J Neuropathol Exp Neurol
Vol. 64, No. 5
May 2005
pp. 420–428

ORIGINAL ARTICLES

Neuropathologic, Biochemical, and Molecular Characterization of the Frontotemporal Dementias

Ryan T. Mott, MD, Dennis W. Dickson, MD, John Q. Trojanowski, MD, PhD, Vicki Zhukareva, PhD, Virginia M. Lee, PhD, Mark Forman, MD, PhD, Vivianna Van Deerlin, MD, PhD, John F. Ervin, BA, Deng-Shun Wang, MD, PhD, Donald E. Schmechel, MD, and Christine M. Hulette, MD

Abstract

The frontotemporal dementias (FTDs) are a heterogeneous group of neurodegenerative disorders that are characterized clinically by dementia, personality changes, language impairment, and occasionally extrapyramidal movement disorders. Historically, the diagnosis and classification of FTDs has been fraught with difficulties, especially with regard to establishing a consensus on the neuropathologic diagnosis. Recently, an international group of scientists participated in a consensus conference to develop such neuropathologic criteria. They recommended a diagnostic classification scheme that incorporated a biochemical analysis of the insoluble tau isoform composition, as well as ubiquitin immunohistochemistry. The use and reliability of this classification system has yet to be examined. In this study, we evaluated 21 cases of FTD. Using traditional histochemical stains and tau protein and ubiquitin immunohistochemistry, we separated each case into one of the following categories: classic Pick disease (PiD; n = 7), corticobasal degeneration (CBD; n = 5), dementia lacking distinctive histopathologic features (DLDH; n = 4), progressive supranuclear palsy (PSP; n = 2), frontotemporal lobar degeneration with motor neuron disease or motor neuron disease-type inclusions (FTLD-MND/MNI; n = 2), and neurofibrillary tangle dementia (NFTD; n = 1). Additionally, we independently categorized each case by the insoluble tau isoform pattern, including 3R (n = 5), 4R (n = 7), 3R/4R (n = 3), and no insoluble tau (n = 6). As suggested by the proposed diagnostic scheme, we found that the insoluble tau isoform patterns correlated strongly with the independently derived histopathologic diagnoses ($p < 0.001$). The data show that cases containing predominantly 3R tau were classic PiD (100%). Cases with predominantly 4R tau were either CBD (71%) or PSP (29%). Cases with both 3R and 4R tau were either a combination of PiD and Alzheimer disease (67%) or NFTD (33%). Finally, cases with no insoluble tau were either DLDH (67%) or FTLD-MND/MNI (33%). To further characterize these cases, we also performed quantitative Western blots for soluble tau, APOE genotyping, and, in selected cases, tau gene sequencing. We show that soluble tau is reduced in DLDH and FTLD-MND/MNI and that APOE4 is overrepresented in PiD and DLDH. We also identified a new family with the R406W mutation and pathology consistent with NFTD. This study validates the recently proposed diagnostic criteria and forms a framework for further refinement of this classification scheme.

Key Words: Apolipoprotein E, Frontotemporal dementia, Frontotemporal lobar degeneration, Microtubule-associated protein tau.

INTRODUCTION

The frontotemporal dementias (FTDs) are a clinically, genetically, and pathologically heterogeneous group of disorders that occur primarily between the ages of 35 and 75 years with an equal incidence in males and females (1). FTDs are universally fatal with a disease duration that typically ranges from 5 to 10 years. Approximately 20% to 40% of patients have a strong family history of dementia. The clinical presentation is variable, with some patients presenting primarily with frontal lobe signs (personality changes) and others showing mostly temporal lobe dysfunction (language impairment). Indeed, 3 major clinical syndromes are recognized, including frontal variant FTD, semantic dementia, and progressive nonfluent aphasia (2, 3). Additionally, patients may present with features indistinguishable from Alzheimer disease (AD) or may display movement abnormalities similar to those seen in Parkinson disease. FTD is also a recognized feature of motor neuron disease (MND), progressive supranuclear palsy (PSP), and corticobasal degeneration (CBD).

Over a century ago, Arnold Pick described several autopsy cases of severe circumscribed atrophy of the frontal and temporal lobes in patients with a clinical history of dementia and language impairment (4–6). Years later, Alzheimer (7) and Altman (8) provided detailed descriptions of the histopathology, including the key features of argyrophilic inclusions within neurons (Pick bodies) and swollen achromatic cells (Pick cells), as well as the absence of senile plaques and tangles. In 1926, Onari and Spatz (9) introduced the eponym Pick disease (PiD) to describe these cases of progressive

From the Departments of Pathology (Neuropathology) (RTM, DES, CMH), and Medicine (Neurology) (JFE, DES, CMH), Duke University Medical Center, Durham, North Carolina; Departments of Neurosciences (DWD, D-SW) and Pathology (DWD), Mayo Clinic, Jacksonville, Florida; and Department of Pathology (Neuropathology) and Laboratory Medicine and the Institute on Aging (JQT, VZ, VML, MF, VVD), University of Pennsylvania, Philadelphia, Pennsylvania.
Supported by NIA AG05128, NINDS N39764, and Glaxo-Smith-Kline (CMH); P30 AG10124 and PO1 AG17586 (JQT); and Mentored Clinical Scientist Award (K08) AG20073 (MF).
Send correspondence and reprint requests to: Christine M. Hulette, MD, Duke University Medical Center, Department of Pathology, Box 3712, Durham, NC 27710; E-mail: hulet001@mc.duke.edu

aphasia and personality deterioration with circumscribed frontotemporal atrophy without plaques and tangles. Decades later, Constantinidis (10) popularized the concept that PiD consists of three neuropathologic subtypes. All 3 subtypes show frontotemporal atrophy to some degree and thus were differentiated based on the presence or absence of Pick bodies and Pick cells. According to this classification scheme, type A PiD contains both Pick bodies and Pick cells, type B contains only Pick cells, and type C does not contain Pick bodies or Pick cells. Although this classification system was initially successful at simplifying the neuropathologic diagnosis of FTDs, recent discoveries have rendered this system incomplete. In particular, the realization that tau protein plays a critical role in many of the FTDs, together with the availability of monoclonal antibodies directed against pathologic forms of this protein, have underscored the necessity for an updated classification system.

Recently, an international group of clinical and basic scientists participated in a consensus conference to develop clinical and neuropathologic criteria for the diagnosis of FTD (1). This panel of experts developed a classification scheme that incorporated the classic neuropathologic findings, immunohistochemical analysis of tau protein and ubiquitin, and biochemical studies into the isoform composition of the insoluble tau deposits. To clarify nomenclature differences between the clinical syndrome of FTD and the neuropathologic findings, they recommended the terminology of frontotemporal lobar degeneration (FTLD) when referring to the pathologic changes. Additionally, they recommended a classification system that comprises 5 distinct neuropathologic categories. Three of these diagnostic categories are differentiated based on the isoform composition of insoluble tau as determined through Western blotting. These categories are separated into those with tau protein deposits containing predominantly 3 microtubule-binding repeats (3R tau), those with predominantly 4 microtubule-binding repeats (4R tau), and cases containing both 3R and 4R tau inclusions. The remaining two neuropathologic categories consist of cases without detectable amounts of insoluble tau. These two categories are separated into cases with or without motor neuron disease-type inclusions in the fascia dentata detected with ubiquitin immunohistochemistry. Recognizing that our knowledge of these disorders is incomplete, the 5 neuropathologic categories incorporate several possible diagnoses. These diagnoses include all of the recognized neuropathologic forms of FTD, including classic PiD, CBD, PSP, neurofibrillary tangle dementia (NFTD), dementia lacking distinctive histopathologic features (DLDH), FTLD with motor neuron disease or motor neuron disease-type inclusions (FTLD-MND/MNI), and frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17).

Given the ever-growing complexity of this group of disorders, the present study was undertaken to evaluate the use and reliability of the newly proposed diagnostic criteria.

MATERIALS AND METHODS

The rapid autopsy program of the Kathleen Price Bryan Brain Bank has been in existence since 1985 (11). The Institutional Review Board at Duke University Medical Center has approved patient recruitment and enrollment procedures. For this study, the brains from 21 patients with a clinicopathologic diagnosis of FTD were selected from the Kathleen Price Bryan Brain Bank. At the time of postmortem examination, the left hemisphere was fixed in 10% buffered formalin, and the right hemisphere was frozen and stored at -70°C for periods of 1 to 14 years. Frozen frontal lobe tissue (approximately 3 g) was removed from each brain for biochemical analysis.

Immunohistochemistry

Paraffin-embedded brain sections (8 μm) were immunostained with antibodies directed against ß-amyloid peptide, AT8 tau, ubiquitin, and α-synuclein. The β-amyloid peptide immunostain required deparaffinized sections, blocked with 2% hydrogen peroxide in methanol for 10 minutes, treated with 95% formic acid (Sigma, St. Louis, MO) for 1 minute, and washed in deionized H_2O. Background staining was blocked with 5% w/v nonfat dry milk in 0.05 M Tris buffer. The monoclonal primary 4G8 antibody (1:1000, 1 μg/mL; Signet, Dedham, MA) was applied and incubated for 45 minutes at 37°C. A biotinylated horse antimouse IgG secondary antibody (1:300 dilution; Vector Labs, Burlingame, CA) was incubated for 20 minutes at 37°C. Horseradish peroxidase-labeled streptavidin (1:800 dilution; Jackson ImmunoResearch Laboratories, West Grove, PA) was incubated for 20 minutes at 37°C. Monoclonal primary antibody AT8 tau (1:40, 5 μg/mL; Innogenetics, Alpharetta, GA) was incubated for 45 minutes at 37°C. Biotinylated horse antimouse IgG secondary antibody (1:300 dilution; Vector Labs) was incubated for 20 minutes at 37°C. Ubiquitin antiserum (1:800; DAKO Corporation, Carpinteria, CA) was applied overnight at room temperature. The reaction was developed using biotinylated swine antirabbit immunoglobulin (1:400; DAKO Corporation) for 30 minutes at 37°C. Fresh horseradish peroxidase-labeled streptavidin (1:500; DAKO Corporation) was incubated for 20 minutes at 37°C. Monoclonal antibody Synuclein-1 (1:100; Transduction Laboratories, Lexington, KY) was applied for 1 hour at 37°C followed by incubations with prediluted biotinylated secondary antibody and prediluted streptavidin–peroxidase antibody (Biomeda, Foster City, CA), each for 20 minutes at 37°C. All sections were developed using diaminobenzidine (Sigma) and counterstained with aqueous hematoxylin (Biomeda).

Western Blot Analysis

Gray matter was carefully dissected from partially thawed frontal lobe samples and homogenized in 200 mg/mL of buffer A (20 mM MES/NaOH at pH 6.8, 80 mM NaCl, 1 mM EGTA, 0.1 mM EDTA, and a cocktail of protease inhibitors). Soluble and sarkosyl-insoluble fractions of tau were prepared as previously described (12, 13). The fractions were boiled in SDS-PAGE sample buffer for 5 minutes and resolved on a 10% SDS-PAGE gel. Proteins were transferred onto nitrocellulose membranes and blotted with WKS44 polyclonal anti-tau antibody (1:1000) at 4°C for overnight. The specific bands were detected using the ECL detection system (Amersham-Pharmacia, Piscataway, NJ). The insoluble tau patterns were noted on each case without prior knowledge of the clinical information or neuropathologic diagnoses. To quantitate soluble tau, the lower portions of the membranes

were probed with anti-α-synuclein monoclonal antibody (LB509, 1:1000). The ratio of the total amount of soluble tau to α-synuclein was then determined using ImageQuant analysis software. This ratio was then presented as a fraction of control brain tissue as previously described (14, 15).

DNA Sequencing for Microtubule-Associated Protein Tau

DNA was isolated from brain tissue using QIAamp DNA Mini Kit (Qiagen, Valencia, CA). Exons 1–5, 7, and 9–13 of microtubule-associated protein tau (MAPT) were amplified from patient genomic DNA (500 ng) using primer pairs described by Poorkaj et al (16) complementary to the intronic regions of MAPT. Amplification reactions contained 1× polymerase chain reaction buffer, 200 nM of each primer, 2.5 U of Taq DNA polymerase (exon 4), or TaqGold DNA polymerase (all other exons) (Applied Biosystems, Foster City, CA) and 200 μM deoxynucleoside triphosphatase (Applied Biosystems) in a final volume of 50 μL. The amplifications using AmpliTaq Gold included a 10-minute activation step at 95°C, followed by 40 cycles of 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 30 seconds with a final 5-minute elongation step at 72°C (all exons except 4 and 7) or 95°C for 30 seconds, 58°C for 45 seconds, and 72°C for 1 minute with a final 9-minute elongation step at 72°C (exon 7). The exon 4 amplification was identical to that of exon 7 without the initial activation step. After amplification, polymerase chain reaction (PCR) products were purified using Qiagen PCR purification Kit (Qiagen) according to the manufacturer's directions. An aliquot of the purified PCR products was electrophoresed on a 1% agarose gel in 1× TBE (Tris-borate-ethylenediamine-tetraacetate) buffer containing ethidium bromide. Approximate DNA concentrations were determined by comparison of the intensity of the gel bands to the equivalent band in a 100-bp DNA ladder (New England Biolabs, Beverly, MA) with known DNA mass for each marker band. Using 100 fmol of each purified fragment, both strands of the products were sequenced using the CEQ dye terminator cycle sequencing kit (Beckman Coulter, Fullerton, CA) on a CEQ 8000 (Beckman Coulter) using both the forward and reverse PCR primers. Sequence analysis was performed with Sequencher software (Gene Codes Corporation, Ann Arbor, MI).

Apolipoprotein E Genotype Determination

Genomic DNA was extracted from fresh-frozen brain using the QIAamp DNA minikit (Qiagen). Amplification was carried out using the "single-day apolipoprotein ε" method (17) with the following modifications. The DNA was amplified using a Hybaid Touchdown thermal cycler (Hybaid, Cambridge, UK). Conditions included an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, 50–60°C touchdown annealing for 30 seconds at 0.5°C per cycle, and 72°C for 45 seconds, with a final extension at 72°C for 10 minutes. The primer sequences used for this amplification were: forward 5′-TAAGCTTGGCACGGCTGTCCAAGGA-3′ and reverse 5′-ACAGAATTCGCCCCGGCCTGGTACAC-3′. After amplification, 5 units of Cfo 1 (Promega, Madison, WI) enzyme and its buffer were added directly to 20 μL of PCR product and incubated at 37°C for 5 hours. The digest was run on a 4.5% agarose gel with 1× TBE giving main fragment sizes of 91, 83, 72, and 48 base pairs.

Statistical Analysis

The data were analyzed using STATGRAPHICS Plus version 5.0 (Manugistics Inc., Rockville, MD). The χ^2 test was used to compare the frequency of insoluble tau isoform patterns among the 6 diagnostic categories. Additionally, the χ^2 test was used to compare the frequency of insoluble isoform patterns seen with specific histopathologic features. Linear regression analysis was used to fit a model to describe the relationship between soluble tau levels (dependent variable) and postmortem interval (independent variable). The p value was calculated using an analysis of variance (ANOVA) table. One-way ANOVA was used to compare soluble tau levels among the diagnostic categories. The Student *t*-test was used with 2-group comparisons. Standardized skewness and kurtosis were used to determine the normality of the data distribution. The Cochran C test, Bartlett test, and Levene test were used to determine equality of variance. The cutoff p value for all tests was set at $\leqslant 0.05$ for statistical significance.

RESULTS

Clinical Findings

In this study, we examined the brains from 21 patients with a clinicopathologic diagnosis of FTD (Table 1). Overall, the mean age at death was 70.0 ± 2.2 years (± 1 standard error of mean) with a range of 56 to 89 years. All patients were white and the majority were female (67%). The data show a trend toward increased age among the female patients (female mean age 72.8 ± 2.8 years; male mean age 64.4 ± 2.5 years; p = 0.068; Student *t*-test). Details of the clinical presentation were available in 16 cases and included dementia with personality deterioration (31%), dementia not otherwise specified (25%), dementia with parkinsonism (19%), dementia with language impairment (13%), dementia with motor neuron disease (6%), and dementia with both personality deterioration and parkinsonism (6%). A significant number of patients had a positive family history of dementia (33%; 6 of 18 cases with sufficient clinical history).

Pathologic Findings

Autopsies were performed in each case, with a mean postmortem interval of 10.8 ± 1.7 hours. The mean brain weight was 892 ± 34 g for the female patients and 1172 ± 37 g for the male patients. The location of cerebral atrophy was variable and included frontotemporal (30%), frontal (20%), temporal (15%), frontoparietal (15%), frontotemporoparietal (10%), and minimal to mild global atrophy (10%). Atrophy of the caudate nucleus was also identified in 40% of the cases.

Classic Pick Disease

Seven of the patients were given the histopathologic diagnosis of classic PiD, including 6 females and one male. The mean age was 75.7 ± 3.7 years. Grossly, all of the classic PiD cases were characterized by severe, circumscribed atrophy that was confined to the frontal and anterior temporal lobes. Two of the cases also displayed significant atrophy of the

TABLE 1. Summary of Clinical, Neuropathologic, and Molecular Findings

Patient	Age (years)	Sex	Clinical Features	Histopathologic Diagnosis	Insoluble tau Pattern	tau Sequence	APOE Genotype
1	68	M	Personality deterioration	PiD	3R	Not done	2,3
2	73	F	Personality deterioration	CBD	4R	Not done	3,3
3	78	F	Dementia not otherwise specified	CBD	4R	Not done	2,3
4	86	F	Not available	PSP	4R	Not done	3,3
5	60	F	Personality deterioration	DLDH	No insoluble tau	Not done	3,4
6	56	M	Mutism	FTLD-MNI	No insoluble tau	Exon 10 polymorphism	2,3
7	79	F	Personality deterioration	PiD	3R	Not done	3,4
8	66	M	Motor neuron disease	FTLD-MND	No insoluble tau	Not done	3,3
9	65	M	Parkinsonism	DLDH	No insoluble tau	Not done	3,3
10	75	F	Not available	PSP	4R	Not done	3,3
11	59	F	Aphasia	PiD	3R	Not done	3,4
12	79	F	Dementia not otherwise specified	PiD and AD	3R/4R	Not done	3,4
13	75	F	Not available	PiD	3R	Not done	3,3
14	65	M	Not available	DLDH	No insoluble tau	Not done	3,4
15	67	F	Dementia not otherwise specified	NFTD	3R/4R	R406W mutation	3,3
16	81	F	Parkinsonism	PiD	3R	Not done	3,3
17	89	F	Not available	PiD and AD	3R/4R	Not done	3,4
18	58	F	Parkinsonism	CBD	4R	Exon 10 5' splice site +16 mutation	3,4
19	75	M	Dementia not otherwise specified	CBD	4R	None	3,3
20	56	M	Personality deterioration	CBD	4R	Not done	2,3
21	60	F	Personality deterioration and parkinsonism	DLDH	No insoluble tau	Exon 11 polymorphism	3,3

PiD, Pick disease; CBD, corticobasal degeneration; PSP, progressive supranuclear palsy; DLDH, dementia lacking distinctive histopathological features; FTLD-MND/MNI, frontotemporal lobar degeneration with motor neuron disease or motor neuron disease-type inclusions; AD, Alzheimer disease; NFTD, neurofibrillary tangle dementia.

caudate nucleus. Histologic and immunohistochemical studies revealed tau-immunoreactive Pick bodies and ballooned neurons in all 7 cases, which were localized to the middle frontal gyrus, superior temporal gyrus, inferior parietal lobule, cingulate gyrus, insula, hippocampal formation, amygdala, caudate nucleus, globus pallidus, and substantia nigra. Two of the cases also had neurofibrillary change and neuritic plaques typical of early-stage AD and were given the combined diagnosis of PiD and AD. Astrocytic plaques, thorn-shaped astrocytes, and ubiquitin-immunoreactive motor neuron disease-type inclusions were absent in all cases.

Independent analysis of the tau isoform composition by Western blotting revealed predominantly 3R tau in the five pure PiD cases (Fig. 1). The 2 cases with combined PiD and AD had both 3R and 4R tau deposits. None of these patients were tested for tau gene mutations. The APOE genotypes included 3/4 (n = 4), 3/3 (n = 2), and 2/3 (n = 1).

Corticobasal Degeneration

Five of the patients were given the histopathologic diagnosis of CBD, including 3 females and 2 males. The mean age was 68.0 ± 4.6 years. Grossly, the brains demonstrated moderate to severe cerebral atrophy that was confined to the frontal (n = 2), temporal (n = 2), and frontoparietal regions (n = 1). Significant atrophy of the caudate nucleus was identified in three cases. All 5 cases had tau-immunoreactive astrocytic plaques in the cortex of the superior frontal gyrus, middle frontal gyrus, and superior temporal gyrus (Fig. 2A). Four cases had tau-immunoreactive thorn-shaped astrocytes that were localized to the white matter of the middle frontal gyrus, superior frontal gyrus, superior temporal gyrus, hippocampal formation, entorhinal cortex, amygdala, caudate nucleus,

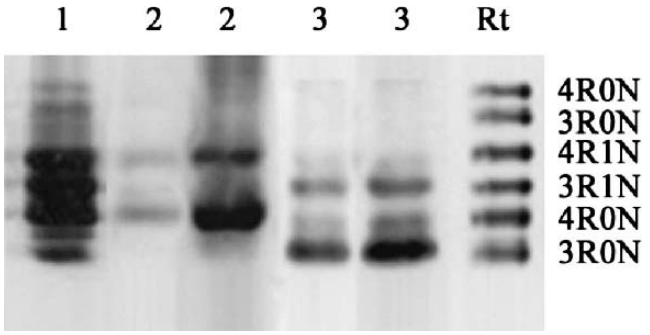

FIGURE 1. Representative Western blot analysis of insoluble tau proteins. Insoluble tau was isolated from frozen tissue samples as described in the ''Materials and Methods'' section. Each sample was dephosphorylated using alkaline phosphatase from *Escherichia coli*. A mixture of recombinant human tau was used as a standard (Rt). Insoluble tau was isolated from 1) Alzheimer disease, showing a mixture of 3R and 4R tau isoforms; 2) corticobasal degeneration (2 regions from a single case), showing predominantly 4R isoforms; and 3) Pick disease (2 regions from a single case), showing predominantly 3R isoforms.

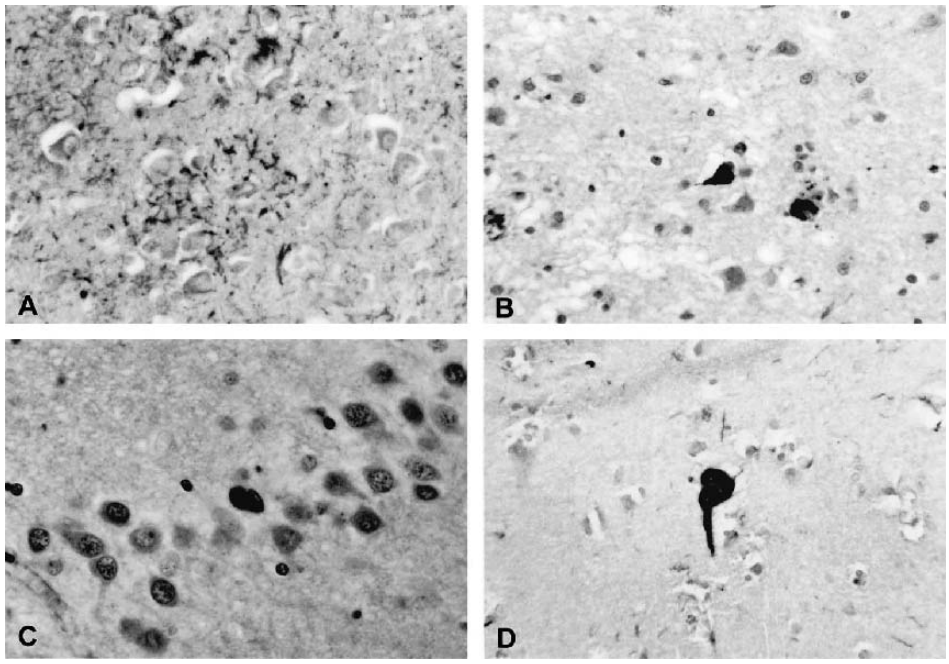

FIGURE 2. (A) Tau-immunoreactive astrocytic plaques were a characteristic feature of corticobasal degeneration (AT8 tau, 400×). **(B)** Tau-immunoreactive thorn-shaped astrocytes were a common finding in progressive supranuclear palsy (AT8 tau, 400×). **(C)** Frontotemporal lobar degeneration with motor neuron disease or motor neuron disease-type inclusions cases were characterized by ubiquitin-immunoreactive inclusions in the dentate neurons of the fascia dentata (ubiquitin, 400×). **(D)** The single case of neurofibrillary tangle dementia was characterized by neurofibrillary change in the absence of glial tau pathology (AT8 tau, 400×). This patient was found to have the R406W mutation.

putamen, globus pallidus, substantia nigra, and thalamus. Neurofibrillary tangles were identified in 4 cases, with a distribution that included the middle frontal gyrus, superior temporal gyrus, hippocampal formation, amygdala, caudate nucleus, putamen, substantia nigra, thalamus, oculomotor nucleus, locus ceruleus, and the pontine nuclei. Ballooned neurons were identified in 2 cases, including in the superior frontal gyrus, middle frontal gyrus, superior temporal gyrus, hippocampal formation, caudate nucleus, putamen, globus pallidus, and thalamus. All cases were negative for Pick bodies, neuritic plaques, and motor neuron disease-type inclusions.

Western blot analysis demonstrated predominantly 4R tau in the insoluble deposits (Fig. 1). Tau gene sequencing was performed in two cases. One of the cases demonstrated the exon 10 5′ splice site +16 mutation. The remaining case was free of mutations or polymorphisms in the tau gene. The APOE genotypes included 2/3 (n = 2), 3/3 (n = 2), and 3/4 (n = 1).

Dementia Lacking Distinctive Histopathologic Features

Four patients, including 2 males and 2 females, were given the histopathologic diagnosis of DLDH. The mean age was 62.5 ± 1.4 years. In each case, there was moderate to severe cerebral atrophy with a frontal (n = 2), frontotemporal (n = 1), and frontoparietal (n = 1) distribution. Two cases had significant atrophy of the caudate nucleus. The histologic features were limited to neuronal loss and gliosis in the grossly affected areas of brain. No tau-immunoreactive inclusions or neurofibrillary changes were identified. Additionally, immunostains for β-amyloid peptide, ubiquitin, and α-synuclein were negative.

Western blot analysis confirmed the absence of insoluble tau. Tau gene sequencing was performed in one case and showed no evidence of mutation. This case did, however, display a polymorphism in exon 11. The APOE genotypes included 3/3 (n = 2) and 3/4 (n = 2).

Progressive Supranuclear Palsy

Two female patients with a mean age of 80.5 ± 5.5 years were given the histopathologic diagnosis of PSP. Both cases were characterized by severe atrophy of the frontal and temporal lobes, moderate atrophy of the parietal lobes, and moderate atrophy of the caudate nuclei and midbrain. Histologically, the frontal, parietal, and temporal lobes displayed significant neuronal loss and gliosis with spongiform changes in the superficial cortex. The hippocampus had mild to moderate neuronal loss throughout the pyramidal cell layer. Both cases demonstrated a dense deposition of tau-immunoreactive thorn-shaped astrocytes in the white matter of the cerebrum and brainstem (Fig. 2B). Although less dense, thorn-shaped astrocytes were also identified in the cortex. Ballooned neurons, Pick bodies, astrocytic plaques, motor neuron disease-type inclusions, and neuritic plaques were absent in both cases. Western blot analysis of the insoluble tau deposits revealed predominantly 4R tau. Tau genetic testing was not performed. Both patients were found to have an APOE genotype of 3/3.

Frontotemporal Lobar Degeneration with Motor Neuron Disease or Motor Neuron Disease-Type Inclusions

Two patients were placed into the category of FTLD with motor neuron disease (FTLD-MND) or with motor neuron disease-type inclusions (FTLD-MNI). One of the patients was a 66-year-old with a clinical history of motor neuron disease and progressive dementia. The other patient was a 56-year-old with a clinical history of mutism and dementia. At autopsy, the patient with motor neuron disease had mild cerebral atrophy. The patient with mutism and

dementia had severe, circumscribed atrophy of the frontal, temporal, and parietal lobes, and moderate atrophy of the caudate nuclei. The only specific histopathologic feature was the presence of ubiquitin-immunoreactive inclusions in the dentate neurons of the hippocampus in both cases (Fig. 2C) and in the anterior motor neurons of the spinal cord in the patient with motor neuron disease. The anterior motor neuron inclusions consisted of hyaline, Lewy body-like inclusions and irregular basophilic inclusions. No upper motor neuron inclusions or Bunina bodies were identified in the motor neuron disease case. The spinal cord was not available for examination in the second case. There was no evidence of tau, ß-amyloid peptide, or α-synuclein pathology in either case.

Western blot analysis confirmed the absence of insoluble tau in both cases. Genetic testing on the 56-year-old patient with dementia and mutism identified a polymorphism in exon 10, but no evidence of mutation. Genetic testing was not performed on the patient with motor neuron disease. The APOE genotypes were 2/3 and 3/3, respectively.

Neurofibrillary Tangle Dementia

One patient was given the histopathologic diagnosis of NFTD. This patient was a 67-year-old white woman who originally presented 13 years before death with an Alzheimer-type dementia. She had a strong family history of dementia affecting her mother, 2 sisters, and 2 uncles from her mother's side. Gross findings included moderate atrophy of the frontal, temporal, and parietal lobes. The caudate nuclei were unremarkable. Histologic examination revealed moderate to severe neuronal loss involving the entorhinal cortex, hippocampus, amygdala, subiculum, medial temporal lobe, and insula. There was severe neurofibrillary change in the amygdala, entorhinal cortex, layer CA1 of the hippocampus, and subiculum (Fig. 2D). The caudate nucleus, putamen, globus pallidus, and substantia nigra showed only mild neuronal loss and gliosis with scattered neurofibrillary tangles. Pertinent negatives included the absence of amyloid plaques, Pick bodies, ballooned neurons, or Lewy bodies. Western blot analysis showed both 3R and 4R tau in the insoluble deposits. Molecular testing revealed the R406W mutation. The patient's APOE genotype was 3/3.

Insoluble Tau: Summary

The insoluble tau isoform patterns correlated strongly with the independently derived histopathologic diagnoses ($p < 0.001$; χ^2 analysis) (Table 2). The data show that cases containing no insoluble tau were either DLDH (67%) or FTLD-MND/MNI (33%). Cases with predominantly 3R tau were exclusively classic PiD (100%). Cases with predominantly 4R tau were either CBD (71%) or PSP (29%). Finally, cases that contained both 3R and 4R isoforms were either a combination of PiD and AD (67%) or NFTD (33%).

The histologic features that correlated with the tau isoform patterns by χ^2 testing included ballooned neurons and 3R tau ($p = 0.006$); Pick bodies and 3R tau ($p < 0.001$); astrocytic plaques and 4R tau ($p = 0.004$); thorn-shaped astrocytes and 4R tau ($p = 0.001$); neurofibrillary tangles and 4R tau ($p = 0.002$); and neuritic plaques and 3R/4R tau ($p = 0.004$).

TABLE 2. Frequency Table for Insoluble tau Pattern by Histopathologic Diagnosis

	PiD	PiD and AD	CBD	PSP	DLDH	FTLD-MND /MNI	NFTD
No insoluble tau	0	0	0	0	4	2	0
3R tau	5	0	0	0	0	0	0
4R tau	0	0	5	2	0	0	0
3R + 4R tau	0	2	0	0	0	0	1

PiD, Pick disease; AD, Alzheimer disease; CBD, corticobasal degeneration; PSP, progressive supranuclear palsy; DLDH, dementia lacking distinctive histopathologic features; FTLD-MND/MNI, frontotemporal lobar degeneration with motor neuron disease or motor neuron disease-type inclusions; NFTD, neurofibrillary tangle dementia.

Soluble Tau: Summary

For each case, the amount of soluble tau was quantitated as a fraction of that found in control brain tissue as previously described (14, 15). The data show that among diagnostic categories, DLDH and FTLD-MND/MNI showed the lowest levels of soluble tau (Fig. 3A), although this did not reach statistical significance ($p = 0.391$; one-way ANOVA). However, if the cases were separated into those with or without insoluble tau, the cases lacking insoluble tau (i.e. DLDH and FTLD-MND/MNI) showed a significantly lower level of soluble tau ($p = 0.045$; Student *t*-test) (Fig. 3B). Although the level of soluble tau was slightly less in cases with a polymorphism in the tau gene (0.34 ± 0.11 vs. 0.52 ± 0.14), this difference failed to reach statistical significance ($p = 0.432$; Student *t*-test). There was not a statistically significant correlation between the soluble tau levels and the postmortem interval ($p = 0.773$; linear regression analysis).

APOE Genotype: Summary

Among all cases, the most common APOE genotype was 3,3 (47.6%), followed by 3,4 (33.3%) and 2,3 (19.1%). The APOE4 allele was identified in patients with PiD (57%), DLDH (50%), and CBD (20%) ($p = 0.394$; χ^2 analysis). Two of the PiD cases with an APOE4 allele also had coexisting AD pathology. The APOE genotypes demonstrated no association with patient age.

DISCUSSION

FTD is the third most common neurodegenerative dementia, behind AD and dementia with Lewy bodies (18, 19). Although this group of neurodegenerative dementias is not uncommon, a simplified classification system has yet to be well-defined. Recent studies into the comparative biochemistry of tau aggregates in neurodegenerative diseases such as FTDs and AD have provided important insight into the pathogenesis of these diseases. Moreover, these studies have provided important tools that may be useful in the diagnostic evaluation of the neurodegenerative tauopathies.

In the human central nervous system, tau protein exists as 6 isoforms that contain either 3 (3R) or 4 (4R) microtubule-binding domains. Excessive phosphorylation of these isoforms leads to pathologic aggregation within neurons and glia. The neurodegenerative diseases that are characterized by tau aggregation such as the FTDs and AD differ in the relative

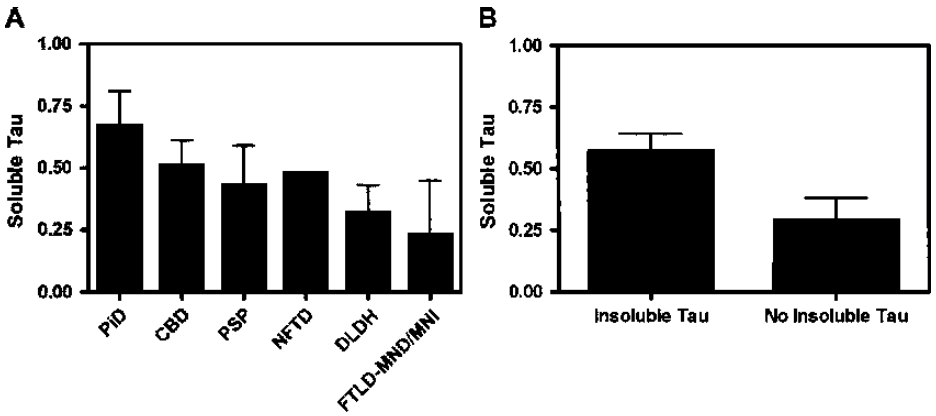

FIGURE 3. Soluble tau was quantitated using Western blot analysis and expressed as a fraction of that seen in control brain tissue. The data represent the mean ± 1 standard error of mean. **(A)** Among the diagnostic categories, dementia lacking distinctive histopathologic features (DLDH) and frontotemporal lobar degeneration with motor neuron disease or motor neuron disease-type inclusions (FTLD-MND/MNI) showed the lowest levels of soluble tau, although this did not reach statistical significance (p = 0.391; one-way analysis of variance). **(B)** Separating the cases based on the presence or absence of insoluble tau revealed that cases lacking insoluble tau (i.e. DLDH and FTLD-MND/MNI) had a significantly lower level of soluble tau (p = 0.045; Student *t*-test).

composition of the 6 tau isoforms. For example, in AD, both the 3R and 4R isoforms are abnormally phosphorylated, leading to aggregation into paired helical filaments that can be detected by Western immunoblotting (20–22). In the FTDs, the composition of abnormally phosphorylated tau varies according to the pathologic subtype and may include predominantly 3R isoforms, predominantly 4R isoforms, or both 3R and 4R isoforms. Additionally, many cases lack insoluble tau deposits. Although numerous studies have shown a correlation between various FTD pathologic subtypes and the tau isoform pattern (20, 22–25), biochemical analysis of tau aggregates has yet to be fully incorporated into the FTD classification system. This is in part the result of the complexity of the FTD nomenclature as well as the lack of consensus on the neuropathologic diagnosis of the FTDs.

In response to these issues, McKhann et al (1) recently proposed a neuropathologic classification scheme that incorporates a biochemical analysis of insoluble tau deposits as well as ubiquitin immunohistochemistry. In the present study, we have demonstrated the use of this system by showing a strong correlation between the insoluble tau isoform composition and the independently derived neuropathologic diagnoses obtained using a histopathologic and immunohistochemical approach. The combination of the biochemical, histopathologic, and immunohistochemical approaches should permit the diagnostic accuracy that is necessary in a research setting. We present a simplified algorithm of this combined approach in Figure 4. We reiterate, however, that the cases with tau-immunoreactive inclusions may in fact represent FTDP-17, because no specific histopathologic findings have been linked to this group of disorders. Additionally, our data show that the cases lacking tau-immunoreactive inclusions (DLDH and FTLD-MND/MNI) may demonstrate polymorphisms in the tau gene or reduced levels of soluble tau, thus not completely excluding a pathologic role for tau in these disorders.

FTDP-17 is a group of autosomal-dominant neurodegenerative syndromes that are characterized by deposition of hyperphosphorylated tau protein within neurons and glia (26–31). The clinical and neuropathologic phenotypes among this group of disorders are variable and may simulate a number of sporadic FTDs. Approximately 80 families with FTDP-17 have been identified, with a total of 31 unique tau gene mutations (19). The described mutations involve exons 1, 9, 10, 11, 12, and 13, as well as the exon 10 5′ splice site. The most common mutations, accounting for 60% of cases, involve exon 10 (P301L and N279K) and the exon 10 5′ splice site (+16) (19). The resulting neuropathologic features, particularly with regard to the localization of tau deposits, vary according to the site of the tau gene mutation. Mutations in exons 1 and 10, and in the exon 10 5′ splice site, tend to cause both neuronal and glial filamentous inclusions; conversely, mutations in exons 9, 11, 12, and 13 cause primarily neuronal inclusions. In the present study, we identified 2 patients with tau gene mutations, including a patient with the exon 10 5′ splice site +16 mutation that comes from a previously described family (32) and a patient with the R406W (exon 13) mutation. There are several reports of mutations in the exon 10 5′ splice site (33–39). The neuropathology produced by these mutations includes neuronal loss and gliosis in the cortex, caudate nucleus, putamen, globus pallidus, amygdala, and numerous brainstem nuclei. Tau-immunoreactive inclusions are identified in both neurons and glial cells, with some cases showing significant white matter pathology. Biochemical studies have shown a tendency to form 4R tau isoforms within the filamentous inclusions (29, 35, 37, 40). The mechanism appears to involve disruption of a stem-loop structure that normally forms over the splice site, which leads to increased affinity for U1 snRNP-binding and a subsequent increase in the incorporation of exon 10 into the tau mRNA (35). The patient with the intron +16 mutation in the present study demonstrated neuropathologic findings indistinguishable from CBD with tau-immunoreactive neuronal inclusions, astrocytic plaques, and thorn-shaped astrocytes, all of which were composed of predominantly 4R tau isoforms. The patient with the R406W mutation represents a newly described family with FTDP-17. Consistent with previous reports of this mutation (35, 41, 42), the primary pathologic findings were those of neuronal loss and gliosis with abundant neurofibrillary tangles and neuropil threads in the absence of glial tau pathology or amyloid deposition. The pathology of this case primarily involved the entorhinal cortex, amygdala, hippocampus, subiculum, medial temporal lobe, and insula. The insoluble

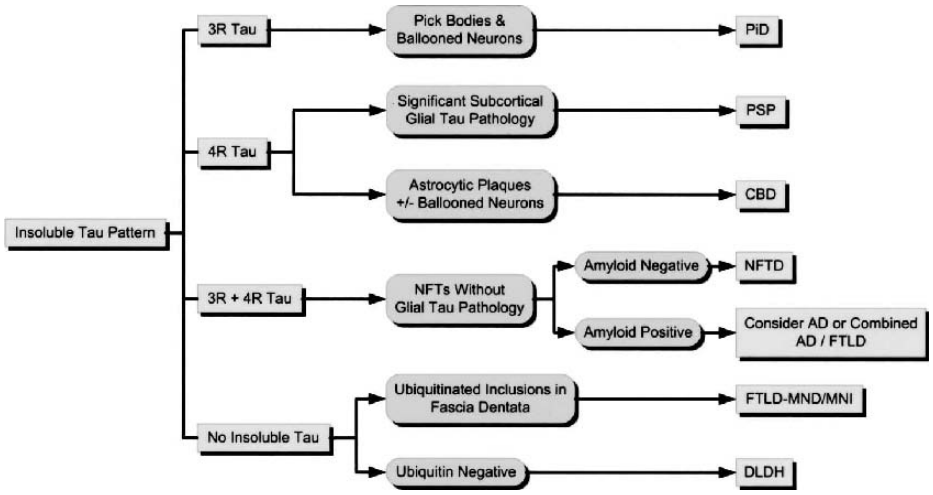

FIGURE 4. Frontotemporal dementia diagnostic algorithm. PiD, Pick disease; CBD, corticobasal degeneration; PSP, progressive supranuclear palsy; NFTD, neurofibrillary tangle dementia; AD, Alzheimer disease; FTLD-MND/MNI, frontotemporal lobar degeneration with motor neuron disease or motor neuron disease-type inclusions; DLDH, dementia lacking distinctive histopathologic features.

tau deposits were composed of both 3R and 4R isoforms, which is consistent with previous reports (29). The molecular mechanisms that result in the observed biochemical abnormality have not been fully elucidated.

The strong association between the APOE epsilon 4 allele and AD is well established (43, 44). On the contrary, no consensus has been reached with regard to the association between the FTDs and APOE genotype. Reports in the literature are often conflicting. For example, there are 3 reports showing an increased frequency of APOE4 in patients with FTD (45–47). Only one of these studies, however, had pathologically confirmed diagnoses (47). The importance of the nonpathologically confirmed findings is difficult to interpret given the potential for coexistence of AD and FTD pathology (as seen in the present study) and the overlap in clinical presentation between these disorders. Additionally, there are numerous studies that show no association between FTD and APOE4 (48–54). Again, only a minority of these cases have pathologic confirmation. In our study, we found that 7 of the 21 patients (33%) had a single APOE4 allele, which is significantly higher than published reports of nondemented controls, which usually lies between 14% and 16% (43, 55). However, 2 of the cases in our series had combined PiD and AD pathology, together with a single APOE4 allele. When these cases were excluded, the APOE4 allele frequency remained elevated at 26%. Further analysis of cases with APOE4 demonstrated an apparent overrepresentation of this allele among patients with DLDH and PiD. These findings support previous studies showing an association between PiD and APOE4 (47). Additionally, the results suggest that APOE4 may be associated with DLDH, a potentially novel finding that needs to be confirmed with larger studies.

In conclusion, the three major findings of this study can be summarized as follows. First, the primary neuropathologic subtypes of FTD can be reproducibly categorized by the isoform composition of insoluble tau. Second, the neuropathologic subtypes of FTD that lack insoluble tau may still show evidence of tau aberrations, including polymorphisms in the tau gene and low levels of soluble tau. Third, the APOE4 allele, which is a risk factor for AD, is overrepresented in the 2 most common neuropathologic subtypes of FTD (i.e. PiD and DLDH). Together, these findings suggest that, although the current classification system is useful and reproducible, more revisions will likely be necessary in the future, particularly with regard to the neuropathologic subtypes lacking clearly discernible tau pathology.

ACKNOWLEDGMENTS

The authors thank the Kathleen Price Bryan Autopsy Program coordinators, Mari Szymanski, RN, C; and Nancy O. Sinclaire, RN, C.

REFERENCES

1. McKhann GM, Albert MS, Grossman M, Miller B, Dickson D, Trojanowski JQ. Clinical and pathologic diagnosis of frontotemporal dementia: Report of the work group on frontotemporal dementia and Pick's disease. Arch Neurol 2001;58:1803–9
2. Hodges JR. Frontotemporal dementia (Pick's disease): Clinical features and assessment. Neurology 2001;56:S6–10.
3. Kertesz A, Munoz DG. Frontotemporal dementia. Med Clin North Am 2002;86:501–18, vi
4. Pick A. Uber die Beziehungen der senilen Hirnatrophie zur Aphasie. Prager Med Wochenschr 1882;17:165–67
5. Pick A. Senile Hernatrophie als Grundlage von Herderscheinungen. Wien Klin Wochenschr 1901;14:403–4
6. Pick A. Zur Symptomatologie der linksseitigen Schlafenlappenatrophie. Monatschr Psychiatr Neurol 1904;16:378–88
7. Alzheimer A. Uber eigenartige Krankheitsfalle des spateren Alters. Z Gesamte Neurol Psychiatrie 1911;4:356–85
8. Altman A. Uber die umschriebene Gehirnatrophie des spateren Alters. Z Neurol Psychiatr 1923;83:610–43
9. Onari K, Spatz H. Anatomische Beitrage zur Lehre von der Pickschen umschriebene-Grosshirnrinden-Atrophie ('Picksche Krankheit'). Z Gesamte Neurol Psychiatrie 1926;101:470–511
10. Constantinidis J, Richard J, Tissot R. Pick's disease. Histological and clinical correlations. Eur Neurol 1974;11:208–17
11. Hulette CM, Welsh-Bohmer KA, Crain B, Szymanski MH, Sinclaire NO, Roses AD. Rapid brain autopsy. The Joseph and Kathleen Bryan Alzheimer's Disease Research Center experience. Arch Pathol Lab Med 1997;121:615–18
12. Liu WK, Le TV, Adamson J, et al. Relationship of the extended tau haplotype to tau biochemistry and neuropathology in progressive supranuclear palsy. Ann Neurol 2001;50:494–502

13. Zhukareva V, Shah K, Uryu K, et al. Biochemical analysis of tau proteins in argyrophilic grain disease, Alzheimer's disease, and Pick's disease: A comparative study. Am J Pathol 2002;161:1135–41
14. Zhukareva V, Sundarraj S, Mann D, et al. Selective reduction of soluble tau proteins in sporadic and familial frontotemporal dementias: An international follow-up study. Acta Neuropathol (Berl) 2003;105:469–76
15. Zhukareva V, Vogelsberg-Ragaglia V, Van Deerlin VM, et al. Loss of brain tau defines novel sporadic and familial tauopathies with frontotemporal dementia. Ann Neurol 2001;49:165–75
16. Poorkaj P, Bird TD, Wijsman E, et al. Tau is a candidate gene for chromosome 17 frontotemporal dementia. Ann Neurol 1998;43:815–25
17. Crook R, Hardy J, Duff K. Single-day apolipoprotein E genotyping. J Neurosci Methods 1994;53:125–27
18. Barker WW, Luis CA, Kashuba A, et al. Relative frequencies of Alzheimer disease, Lewy body, vascular and frontotemporal dementia, and hippocampal sclerosis in the State of Florida Brain Bank. Alzheimer Dis Assoc Disord 2002;16:203–12
19. Dickson DW, Bergeron C, Hauw JJ, Jellinger KA, Lantos PL, Mizusawa H, eds. *Neurodegeneration: The Molecular Pathology of Dementia and Movement Disorders.* Basel, Switzerland: ISN Neuropath Press, 2003
20. Buee L, Delacourte A. Comparative biochemistry of tau in progressive supranuclear palsy, corticobasal degeneration, FTDP-17 and Pick's disease. Brain Pathol 1999;9:681–93
21. Flament S, Delacourte A. Abnormal tau species are produced during Alzheimer's disease neurodegenerating process. FEBS Lett 1989;247: 213–16
22. Flament S, Delacourte A, Verny M, Hauw JJ, Javoy-Agid F. Abnormal tau proteins in progressive supranuclear palsy. Similarities and differences with the neurofibrillary degeneration of the Alzheimer type. Acta Neuropathol (Berl) 1991;81:591–96
23. Delacourte A, Robitaille Y, Sergeant N, et al. Specific pathologic tau protein variants characterize Pick's disease. J Neuropathol Exp Neurol 1996;55:159–68
24. Forman MS, Zhukareva V, Bergeron C, et al. Signature tau neuropathology in gray and white matter of corticobasal degeneration. Am J Pathol 2002;160:2045–53
25. Arai T, Ikeda K, Akiyama H, et al. 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) 2001;101:167–73
26. Hutton M. Molecular genetics of chromosome 17 tauopathies. Ann NY Acad Sci 2000;920:63–73
27. Hutton M. Missense and splice site mutations in tau associated with FTDP-17: Multiple pathogenic mechanisms. Neurology 2001;56:S21–25
28. Ingram EM, Spillantini MG. Tau gene mutations: Dissecting the pathogenesis of FTDP-17. Trends Mol Med 2002;8:555–62
29. Lee VM, Goedert M, Trojanowski JQ. Neurodegenerative tauopathies. Annu Rev Neurosci 2001;24:1121–59
30. Tolnay M, Probst A. Frontotemporal lobar degeneration—tau as a pied piper? Neurogenetics 2002;4:63–75
31. Yoshiyama Y, Lee VM, Trojanowski JQ. Frontotemporal dementia and tauopathy. Curr Neurol Neurosci Rep 2001;1:413–21
32. Yamaoka LH, Welsh-Bohmer KA, Hulette CM, et al. Linkage of frontotemporal dementia to chromosome 17: Clinical and neuropathologic characterization of phenotype. Am J Hum Genet 1996;59:1306–12
33. D'Souza I, Schellenberg GD. tau exon 10 expression involves a bipartite intron 10 regulatory sequence and weak 5′ and 3′ splice sites. J Biol Chem 2002;277:26587–99
34. Goedert M, Spillantini MG, Crowther RA, et al. tau gene mutation in familial progressive subcortical gliosis. Nat Med 1999;5:454–57
35. Hutton M, Lendon CL, Rizzu P, et al. Association of missense and 5′-splice-site mutations in tau with the inherited dementia FTDP-17. Nature 1998;393:702–5
36. Miyamoto K, Kowalska A, Hasegawa M, et al. Familial frontotemporal dementia and parkinsonism with a novel mutation at an intron 10+11-splice site in the tau gene. Ann Neurol 2001;50:117–20
37. Spillantini MG, Murrell JR, Goedert M, Farlow MR, Klug A, Ghetti B. Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. Proc Natl Acad Sci U S A 1998;95:7737–41
38. Stanford PM, Shepherd CE, Halliday GM, et al. Mutations in the tau gene that cause an increase in three repeat tau and frontotemporal dementia. Brain 2003;126:814–26
39. Yasuda M, Takamatsu J, D'Souza I, et al. A novel mutation at position +12 in the intron following exon 10 of the tau gene in familial frontotemporal dementia (FTD-Kumamoto). Ann Neurol 2000;47:422–29
40. Reed LA, Wszolek ZK, Hutton M. Phenotypic correlations in FTDP-17. Neurobiol Aging 2001;22:89–107
41. Reed LA, Grabowski TJ, Schmidt ML, et al. Autosomal dominant dementia with widespread neurofibrillary tangles. Ann Neurol 1997;42: 564–72
42. Saito Y, Geyer A, Sasaki R, et al. Early-onset, rapidly progressive familial tauopathy with R406W mutation. Neurology 2002;58:811–13
43. Saunders AM, Strittmatter WJ, Schmechel D, et al. Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. Neurology 1993;43:1467–72
44. Strittmatter WJ, Saunders AM, Schmechel D, et al. Apolipoprotein E: High-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. Proc Natl Acad Sci USA 1993;90:1977–81
45. Fabre SF, Forsell C, Viitanen M, et al. Clinic-based cases with frontotemporal dementia show increased cerebrospinal fluid tau and high apolipoprotein E epsilon4 frequency, but no tau gene mutations. Exp Neurol 2001;168:413–18
46. Gustafson L, Abrahamson M, Grubb A, Nilsson K, Fex G. Apolipoprotein-E genotyping in Alzheimer's disease and frontotemporal dementia. Dement Geriatr Cogn Disord 1997;8:240–43
47. Kalman J, Juhasz A, Majtenyi K, et al. Apolipoprotein E polymorphism in Pick's disease and in Huntington's disease. Neurobiol Aging 2000;21: 555–58
48. Geschwind D, Karrim J, Nelson SF, Miller B. The apolipoprotein E epsilon4 allele is not a significant risk factor for frontotemporal dementia. Ann Neurol 1998;44:134–38
49. Hughes A, Mann D, Pickering-Brown S. tau haplotype frequency in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Exp Neurol 2003;181:12–16
50. Kowalska A, Asada T, Arima K, et al. Genetic analysis in patients with familial and sporadic frontotemporal dementia: Two tau mutations in only familial cases and no association with apolipoprotein epsilon4. Dement Geriatr Cogn Disord 2001;12:387–92
51. Minthon L, Hesse C, Sjogren M, Englund E, Gustafson L, Blennow K. The apolipoprotein E epsilon4 allele frequency is normal in frontotemporal dementia, but correlates with age at onset of disease. Neurosci Lett 1997;226:65–67
52. Pickering-Brown SM, Owen F, Isaacs A, et al. Apolipoprotein E epsilon4 allele has no effect on age at onset or duration of disease in cases of frontotemporal dementia with Pick- or microvacuolar-type histology. Exp Neurol 2000;163:452–56
53. Riemenschneider M, Diehl J, Muller U, Forstl H, Kurz A. Apolipoprotein E polymorphism in German patients with frontotemporal degeneration. J Neurol Neurosurg Psychiatry 2002;72:639–41
54. Verpillat P, Camuzat A, Hannequin D, et al. Apolipoprotein E gene in frontotemporal dementia: An association study and meta-analysis. Eur J Hum Genet 2002;10:399–405
55. Houlden H, Crook R, Backhovens H, et al. ApoE genotype is a risk factor in nonpresenilin early-onset Alzheimer's disease families. Am J Med Genet 1998;81:117–21