG, CB, PRT, TA, PA, FA, NPT, AX, T	3R/4R	H2H2
8	AG	AG, PRT, PA, AX, T	3R/4R	H1H2
9	AG	AG, PRT, TA, ThA, PA, FA, NPT, AX, T	3R/4R	H2H2
10	AG, NI, PRT, CB	AG, PRT, PA, AX, T	3R/4R	H1H1
11	AG, CB, TA	AG, CB, PRT, TA, PA, FA, NPT, AX, T	3R/4R	H1H1
12	Negative	Negative	Negative	H2H2
13	AG, PRT, TA, ThA, CB, APL	AG, PRT, CB, TA, ThA, PA, FA, NPT, AX, T	3R/4R	H1H1
14	Negative	Negative	Negative	H1H2

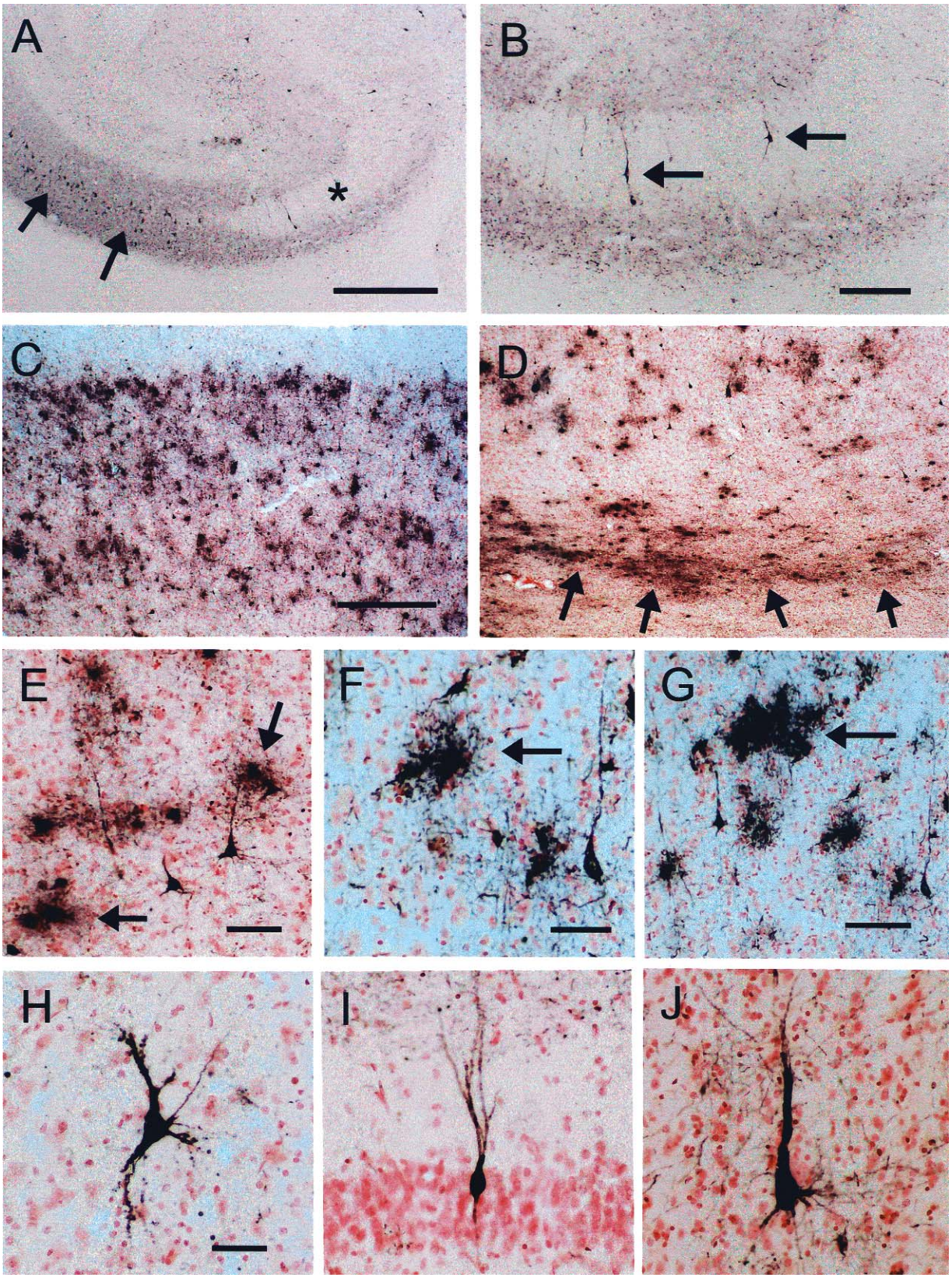
Table 4. *Presence and characteristics of abnormal tau accumulation in hippocampal sclerosis cases.* Alzheimer-type changes, ie, Neurofibrillary tangles, neuritic plaques, are excluded (see Table 3). AG = argyrophilic grains; NI = solid neuronal inclusions (not tangles); NPT = neuropil threads; CB = coiled bodies; PRT = pretangle neurons; TA = tufted astrocytes; PA = protoplasmic astrocytes; ThA = thorned astrocytes; FA = fibrous astrocytes; APL = astrocytic plaques; AX = axon-like; T = terminal-like.

grains, which were mostly localized to the insula, amygdala, hippocampus, parahippocampal and fusiform gyri, were present in 8 cases (Figure 1C, D, E). Neuronal Gallyas-positive structures, resembling "pretangles" or the small neuronal inclusions typical of corticobasal degeneration, were seen in neocortical regions of 3 cases; one case had rare Pick-body-like neuronal inclusions (Figure 1F) and one case displayed frequent small tangles in the granule cells of the dentate gyrus (Figure 1B). Gallyas-positive glial structures were apparent in 4 cases; these were present in neocortical and limbic areas as well as cerebral white matter, caudate nucleus and putamen. Coiled bodies (Figure 1I) were most frequently seen (4 cases), followed by tufted astrocytes (3 cases; Figure 1G), thorned astrocytes (one case; Figure 1H) and astrocytic plaques (one case). These Gallyas-positive structures were similar to those which have previously been described in cases of PSP, CBD and FTDP-17 (12, 33).

Four cases had microscopically-verified old cerebral infarcts. In 3 these were small and few in number, and were located in the basal ganglia, inferior temporal neocortex or cerebellar cortex. In one case there were frequent old cortical microinfarcts and one large old infarct of the inferior surfaces of the occipital and temporal lobe, consistent with a posterior cerebral artery occlusion. Cerebral white matter rarefaction was present in 6 of the 14 cases.

Neuropathologic scores and diagnoses. Neuropathology scores and diagnoses are summarized in Table 3. All cases received the diagnosis of hippocampal sclerosis. Six cases met CERAD criteria (39) for probable or definite AD; of these, 2 were "definite AD" and 4 were "probable AD". Neurofibrillary tangle distribution was staged by the method of Braak (8); 3 cases were Braak stage V or VI, 6 were stage III or IV and 5 were stage I or II. Three cases received National Institute on Aging-Reagan Institute (NIA-RI) (28) designa-

Figure 2. (Opposing page) All panels are from 40 μm fixed frozen sections stained immunohistochemically with the AT8 antibody. Sections C-J were counterstained with Neutral Red. (**A**) Low magnification photomicrograph of the hippocampus, showing tau-reactive pyramidal neurons in the CA2 subfield (arrows). The asterisk marks area CA3. Calibration bar equals 2 mm. (**B**) The arrows point to tau-immunoreactive pyramidal cells in area CA3. Calibration bar equals one mm. (**C**) Low magnification photomicrograph of cingulate gyrus cortex, showing pattern of tau-immunoreactive astrocytic clusters interspersed with positive neurons. Calibration bar equals 2 mm and serves for D as well. (**D**) Low magnification photomicrograph of inferior temporal gyrus cortex, showing band of dense astrocytic staining in the white matter immediately underlying the cortex (arrows). (**E**) Tau-immunoreactive pyramidal neurons and protoplasmic astrocytes (arrows) in cortex of superior frontal gyrus. Calibration bar equals 100 μm. (**F**) Tau-immunoreactive pyramidal neuron and protoplasmic astrocytes (arrow) in cortex of superior frontal gyrus. Calibration bar equals 50 μm. (**G**) A cluster of tau-immunoreactive protoplasmic astrocytes (arrow) in cortex of superior frontal gyrus. Calibration bar equals 100 μm. (**H**) Modified pyramidal cell in CA4 region of hippocampus. Calibration bar equals 25 μm and also serves for I and J. (**I**) Tau-immunoreactive dentate granule cell neuron. (**J**) Tau-immunoreactive pyramidal neuron in cortex of superior frontal gyrus.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tions of "high probability" that dementia was due to AD; the remaining cases were "intermediate," "low," or "zero." Three cases were diagnosed with Parkinson's disease due to their initial presentation with signs of parkinsonism and the presence of Lewy bodies at autopsy. One of these also met diagnostic criteria for progressive supranuclear palsy (21) due to the presence of neurofibrillary tangles in the putamen, globus pallidus, subthalamic nucleus, substantia nigra, and dentate nucleus as well as the presence of tufted astrocytes in the cerebral cortex and putamen and grumose degeneration in the dentate nucleus. One case was diagnosed with vascular dementia due to the presence of frequent old cortical microinfarcts, a large temporal and occipital lobe infarct and severe cerebral white matter rarefaction.

Immunohistochemical staining for tau protein. Features of abnormal tau accumulation are summarized in Table 4. Staining with the AT8, PHF-1 and Alz-50 antibodies was qualitatively similar, but the AT8 antibody revealed much higher densities of immunoreactive structures than that seen with the other 2 antibodies. The following description and figures are therefore derived from staining obtained with the AT8 antibody.

Twelve of the 14 cases showed similar staining patterns, with frequent tau-positive structures similar to those described for FTDP-17 cases. Two cases showed only rare AT8-positive structures. Positive tau staining was most frequently present in frontal, cingulate, insular and temporal association neocortex, as well as the hippocampus and parahippocampal gyrus. Other areas affected included cerebral white matter, thalamus and putamen. The amygdala, parietal and occipital lobe cortex, cerebellum and brainstem regions were not assessed consistently for tau staining.

Affected regions showed frequent AT8-positive neuronal structures. Neuronal staining most frequently was of the "pre-tangle" type or had a Golgi-like appearance, with diffuse cytoplasmic staining of the perikarya and dendrites (Figure 2A-J). Affected neurons were often of the pyramidal type; pyramidal neurons in hippocampal subfields CA4 through CA1 (when proximal CA1 regions were not sclerotic) were often heavily affected (Figure 2A, B, H). Small multipolar and granular neuronal morphologies were also seen. In several cases some dentate granule cells were diffusely and strongly stained, including their apical dendrites rising into the molecular layer (Figure 2I). Neurofibrillary tangles of AD type were rare to absent in neocortical areas except in the 3 cases which had coincident AD with Braak stage V or VI. Rare Pick body-like inclusions were seen in the frontal cortex of one case. Grains identical to those seen with the Gallyas stain (argyrophilic grains) were seen in all 8 cases which had Gallyas-positive grains. Dots, tiny grains and thread-like staining, resembling axons and presynaptic terminals, were even more frequently present, especially in the hippocampal formation, subiculum, entorhinal and transentorhinal cortex. Sclerotic regions of CA1 always contained dense fields of these.

Positive glial staining included forms consistent with oligodendroglial and astrocytic morphologies (13, 33). Coiled bodies, thought to be contained within oligodendroglial cell bodies, were often present in large numbers in both gray and white matter (Figure 3F). Astrocytic-like staining patterns included tufted (Figure 3E), thorned, fibrous (Figure 3D) and protoplasmic forms (Figures 2E, F, G; 3A, B, C). The latter were particularly prominent, often forming densely-packed clusters (Figure 2C, D, G), giving the impression, at low magnification, that these were reactions to neuritic plaques, as has been described previously for fibrous astrocytes in aging and Alzheimer's disease (4). However, this possibility was ruled out since these clusters of protoplasmic astrocytes were also densely present in cases which lacked diffuse or neuritic plaques. Although protoplasmic astrocytes have not been previously described as being a feature of the tauopathies, we decided these astrocytes were best described as protoplasmic astrocytes since their processes have numerous knobby spines, similar to those originally described by Cajal (11). Fibrous astrocytes have smooth processes without spines. Also, fibrous astrocytes stain well with antibodies against glial fibrillary acidic protein (GFAP), while protoplasmic astrocytes are not detected with GFAP immunocytochemistry. Double-staining with antibodies to glial fibrillary acidic protein (not shown) showed that only very rare astrocytes of protoplasmic morphology were double-labeled; this is consistent with the known relative lack of GFAP in protoplasmic astrocytes. In several cases a dense band of immunoreactive fibrous astrocytes was present in the subneocortical U-fibre region of the white matter (Figure 2D). Astrocytic plaques similar to those seen in corticobasal degeneration were seen in 2 cases (not shown).

Apolipoprotein E genotyping. Apolipoprotein E genotype was determined for all cases. There were 8 cases with the 3/3 genotype (57%), 4 with 3/4 (29%) and 2 with 2/3 (14%).

Tau gene sequence findings. No tau gene mutations were discovered in any of the 14 cases. Seven cases had

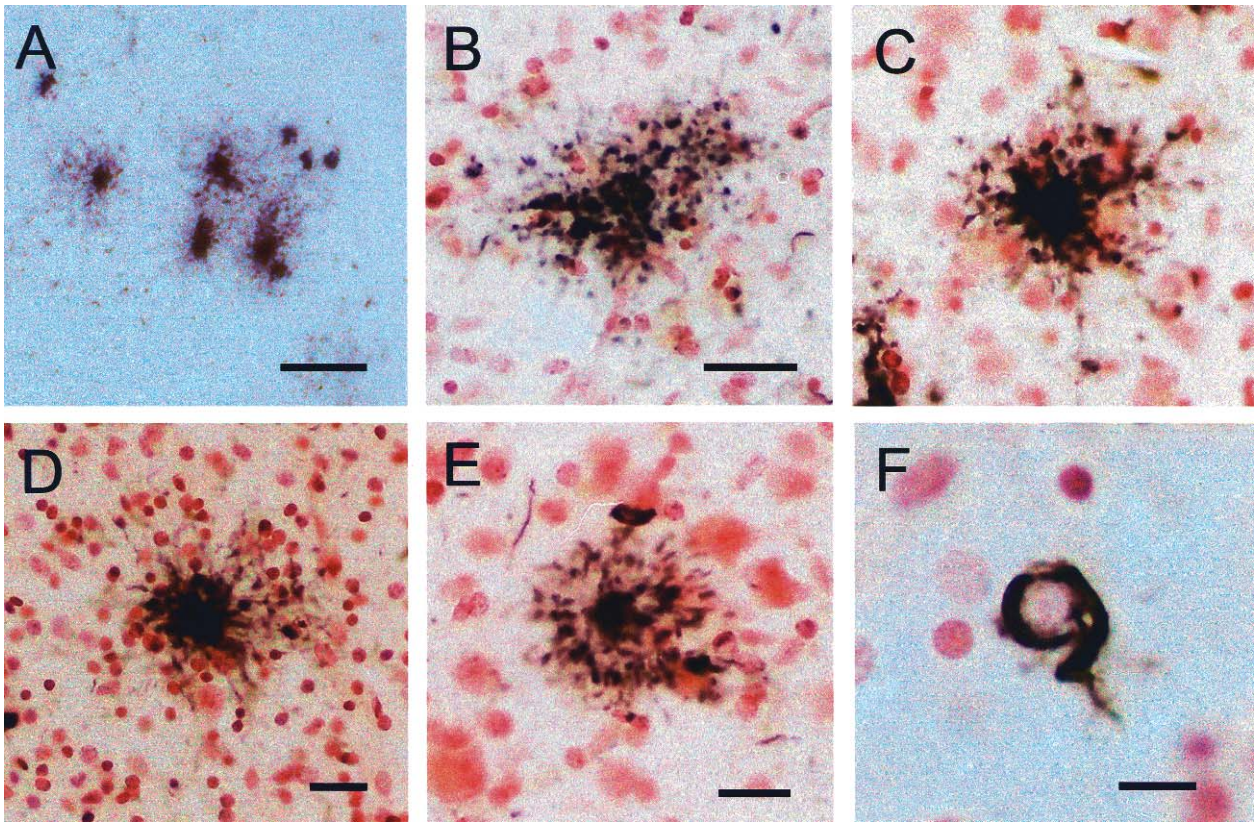

Figure 3. Tau-immunoreactive glial structures in 40 μm fixed frozen sections stained with the AT8 antibody; sections B-F were counterstained with Neutral Red. (**A**) Tau-immunoreactive protoplasmic astrocytes with a "hairy" morphology, located in cortex of cingulate gyrus. Calibration bar equals 100 μm. (**B**) and (**C**) Protoplasmic astrocytes in cortex of middle frontal gyrus and inferior temporal gyrus, respectively. Calibration bar equals 50 μm. (**D**) Tau-immunoreactive astrocyte with "hairy" fibrous morphology in white matter of frontal lobe. Calibration bar equals 30 μm. (**E**) Tufted astrocyte in cortex of superior frontal gyrus. Calibration bar equals 20 μm. (**F**) Coiled body in white matter of parahippocampal gyrus. Calibration bar equals 10 μm.

the H1H1 haplotype (50%), 4 were H1H2 (29%) and 3 were H2H2 (21%).

Tau immunoblot findings. The pattern and intensity of bands representing sarkosyl-soluble tau protein did not differ appreciably from that seen in normal control cases. Ten of the 12 HS cases analyzed, however, had significant amounts of sarkosyl-insoluble tau protein. The cases which lacked sarkosyl-insoluble tau were also negative for significant tauopathy with AT8 immunohistochemistry. Western blots showed that sarkosyl-insoluble tau consisted of a mixture of 3- and 4-repeat isoforms, with a pattern similar to that seen in Alzheimer's disease (Figure 4). This consists of 3 major bands at approximately 55, 64, and 69 kD.

Discussion

The results of this study suggest that a very high proportion of cases with HSD have a generalized cerebral tauopathy whose pathology resembles that which has been described for FTDP-17 families. Screening for known tau mutations was negative. Therefore, it appears that hippocampal sclerosis dementia with tauopathy (HSDT) is a sporadic tauopathy, like progressive supranuclear palsy (PSP), corticobasal degeneration (CBD) and Pick's disease. In our autopsy population, HSDT is slightly less common than PSP (12 cases of HSDT versus 16 of PSP over the same time period) and at least 10 times more common than CBD and Pick's disease (one case of each).

Clinically, HSDT resembles FTDP-17 in that the syndrome is often characterized by a presentation with memory deficits and frontal lobe symptoms with later progression to dementia and parkinsonism. Memory impairment, typically of short-term memory, has been described as an early sign in FTDP-17 in several publications (37, 48) but sometimes this may not occur until later stages (36). In this and other series of HS demen-

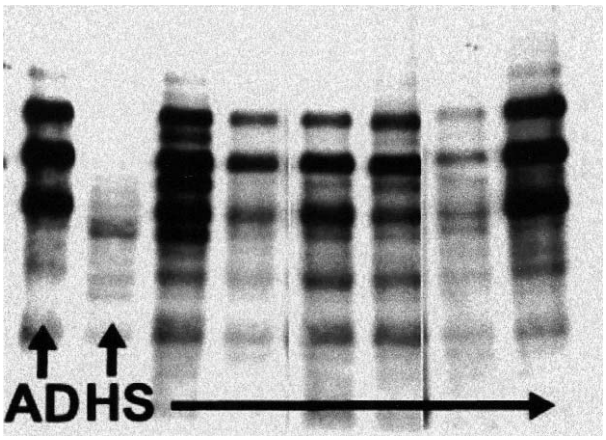

Figure 4. Western blot of sarkosyl-insoluble tau protein reacted with the WKS44 antibody. HS cases are compared with an AD case. Of 12 HS cases analyzed, 10 had significant amounts of sarkosyl-insoluble tau (eg, the 6 cases represented by the 6 lanes on the right side of the panel). Two cases had very low amounts of sarkosyl-insoluble tau (eg, the case represented in the lane adjacent to the AD case). The pattern of bands in HS cases with sarkosyl-insoluble tau resembled that seen in typical AD cases, with 3 major bands at approximately 55, 64 and 69 kD (eg, 3 strongest bands in the AD case). This is consistent with a mixture of 3- and 4-repeat tau.

tia cases, early short-term memory impairment is very common (1, 15, 17, 35, 41) .

Frontal lobe signs in FTDP-17 have included disinhibition, personality changes, aggressive behavior, apathy, lack of personal hygiene, hyperorality, repetitive or compulsive behavior, word-finding difficulty and lack of judgement (40, 45, 37). Similar symptoms have been described as being common presentations of HSD, including disinhibition, odd behavior and social withdrawal (41, 52). In this study, we found that the majority of HSD cases had frontal lobe signs; 4 cases presented with frontal lobe signs while another 4 developed these later.

Parkinsonism is a frequent but usually later occurrence in FTDP-17 cases (37, 48 49) and typically consists of rigidity, bradykinesia and postural instability without a parkinsonian resting tremor. Parkinsonism was not reported to be a common finding in previous publications concerning HSD. One study found bradykinesia and an abnormal gait with poor balance in 8 of the 26 cases, with 6 of these affected by frequent falls (17). We have found parkinsonism to be a prominent and common feature of HSD, with 7 of the 14 cases affected; in addition, 7 cases had a history of frequent falls.

Another common clinical occurrence in both FTDP-17 (19, 48, 49) and HSD (41) is dysphasia, usually of the motor type, which may progress to mutism. In this study, 8 of the 14 of the HSD cases had dysphasic symptoms.

Neuropsychological test results are consistent with the theoretical effects of combined degeneration of the hippocampus and frontal neocortex. While there were too few cases with available testing to draw any definitive conclusions, a general pattern of memory and expressive language deficits, followed by deterioration in some frontally mediated abilities such as sequential set shifting (eg, Trails B) was observed. This pattern seems to hold regardless of a variety of complicating conditions. Pathologically, the major distinction between HSDT and FTDP-17 is the presence of hippocampal sclerosis in the former. A review of FTDP-17 cases (44) states that hippocampal neuron loss is usually mild. Two cases, however, have been reported to have had either severe neuron loss in the hippocampus and subiculum (16) or marked atrophy of the hippocampus and parahippocampal gyrus (40) while another case had a heavily gliotic perforant pathway termination in the dentate gyrus molecular layer (37).

The immunohistochemical morphology of abnormal tau deposits in HSDT closely resembles that which has been reported for FTDP-17, although in both conditions there may be considerable variability from case to case (34). Generally, in both conditions, tau immunoreactivity differs from Alzheimer's disease in that many of the stained structures are glial rather than neuronal. Glial deposits in FDTP-17 are reportedly most common in oligodendrocytes, represented by the coiled body (10, 16, 29, 40, 42, 48, 49); these are also common in HSDT. Astrocytic structures in FTDP-17 and HSDT resemble the tufted astrocytes typical of progressive supranuclear palsy (10, 19) and/or, but much less commonly, the astrocytic plaques characteristic of corticobasal degeneration (19, 38, 42). Common in both conditions is a prominent band of tau-positive astrocytes in the subcortical U-fiber region (16, 19). A feature of HSDT which has not been described in FTDP-17 is the presence of numerous tau-positive astrocytes with a protoplasmic morphology, which often aggregate into large clusters.

In both FTDP-17 and HSDT, neuronal staining usually takes the form of diffuse or granular staining of the perikarya, in the absence of typical silver-positive neurofibrillary tangles (10, 29, 47, 48). This staining, when intense, has the appearance of a Golgi stain. Thread- and dot-like structures, presumably representing neuronal axons, dendrites and presynaptic terminals, are also present in both conditions. Published cases of HSD and FTDP-17 have not specified whether argyrophilic

grains were present; we have found that more than half of HSDT cases possess this change.

Biochemically, the tauopathies are characterized by the presence of hyperphosphorylated, sarkosyl-insoluble tau protein. In FTDP-17, tau isoforms with 4-repeats predominate in cases which have mutations in or around exon 10 while mutations at other sites have a mix of 3- and 4-repeat tau. It has been postulated that the exon 10 splice site mutations disrupt alternative splicing of tau messenger mRNA (54). In CBD and PSP, 4 repeat isoforms predominate while in AD there is a mix of 3- and 4-repeat forms (9). We have found that HSDT cases have a mix of 3- and 4-repeat tau. The presence of argyrophilic grains in 8 of the 12 HSDT cases suggests, however, that 4-repeat tau may predominate within some neuronal subsets, as a recent report has documented that argyrophilic grains contain mostly 4-repeat tau (51).

No tau mutations were found in any of the 14 cases of HSD. As 5 of our cases had a strong family history, there is a possibility that we may have missed mutations that may exist outside of the tau gene regions examined, or that mutations in genes other than tau may be responsible for familial cases. For example, apparently pathogenic tau mutations have recently been described in exon 1 (22), which had previously not been thought to harbor tau mutations. The fact that these mutations are very rare suggests, however, that HSDT—like PSP, CBD, and Pick's disease—may be a sporadic condition. Two other novel sporadic tauopathies have recently been described as single case reports (5, 6).

Some reports have suggested that certain tau and apolipoprotein E polymorphisms may be associated with specific tauopathies. The H1 tau haplotype has a statistically significant association with PSP and CBD (18, 23, 25) while the apoE ϵ4 allele may be associated with Pick's disease (32, 39, 43). Sporadic cases of frontotemporal degeneration with anomic aphasia have increased H2 and ϵ4 frequencies (30, 44). In the HSD cases of this series, there was a predominance of the H1 haplotype and an increased frequency of the ϵ4 allele (29%), relative to the general population (published figures usually find a general population prevalence of about 15%). Other studies have found ϵ4 frequencies of 12% (50) and 31% (33) in HSD cases.

Most investigators have assumed that HSD is caused by a global hypoxic-ischemic episode, since it is well established in both experimental animals and human case reports that CA1 neurons may undergo selective cell death after such an event. The literature on HSD, however, documents only 2 such occurrences (dementia developed after myocardial infarctions) (52) among 56 reported cases. It has been speculated that subclinical (15) or occult hypoxic-ischemic events, such as might occur during general anesthesia, may be responsible for the HS that occurs in HSD (17).

The evidence that HSD represents a vascular condition is therefore indirect, relying on the frequent presence of vascular risk factors. A cardio- or cerebrovascular disease history is common in HS dementia patients (15, 17, 35). In one study, 7 (87.5%) of the 8 HS dementia cases had medical histories of cardio- or cerebrovascular disease, compared to only 16% of a control series of AD cases (15). Another study found a clinical history of stroke in 56% of HSD cases as compared with 25% of a control AD series (35); autopsy findings failed to confirm an increased prevalence of infarctions in the HS cases, however. Neither historical or pathologic evidence of cardio- or cerebrovascular disease correlated with the presence or absence of HS in yet another study. In the present study, 76% of HSD cases had a cardiovascular disease history; we did not compare this to a control series. Much larger numbers of HSD and appropriate control cases will be required to adequately address this association in a statistical manner, but the lack of a definite history of a global hypoxic-ischemic event in the overwhelming majority of HSD cases casts considerable doubt on vascular theories of its pathogenesis.

The presence of a widespread tauopathy in HSDT cases suggests that this is a neurodegenerative condition rather than a vascular one. The finding of marked frontal and temporal lobe gyral atrophy and gliosis in most cases suggests that the process is not limited to the hippocampus and adjacent structures. Since hippocampal pyramidal neurons, including those in proximal portions of CA1 that were not sclerotic, were heavily affected by abnormal tau deposition in our series, it seems reasonable to postulate that the extreme loss of CA1 neurons in HS is caused by this. Sclerotic regions did not contain ghost tangles as in AD, but often had high densities of grains, dots and threads. The evidence from human tau mutations, tau transgenic mice and flies is highly suggestive that abnormal accumulation of tau protein is neurotoxic (27, 36) and that neuronal death can occur without the formation of neurofibrillary tangles (45). Neuronal tau deposits in HSDT may cause cell death without forming neurofibrillary tangles, leaving no markers as evidence of their former presence. This may explain the complete loss of CA1 neurons in the absence of ghost tangles.

Two of the 14 HSD cases in this series did not have a tauopathy. The clinical and pathologic profile of these

2 cases did not differ in any obvious way from the cases with tauopathies. Although one of the non-tauopathy cases was non-demented (having only an action tremor and postural instability), another of the cases with tauopathy was also non-demented (but had clinical parkinsonism and memory impairment). The other non-tauopathy case presented with memory impairment and disinhibition, progressing to dementia and aphasia. This syndrome is typical of the tauopathy cases as well. Both non-tauopathy cases had only unilateral HS, but 2 of the 12 tauopathy cases also had only unilateral HS. In the absence of tauopathy, the etiology of HS remains unclear, but it appears that these cases are much less common than HS with tauopathy.

In this series of cases as well as in previous series (15, 17, 35), HSD cases often also met diagnostic criteria for AD. Six of the 14 cases in this series met CERAD criteria for probable or definite AD; in 3 other series 9 of 13 (17), 5 of 8 (15) and 5 of 16 (35) met pathologic criteria for AD. In all of these, the majority of cases were "plaque-only" AD. In our series also, more cases were "plaque-only" AD (4 cases), with only 3 of the 7 possessing substantial numbers of neocortical tangles. The high prevalence of AD-type changes in HSD suggests that one condition or both may increase the risk of developing the other.

Comparison of the clinical features of HSD and AD emphasizes that it is very difficult, if not impossible, to distinguish between the two diseases clinically (1, 15, 35). One group found that HSD cases perform significantly better on Trails A than AD cases, but only on the initial visit (35). As HS in the setting of temporal lobe epilepsy can be readily detected with MR scans, this may offer some hope of distinguishing HSD from AD, particularly if hippocampal atrophy occurs earlier or is significantly more severe in the former condition. Only 5 of the 14 cases in our series received neuroimaging, which included MRI (3 cases), PET (1 case), SPECT (1 case) and CT (3 cases). The reports from these did not, however, comment on hippocampal size.

In summary, HSDT is a multisystem sporadic tauopathy that is associated with memory impairment, dementia and parkinsonism. It is probably the third most common tauopathy, after Alzheimer's disease and progressive supranuclear palsy.

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References

1. Ala TA, Beh GO, Frey WH (2000) Pure hippocampal sclerosis: a rare cause of dementia mimicking Alzheimer's disease. *Neurology* 54:843-848.
2. Arai T, Ikeda K, Akiyama H, Shikamoto Y, Tsuchiya K, Yagishita S, Beach T, Rogers J, Schwab C, McGeer PL (2001) Distinct isoforms of tau aggregated in neurons and glial cells in brains of patients with Pick's disease, corticobasal degeneration and progressive supranuclear palsy. *Acta Neuropathol (Berl)* 101:167-173.
3. Beach TG, Tago H, Nagai T, Kimura H, McGeer PL, McGeer EG (1987) Perfusion-fixation of the human brain for immunohistochemistry: comparison with immersion-fixation. *J Neurosci Methods* 19:183-192.
4. Beach TG, Walker R, McGeer EG (1989) Patterns of gliosis in Alzheimer's disease and aging cerebrum. *Glia* 2:420-436.
5. Berry RW, Quinn B, Johnson N, Cochran EJ, Ghosal N, Binder LL (2001) Pathological glial tau accumulations in neurodegenerative disease: review and case report. *Neurochem Int* 39:469-479.
6. Bigio EH, Lipton AM, Yen SH, Hutton ML, Baker M, Nacharaju P, White CL, Davies P, Lin W, Dickson DW (2001) Frontal lobe dementia with novel tauopathy: sporadic multiple system tauopathy with dementia. *J Neuropathol Exp Neurol* 60:328-341.
7. Braak H, Braak E (1991) Demonstration of amyloid deposits and neurofibrillary changes in whole brain sections. *Brain Pathol* 1:213-216.
8. Braak H, Braak E (1991) Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol (Berl)* 82:239-259.
9. Buee L, Delacourte A (1999) Comparative biochemistry of tau in progressive supranuclear palsy, corticobasal degeneration, FTDP-17 and Pick's disease. *Brain Pathol* 9:681-693.
10. Bugiani O, Murrell JR, Giaccone G, Hasegawa M, Ghigo G, Tabaton M, Morbin M, Primavera A, Carella F, Solaro C, Grisoli M, Savoiardo M, Spillantini MG, Tagliavini F, Goedert M, Ghetti B (1999) Frontotemporal dementia and corticobasal degeneration in a family with a P301S mutation in tau. *J Neuropathol Exp Neurol* 58:667-677.
11. Cajal, SRy (1984) Neuroglia. In: *The Neuron and the Glial Cell. Translation of "Textura del Sistema Nervioso del Hombre y de los Vertebrados (1937)*", de la Torre J, Gibson WC (eds. and translation), pp. 263-290, Charles C. Thomas: Illinois.
12. Chin SS, Goldman JE (1996) Glial inclusions in CNS degenerative diseases. *J Neuropathol Exp Neurol* 55:499-508.
13. Clark AW, White CL, Manz HJ, Parhad IM, Curry B, Whitehouse PJ, Lehmann J, Coyle JT (1986) Primary degenerative dementia without Alzheimer pathology. *Can J Neurol Sci* 13:462-470.

14. Clark LN, Poorkaj P, Wszolek Z, Geschwind DH, Nasreddine ZS, Miller B, Li D, Payami H, Awert F, Markopoulou K, Andreadis A, D'Souza I, Lee VM, Reed L, Trojanowski JQ, Zhukareva V, Bird T, Schellenberg G, Wilhelmsen KC (1998) Pathogenic implications of mutations in the tau gene in pallido-ponto-nigral degeneration and related neurodegenerative disorders linked to chromosome 17. *Proc Natl Acad Sci U S A* 95:13103-13107.
15. Corey-Bloom J, Sabbagh MN, Bondi MW, Hansen L, Alford MF, Masliah E, Thal LJ (1997) Hippocampal sclerosis contributes to dementia in the elderly. *Neurology* 48:154-160.
16. Delisle MB, Murrell JR, Richardson R, Trofatter JA, Rascol O, Soulages X, Mohr M, Calvas P, Ghetti B (1999) A mutation at codon 279 (N279K) in exon 10 of the Tau gene causes a tauopathy with dementia and supranuclear palsy. *Acta Neuropathol (Berl)* 98:62-77.
17. Dickson DW, Davies P, Bevona C, Van Hoeven KH, Factor SM, Grober E, Aronson MK, Crystal HA (1994) Hippocampal sclerosis: a common pathological feature of dementia in very old (> or = 80 years of age) humans. *Acta Neuropathol (Berl)* 88:212-221.
18. Ezquerra M, Pastor P, Valldeoriola F, Molinuevo JL, Blesa R, Tolosa E, Oliva R (1999) Identification of a novel polymorphism in the promoter region of the tau gene highly associated to progressive supranuclear palsy in humans. *Neurosci Lett* 275:183-186.
19. Goedert M, Spillantini MG, Crowther RA, Chen SG, Parchi P, Tabaton M, Lanska DJ, Markesbery WR, Wilhelmsen KC, Dickson DW, Petersen RB, Gambetti P (1999) Tau gene mutation in familial progressive subcortical gliosis. *Nat Med* 5:454-457.
20. Greenberg SG, Davies P, Schein JD, Binder LI (1992) Hydrofluoric acid-treated tau PHF proteins display the same biochemical properties as normal tau. *J Biol Chem* 267:564-569.
21. Hauw JJ, Daniel SE, Dickson D, Horoupian DS, Jellinger K, Lantos PL, McKee A, Tabaton M, Litvan I (1994) Preliminary NINDS neuropathologic criteria for Steele-Richardson-Olszewski syndrome (progressive supranuclear palsy). *Neurology* 44:2015-2019.
22. Hayashi S, Toyoshima Y, Hasegawa M, Umeda Y, Wakabayashi K, Tokiguchi S, Iwatsubo T, Takahashi H (2002) Late-onset frontotemporal dementia with a novel exon 1 (Arg5His) tau gene mutation. *Ann Neurol* 51:525-530.
23. Higgins JJ, Golbe LI, De Biase A, Jankovic J, Factor SA, Adler RL (2000) An extended 5'-tau susceptibility haplotype in progressive supranuclear palsy. *Neurology* 55:1364-1367.
24. Hixson JE, Vernier DT (1990) Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with HhaI. *J Lipid Res* 31:545-548.
25. Houlden H, Baker M, Morris HR, MacDonald N, Pickering-Brown S, Adamson J, Lees AJ, Rossor MN, Quinn NP, Kertesz A, Khan MN, Hardy J, Lantos PL, George-Hyslop P, Munoz DG, Mann D, Lang AE, Bergeron C, Bigio EH, Litvan I, Bhatia KP, Dickson D, Wood NW, Hutton M (2001) Corticobasal degeneration and progressive supranuclear palsy share a common tau haplotype. *Neurology* 56:1702-1706.
26. Hutton M (2001) Missense and splice site mutations in tau associated with FTDP-17: multiple pathogenic mechanisms. *Neurology* 56:S21-S25.
27. Hutton M (2001) Missense and splice site mutations in tau associated with FTDP-17: multiple pathogenic mechanisms. *Neurology* 56:S21-S25.
28. Hyman BT, Trojanowski JQ (1997) Consensus recommendations for the postmortem diagnosis of Alzheimer disease from the National Institute on Aging and the Reagan Institute Working Group on diagnostic criteria for the neuropathological assessment of Alzheimer disease. *J Neuropathol Exp Neurol* 56:1095-1097.
29. Iijima M, Tabira T, Poorkaj P, Schellenberg GD, Trojanowski JQ, Lee VM, Schmidt ML, Takahashi K, Nabika T, Matsumoto T, Yamashita Y, Yoshioka S, Ishino H (1999) A distinct familial presenile dementia with a novel missense mutation in the tau gene. *Neuroreport* 10:497-501.
30. Ingelson M, Fabre SF, Lilius L, Andersen C, Viitanen M, Almkvist O, Wahlund LO, Lannfelt L (2001) Increased risk for frontotemporal dementia through interaction between tau polymorphisms and apolipoprotein E epsilon4. *Neuroreport* 12:905-909.
31. Jellinger KA (1994) Hippocampal sclerosis: a common pathological feature of dementia in very old humans. *Acta Neuropathol (Berl)* 88:-599.
32. Kalman J, Juhasz A, Majtenyi K, Rimanoczy A, Jakab K, Gardian G, Rasko I, Janka Z (2000) Apolipoprotein E polymorphism in Pick's disease and in Huntington's disease. *Neurobiol Aging* 21:555-558.
33. Komori T (1999) Tau-positive glial inclusions in progressive supranuclear palsy, corticobasal degeneration and Pick's disease. *Brain Pathol* 9:663-679.
34. Lantos PL, Cairns NJ, Khan MN, King A, Revesz T, Janssen JC, Morris H, Rossor MN (2002) Neuropathologic variation in frontotemporal dementia due to the intronic tau 10(+16) mutation. *Neurology* 58:1169-1175.
35. Leverenz JB, Agustin CM, Tsuang D, Peskind ER, Edland SD, Nochlin D, DiGiacomo L, Bowen JD, McCormick WC, Teri L, Raskind MA, Kukull WA, Larson EB (2002) Clinical and neuropathological characteristics of hippocampal sclerosis: a community-based study. *Arch Neurol* 59:1099-1106.
36. Lewis J, Dickson DW, Lin WL, Chisholm L, Corral A, Jones G, Yen SH, Sahara N, Skipper L, Yager D, Eckman C, Hardy J, Hutton M, McGowan E (2001) Enhanced neurofibrillary degeneration in transgenic mice expressing mutant tau and APP. *Science* 293:1487-1491.
37. Lynch T, Sano M, Marder KS, Bell KL, Foster NL, Defendini RF, Sima AA, Keohane C, Nygaard TG, Fahn S (1994) Clinical characteristics of a family with chromosome 17-linked disinhibition-dementia-parkinsonism-amyotrophy complex. *Neurology* 44:1878-1884.
38. Mirra SS, Murrell JR, Gearing M, Spillantini MG, Goedert M, Crowther RA, Levey AI, Jones R, Green J, Shoffner JM, Wainer BH, Schmidt ML, Trojanowski JQ, Ghetti B (1999) Tau pathology in a family with dementia and a P301L mutation in tau. *J Neuropathol Exp Neurol* 58:335-345.

39. Morris HR, Schrag A, Nath U, Burn D, Quinn NP, Daniel S, Wood NW, Lees AJ (2001) Effect of ApoE and tau on age of onset of progressive supranuclear palsy and multiple system atrophy. *Neurosci Lett* 312:118-120.
40. Nasreddine ZS, Loginov M, Clark LN, Lamarche J, Miller BL, Lamontagne A, Zhukareva V, Lee VM, Wilhelmsen KC, Geschwind DH (1999) From genotype to phenotype: a clinical pathological, and biochemical investigation of frontotemporal dementia and parkinsonism (FTDP-17) caused by the P301L tau mutation. *Ann Neurol* 45:704-715.
41. Rasmusson DX, Brandt J, Steele C, Hedreen JC, Troncoso JC, Folstein MF (1996) Accuracy of clinical diagnosis of Alzheimer disease and clinical features of patients with non-Alzheimer disease neuropathology. *Alzheimer Dis Assoc Disord* 10:180-188.
42. Reed LA, Schmidt ML, Wszolek ZK, Balin BJ, Soontornniyomkij V, Lee VM, Trojanowski JQ, Schelper RL (1998) The neuropathology of a chromosome 17-linked autosomal dominant parkinsonism and dementia ("pallidoponto-nigral degeneration"). *J Neuropathol Exp Neurol* 57:588-601.
43. Schneider JA, Gearing M, Robbins RS, de l'Aune W, Mirra SS (1995) Apolipoprotein E genotype in diverse neurodegenerative disorders. *Ann Neurol* 38:131-135.
44. Short RA, Graff-Radford NR, Adamson J, Baker M, Hutton M (2002) Differences in tau and apolipoprotein E polymorphism frequencies in sporadic frontotemporal lobar degeneration syndromes. *Arch Neurol* 59:611-615.
45. de Silva R, Farrer M (2002) Tau neurotoxicity without the lesions: a fly challenges a tangled web. *TINS* 25:327-329.
46. Spillantini MG, Bird TD, Ghetti B (1998) Frontotemporal dementia and Parkinsonism linked to chromosome 17: a new group of tauopathies. *Brain Pathol* 8:387-402.
47. Spillantini MG, Crowther RA, Kamphorst W, Heutink P, van Swieten JC (1998) Tau pathology in two Dutch families with mutations in the microtubule-binding region of tau. *Am J Pathol* 153:1359-1363.
48. Spillantini MG, Goedert M, Crowther RA, Murrell JR, Farlow MR, Ghetti B (1997) Familial multiple system tauopathy with presenile dementia: a disease with abundant neuronal and glial tau filaments. *Proc Natl Acad Sci U S A* 94:4113-4118.
49. Spillantini MG, Murrell JR, Goedert M, Farlow MR, Klug A, Ghetti B (1998) Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. *Proc Natl Acad Sci U S A* 95:7737-7741.
50. Sulkava R, Haltia M, Paetau A, Wikstrom J, Palo J (1983) Accuracy of clinical diagnosis in primary degenerative dementia: correlation with neuropathological findings. *J Neurol Neurosurg Psychiatry* 46:9-13.
51. Togo T, Sahara N, Yen SH, Cookson N, Ishizawa T, Hutton M, de Silva R, Lees A, Dickson DW (2002) Argyrophilic grain disease is a sporadic 4-repeat tauopathy. *J Neuropathol Exp Neurol* 61:547-556.
52. Troncoso JC, Kawas CH, Chang CK, Folstein MF, Hedreen JC (1996) Lack of association of the apoE4 allele with hippocampal sclerosis dementia. *Neurosci Lett* 204:138-140.
53. Volpe BT, Petito CK (1985) Dementia with bilateral medial temporal lobe ischemia. *Neurology* 35:1793-1797.
54. Yen SH, Hutton M, DeTure M, Ko LW, Nacharaju P (1999) Fibrillogenesis of tau: insights from tau missense mutations in FTDP-17. *Brain Pathol* 9:695-705.
55. Zweig RM, Schegg KM, Peacock JH, Melarkey D (1989) A case of Alzheimer's disease and hippocampal sclerosis with normal cholinergic activity in basal forebrain, neocortex, and hippocampus. *Neurology* 39:288-290.