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Familial frontotemporal dementia with ubiquitin-positive inclusions is linked to chromosome 17q21–22

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Summary

Hereditary frontotemporal dementia (FTD) is an autosomal dominant neurodegenerative disorder that is associated with mutations in the *tau* gene and with the pathological accumulation of hyperphosphorylated tau protein in affected brain cells in about a quarter of cases. However, most FTD families have no demonstrable tau mutations. Here we describe the clinical and neuropathological features of a large family with hereditary FTD. Genetic analysis showed strong evidence for linkage to chromosome 17q21–22 (maximum lod score 3.46, $\theta = 0$ for marker D17S950), but mutations in the *tau* gene were not found. Clinical symptoms, neuropsychological deficits and neuroimaging findings of affected family members were similar to sporadic and tau-related FTD. The mean age at onset was 61.2 years, with loss of initiative and decreased spontaneous speech as the most prominent presenting symptoms. Pathological examination of the brains of two affected family members showed non-specific neuronal degeneration with dense cytoplasmic ubiquitin-positive inclusions in neurones of the second layer of the frontotemporal cortex and dentate gyrus of the hippocampus. In a number of neurones these inclusions appeared to be located inside the nucleus, although due to the small number of these inclusions this localization could not be confirmed by electron microscopy. The inclusions were not stained by tau, α-synuclein or polyglutamine antibodies. Biochemical analysis of soluble tau did not reveal abnormalities in tau isoform distribution and analysis of mRNA showed the presence of both three- and four-repeat transcripts. This is the first report of ubiquitin-positive, tau-negative inclusions in an FTD family with significant linkage to chromosome 17q21–22. Further characterization of the ubiquitin-positive inclusions may clarify the neurodegenerative pathways involved in this subtype of FTD.

Keywords: familial frontotemporal dementia; FTDP-17; ubiquitin-positive inclusions

Abbreviations: FTD = frontotemporal dementia; FTDP-17 = frontotemporal dementia and parkinsonism linked to chromosome 17; GFAP = glial fibrillary acidic protein; HMPAO = 99mTc-hexamethyl propyleneamine oxime; IBZM = [^{123}I]iodobenzamide; MC = monoclonal; PC = polyclonal; RT-PCR = reverse transcriptase–polymerase chain reaction; SPECT = single photon emission computed tomography

Introduction

Hereditary frontotemporal dementia (FTD) is a genetically heterogeneous disorder. *Tau* mutations were first identified in several families with FTD and parkinsonism linked to chromosome 17 (FTDP-17) (Foster *et al.*, 1997; Hutton *et al.*, 1998; Poorkaj *et al.*, 1998; Spillantini *et al.*, 1998*c*) and subsequently in patients presenting with other clinical phenotypes, including progressive supranuclear palsy and corticobasal degeneration (Bugiani *et al.*, 1999; Delisle *et al.*, 1999; Stanford *et al.*, 2000). All families with *tau* mutations have in common the accumulation of hyperphosphorylated tau protein in affected neurones or glial cells (Spillantini *et al.*, 1998*a*; van Swieten *et al.*, 1999). However, at least three FTDP-17 families have not shown *tau* mutations, despite significant linkage to the *tau*-containing region on

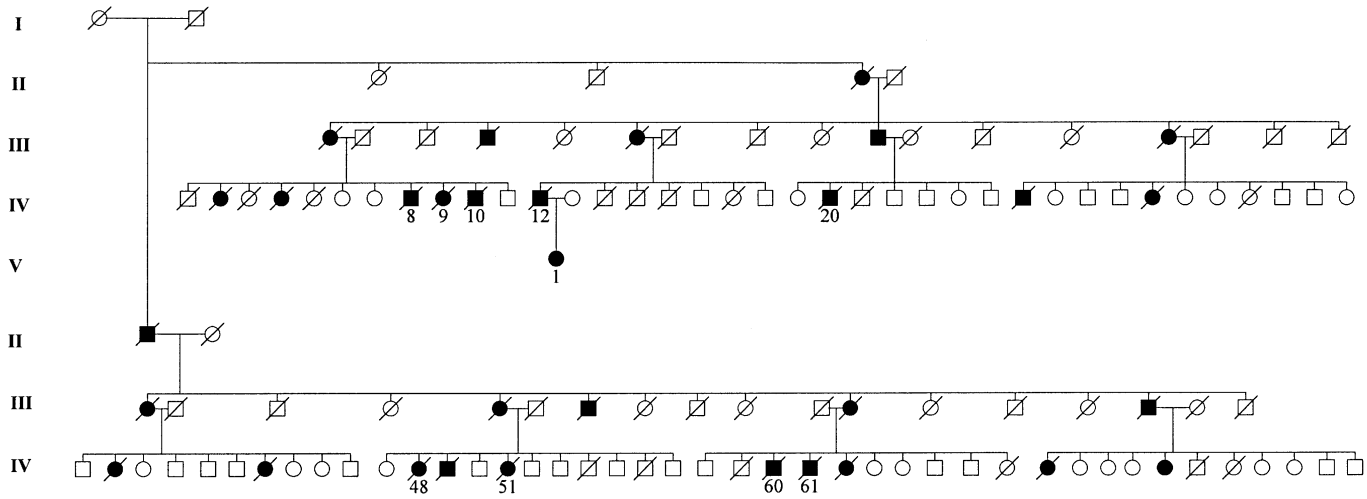

Fig. 1 Pedigree of family HFTD3.

chromosome 17q21–22 (Froelich *et al.*, 1997; Heutink *et al.*, 1997; Lendon *et al.*, 1998). Linkage to another locus on the centromeric region of chromosome 3 has been reported previously in a single Danish FTD kindred (Brown, 1998). Recently, locus heterogeneity in FTD has been emphasized by the identification of a new locus on chromosome 9q21–22 in families with amyotrophic lateral sclerosis and FTD-type dementia (Hosler *et al.*, 2000).

Two FTD families have shown ubiquitin-positive, tau-negative inclusions, which suggests an alternative pathophysiological mechanism (Kertesz *et al.*, 2000; Kovari *et al.*, 2000). Ubiquitin-positive inclusions have classically been associated with FTD with motor neurone disease, although recent studies have shown similar inclusions in sporadic cases of FTD without motor neurone disease (Jackson *et al.*, 1996) and in semantic dementia (Rossor *et al.*, 2000). These ubiquitin-positive cytoplasmic inclusions are present consistently in the superficial layers of the frontotemporal cortex and dentate gyrus of the hippocampus, and have to be distinguished from the ubiquitinated neurites that can be found in nearly all cases of FTD (Iseki *et al.*, 1998; Kinoshita *et al.*, 1997).

Here we describe the clinical and neuropathological features of a large Dutch FTD family (family HFTD3) reported earlier (Heutink *et al.*, 1997), which is characterized by ubiquitin-positive, tau-negative inclusions. Genetic analysis revealed strong evidence for linkage to the tau-containing region of chromosome 17q21–22, but mutations in the *tau* gene were not identified.

Methods

Clinical data

This four-generation FTD family with 32 affected members (19 women, 13 men) shows an autosomal dominant pattern of inheritance (Fig. 1) and has been reported briefly (Heutink *et al.*, 1997). Clinical information was obtained by interviewing relatives of patients, neurological examination of living patients (six patients) and reviewing the medical records (including hard copies of neuroimaging studies). The diagnosis of FTD according to the criteria of the Lund and Manchester groups (Lund and Manchester Groups, 1994; Neary *et al.*, 1998) was established in 10 patients, and unspecified dementia was diagnosed in the remaining 22 patients because of limited clinical information. Extensive psychometric testing was done at our department in two patients and included the assessment of language functions, intelligence, attention and concentration, memory, executive functions, abstract thinking and visuoconstructive abilities. Neuropsychological evaluation was done earlier elsewhere in five patients. CT was available in six patients, MRI in one patient, single photon emission computed tomography (SPECT) scanning with 99mTc-hexamethyl propyleneamine oxime (HMPAO) in three patients and with [^{123}I]iodobenzamide (IBZM) scanning in one patient. Brain autopsy with neuropathological verification of the clinical diagnosis was performed in two patients. The Medical Ethics Committee of the University Hospital of Rotterdam approved the study. The spouse or a first-degree relative of each patient gave informed consent for blood sampling for DNA studies.

Genetic studies

We have reported mildly positive lod-scores for chromosome 17 markers for this family previously, but these scores did not reach significance (Heutink *et al.*, 1997). After ascertainment of additional affected relatives, we repeated the analysis on all available family members.

Genomic DNA was isolated from peripheral blood as described by Miller and colleagues (Miller *et al.*, 1988). The short tandem repeat polymorphisms D17S945, D17S953, D17S946, D17S934, D17S951, D17S950 and D17S791 for chromosome 17q21–22 were selected on the basis of previous linkage results (Heutink *et al.*, 1997) and D3S1598, D3S3695,

D3S3681, D3S1603 and D3S3574 for the pericentromeric region of chromosome 3 were selected on the basis of a report of linkage in familial FTD (Brown, 1998). Genomic DNA (25 ng) was amplified in 10 μl polymerase chain reactions (PCR) containing 1X GeneAmp PCR Gold Buffer, 1.5 mM MgCl, 25 ng of fluorescent forward primer, 25 ng unlabelled reverse primer and 0.4 U of AmpliTaq Gold DNA polymerase. Initial denaturation was 15 min at 95°C followed by 32 cycles of 30 s denaturation at 95°C, 30 s annealing at 55°C and 90 s extension at 72°C. Reactions were prepared using a Beckman Biomeck 2000 robot system and performed in 384-well plates covered with sealing lids (Costar 6557; 6555). A GeneAmp PCR System 9700 (Applied Biosystems, Foster City, Calif., USA) equipped with dual 384-well plates was used for amplification. PCR products were pooled and loaded on an ABI 377 automated sequencer (filter set D; 5% denaturing FMC LongRanger acrylamide gel), data were analysed using ABI GeneScan 3.1 and ABI Genotyper 2.1 software.

Two-point linkage analysis was performed using the Mlink and Ilink programs of the Linkage package, version 5.1 (Lathrop and Lalouel, 1984). Maximum lod and location scores were calculated for each marker using an affected-only analysis. Unaffected family members were typed as unknown. A gene frequency of 1 : 10 000, no phenocopies and equal allele frequencies of the genotyped markers were used in the calculations. Changing allele frequencies of the polymorphic markers did not alter the lod and location scores significantly. Multipoint analysis was performed by subsequent three-point linkage analysis on all markers tested.

Exons 1, 2, 3, 4, 5, 7, 9, 10, 11, 12 and 13 of the *tau* gene were amplified using specific primers derived from the 5′ and 3′ intronic sequences (Rizzu *et al.*, 1999). The annealing temperature for all primer pairs was 58°C. Amplification conditions were as follows: reaction volume was 50 μl with a final concentration of 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM dNTPs, *Taq* polymerase at 1.5 U/50 μl, primers at 25 pmol/μl and 50 ng of template genomic DNA. The PCR reactions were analysed on a 2% agarose gel to verify the size and quantity of the PCR product. PCR products were analysed subsequently by direct sequence analysis of the PCR products on an automated DNA sequencer (ABI 377) using the BigDye terminator cycle sequencing kit (Applied Biosystems).

Neuropathology

A brain autopsy was performed by the Netherlands Brain Bank on two patients (IV:10 and IV:60). Informed consent was obtained from the patients' next of kin before autopsy for the use of the tissue for diagnostic purposes as well as for scientific research. After fresh dissection of various brain regions, tissue blocks were either frozen rapidly in liquid nitrogen and stored at –80°C (Patient IV:60 only) or fixed in formalin and embedded in paraffin. Formalin-fixed, paraffin-embedded sections from all cortical regions, the hippocampus and parahippocampal gyrus, amygdala, substantia nigra, basal ganglia, thalamus, cerebellum and brainstem were processed for routine staining (haematoxylin and eosin, Bodian, methenamine silver and congo red) and immunohistochemistry. A conventional avidin–biotin–peroxidase complex method (Zymed Laboratories, San Francisco, Calif., USA) was used, with diaminobenzidine as the chromogen. Slides were counterstained with Mayer's haematoxylin and mounted in Entellan.

We used monoclonal (MC) and polyclonal (PC) antibodies raised against tau protein, both phosphorylation-dependent [AT8 (MC), 1 : 40, Innogenetics, Gent, Belgium; AT180 (MC), 1 : 500, Innogenetics; AT270 (MC), 1 : 500, Innogenetics; PHF1 (MC), 1 : 500, gift from Peter Davies, Albert Einstein College of Medicine, New York, NY, USA; MC1 (MC), 1 : 25, gift from Peter Davies] and phosphorylation-independent [T14 (MC), 1 : 100, Zymed Laboratories; BR01 (MC), 1 : 500, Innogenetics; tau2 (MC), 1 : 100, Sigma, St Louis, Mo., USA], as well as antibodies against ubiquitin (PC, 1 : 500, Dako, Glostrup, Denmark), polyglutamine (1C2, MC, 1 : 1000, Chemicon, Temecula, Calif., USA), human leucocyte antigen DR (HLA-DR) (MC, 1 : 100, Dako), αB-crystallin (PC, 1 : 500, Novocastra Laboratories, Newcastle-upon-Tyne, UK), β-amyloid (βA4, MC, 1 : 100, Dako), α-, β- and γ-synuclein [PER 4, 3 and 5, 1 : 500, M. G. Spillantini, Cambridge, UK (Spillantini *et al.*, 1998*b*)], β-tubulin (TUB 2.1, MC, 1 : 500, Sigma), glial fibrillary acidic protein (GFAP) (MC, 1 : 500, Dako), synaptophysin (PC, 1 : 100, Dako), microtubule associated protein (MAP2) (MC, 1 : 100, Boehringer, Mannheim, Germany), neurofilament (SMI-32, 1 : 1000, Sternberger Monoclonals, Lutherville, Md., USA), neuroserpin (1 : 500, M. G. Spillantini), actin (MC, 1 : 25, Dako), neurogranin (1 : 500, Chemicon), strathmin (1 : 500, Calbiochem, San Diego, Calif., USA), heparan sulphate (1 : 250, Seikagaku Amerika, Rockville, Md., USA) and parkin (1 : 500, Chemicon). Heat-induced antigen retrieval was performed by heating slides at 80°C for 30 min in 0.1 M sodium citrate buffer at pH 7.7 for several antibodies (polyglutamine, HLA-DR, β-tubulin, synaptophysin, MAP2, GFAP, T14, BR01 and tau2). Tissue sections were pretreated with 90% formic acid for 5 min before incubation with βA4 antibody.

Biochemical studies

Soluble tau was extracted with 2.5% perchloric acid as described previously (Goedert and Jakes, 1990) and blotted onto Immobilon P (Millipore, Bedford, Mass., USA). Blots were incubated overnight at 4°C with phosphorylation-independent anti-tau antibodies (BR133 and BR134; Spillantini *et al.*, 1998*a*) and stained using the biotin–avidin Vectastain system (Vector Laboratories, Burlingame, Calif., USA). Sarkosyl-insoluble extracts were run on 10% SDS-PAGE (sodium dodecylsulphate–polyacrylamide gel electrophoresis) gels and blotted onto Immobilon P (Millipore) and processed for immunoblotting as indicated above. Electron

Table 1 *Clinical features and neuroimaging of 10 affected family members*

Patient	Sex	Age at onset (years)	Duration (years)	Initial complaints	Further symptoms	Neuroimaging	Duration at imaging
IV:8	M	58	13	Memory problems	Agitation, mutism, hyperorality	NA	–
IV:9	F	63	6	Restlessness	Roaming, hyperorality, loss of decorum	NA†	–
IV:10	M	57	9	Lack of interest, agitation	Restlessness, roaming, hyperorality	FT mild atrophy	3
IV:12	M	57	6	Personality change	Disinterest, hyperorality, perseveration	NA	–
IV:20	M	57	10	Behavioural change	Apathy, mutism, parkinsonism	NA	–
IV:48	F	71	7	Loss of initiative	Reduced speech, hyperorality	T mild atrophy, R>L	3
IV:51	F	64	7	Loss of initiative	Memory problems, hyperorality	FT mild atrophy	1
IV:60	M	70	6	Personality change	Disinterest, decreased speech	F>T severe atrophy, L>R†	5
IV:61	M	62	13	Confabulations	Aggression, hyperorality	FT mild atrophy	2
V:1	F	53	1*	Memory problems	Apathy, disinterest, parkinsonism	FT moderate atrophy†	1

M = male; F = female; FT = frontotemporal; T = temporal; F>T = frontal atrophy more severe than temporal atrophy; R = right; L = left; NA = not available. *Ongoing. †SPECT scan performed and showing hypoperfusion of anterior parts of the brain.

microscopy was used to evaluate the presence of sarkosyl-insoluble tau filaments as described previously (Crowther, 1991).

Total RNA was isolated from frontal cortex tissue of Patient IV:60 and two healthy control brains using the RNAzolB kit (Campro Scientific, Berlin, Germany) according to the manufacturer's specifications. Reverse transcription (RT)-PCR was performed using the Superscript Pre-amplification System (Life Technologies, Gaithersburg, Md., USA) on 5 μg of brain RNA with both oligo(dT) and random hexamer primers. PCR was performed between exon 9 (forward, 5′-ATCGCAGCGGCTACAGCAG-3′) and exon 11 (reverse, 5′-TGGTTTATGATGGATGGATGTTGCCT-3′). PCR products (30 cycles) were resolved on 2% agarose gel and visualized with ethidium bromide.

Results

Clinical features

The mean age at onset of symptoms in the 10 patients with probable FTD was 61.2 years (range 53–71 years). The mean duration of symptoms until the time of death was 8.6 years ($n = 9$); one patient was still alive at the time of investigation. The average age at death of all affected family members ($n = 30$) was 69.1 years. There was no difference between the age at death of men (69.7 years) and women (68.6 years) or evidence of anticipation in consecutive generations.

Loss of initiative and decreased spontaneous speech were the most prominent clinical features. The patients withdrew socially and lost interest in their family and environment. Restlessness and agitation were often reported at late stages of the disease. Patients did not show any concern or insight as to their illness, except at the earliest stage in one patient. Memory problems were inconsistent and often related to decreased attention span. Focal neurological deficits were absent, whereas primitive reflexes were seen at a late stage in all patients examined. Bradykinesia and cogwheel rigidity were found in three patients, in one at a very late stage of the disease. The two patients with early symptoms (IV:20 and V:1) were both treated with levodopa, which resulted in only a partial and transient response in Patient V:1. Signs of motor neurone disease, such as muscle weakness, hyperreflexia and fasciculations, were absent. No epileptic seizures or myoclonic movements were observed or mentioned in the medical records of any of the patients. The main clinical features and neuroimaging findings are summarized in Table 1.

Extensive neuropsychological evaluation in two patients (1 and 2 years after onset, respectively) showed evidence for cognitive impairment compatible with the diagnosis of FTD. Both patients were cooperative but lacked insight about their performance and were easily distracted as a result of decreased attention and stimulus-bound behaviour. Reduced word fluency, impaired abstract thinking, perseverations and reduced mental flexibility were also evident. Except for mild naming difficulties, no evident signs of language disturbance were apparent. Memory and visuospatial functions were both relatively spared. Neuropsychological results for the other patients, using various test batteries, were also consistent with frontal lobe dysfunction.

Structural neuroimaging, available in six patients, showed mild atrophy (frontotemporal in three, temporal in one) in four patients (1, 2, 3 and 3 years after onset) and moderate to severe frontotemporal atrophy in two patients (1 and 5 years after onset). Two of these patients showed mild asymmetry of atrophy. An HMPAO-SPECT scan was available for three patients and showed anterior hypoperfusion in all three, with a mildly asymmetrical pattern in two patients. IBZM-SPECT was performed in one patient (V:1) and showed an asymmetrical reduction in dopamine D_2 receptors in the striatum.

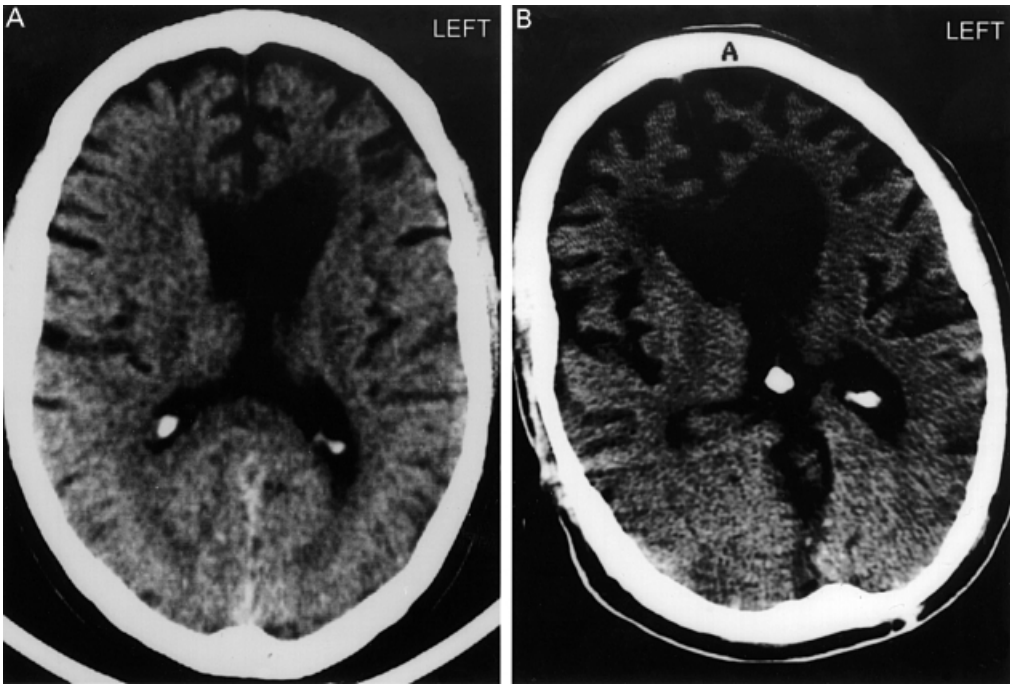

Fig. 2 CT scans of Patient IV:60. (**A**) Transverse image 2 years after onset showing mild frontotemporal atrophy, which is slightly more prominent on the left than on the right. (**B**) Transverse image showing progression of atrophy 5 years after onset of symptoms.

Illustrative cases

Patient IV:60

This patient became unable to manage his farm at age 70 and his son had to take over. He showed lack of initiative and did not respond adequately in difficult situations. Spontaneous speech decreased and he lost interest in his family and friends. He did not care sufficiently for his handicapped wife and quarrelled with her about trivialities. He spent most of his time at home sitting in a chair and tapping the armrest with his fingers continuously. He showed no signs of disinhibition and his eating habits remained unchanged. Neuropsychological evaluation 2 years after onset showed mild naming difficulties, problems with abstract thinking, perseverative and impulsive behaviour, mild visuospatial impairment and relatively intact memory functions. A CT scan at that time showed mild atrophy of both the frontal and temporal lobes, which was slightly more prominent in the left hemisphere (Fig. 2A). SPECT showed evident hypoperfusion of the anterior parts of both cerebral hemispheres, which was more pronounced on the left than on the right. His condition deteriorated during the next 3 years. He developed mutism, bradykinesia, rigidity without resting tremor, and swallowing difficulties. A second CT scan 5 years after disease onset showed moderate to severe atrophy of the same regions (Fig. 2B). The patient died at the age of 76 years, probably from ischaemic heart disease, and an autopsy was done.

Patient V:1

This 53-year-old woman complained of memory problems and difficulty in scheduling her work as a specialized nurse. Her colleagues found her alertness reduced in complicated situations. Her husband noted social withdrawal by neglecting telephone calls and appointments with friends. She called her daughter several times a day with identical questions. She was restless and made long trips on foot (several miles a day) or by bicycle (up to 40 miles a day) without losing her way. She was caught twice for shoplifting in a supermarket. An MRI scan of the brain showed symmetrical frontotemporal atrophy (Fig. 3A and B), with corresponding anterior hypoperfusion on SPECT scan. Psychometric evaluation showed decreased attention and concentration, perseveration, impairment of abstract thinking and concept-shifting, and relatively intact memory and visuospatial functions. One year after onset, the patient still showed some insight as to her illness, and felt depressed about her deficits. Furthermore, she complained of fatigue and stiffness in both legs and her right hand. Neurological examination showed a masked face, mild bradykinesia and cogwheel rigidity in both extremities, without postural instability. IBZM-SPECT showed a reduction in striatal dopamine D2 receptors, greater on the left than on the right (Fig. 3C). The symptoms showed a subjective partial and transient response to levodopa treatment.

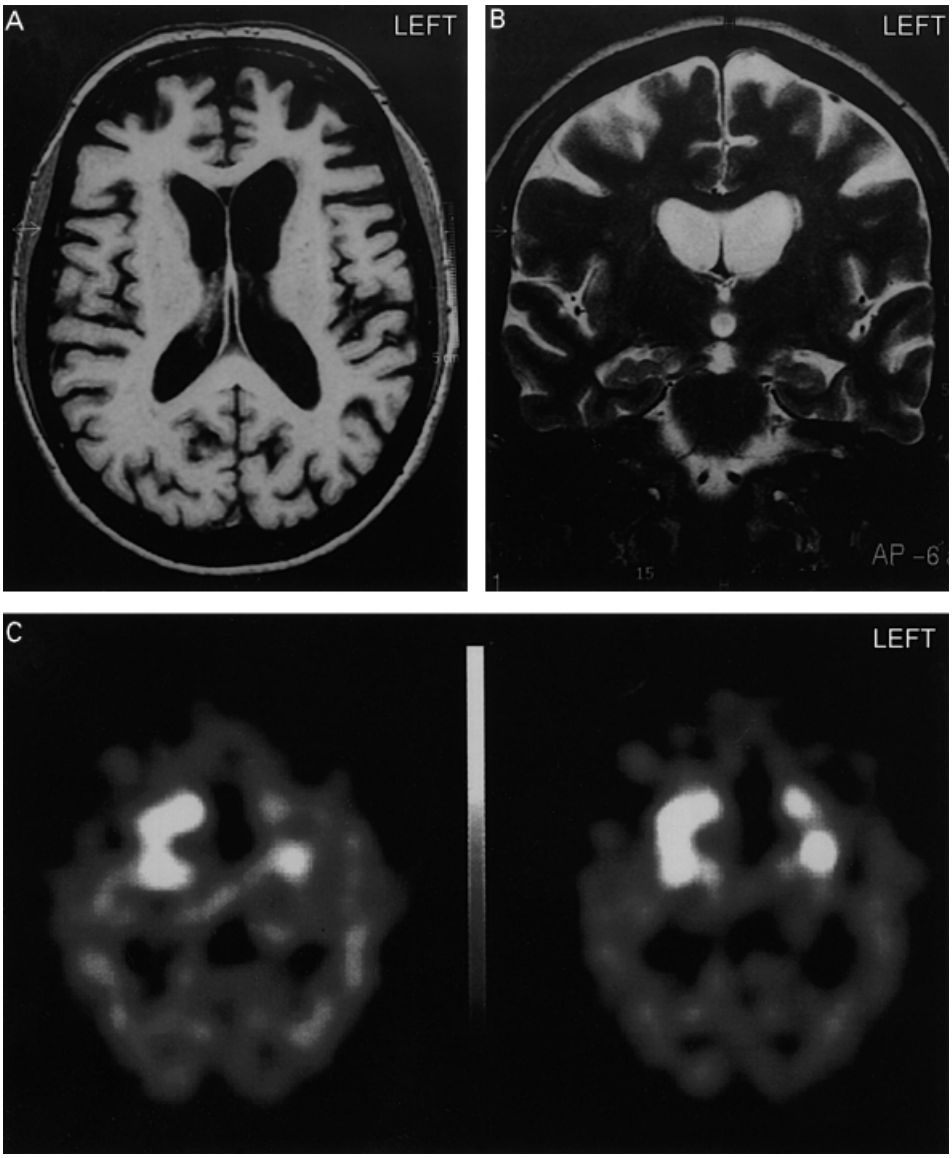

Fig. 3 MRI scans of Patient V:1. (**A**) T_1-weighted transverse image showing moderate frontotemporal atrophy 1 year after disease onset. (**B**) T_2-weighted coronal image made at the same time as that illustrated in **A**, showing additional mild atrophy of the temporal lobes. (**C**) Reduction in striatal dopamine D_2 receptors on IBZM-SPECT. The reduction is greater on the left than on the right.

Genetic studies

Positive lod scores for all chromosome 17 markers were obtained, with a maximum lod score of 2.5 with marker D17S950 ($\theta = 0$). Multipoint analysis resulted in a maximum lod score of 3.46 at D17S950 ($\theta = 0$). Haplotype analysis revealed recombination events with markers D17S945 and D17S953 but not with marker D17S791. Therefore, the microtubule-associated protein *tau* gene is located within the critical region for this family. Mutation analysis of the complete coding region and intron–exon boundaries did not reveal a pathogenic mutation in this gene. Two-point lod scores for all chromosome 3 markers did not support linkage to this region, although none of the markers definitely excluded linkage.

Neuropathology

Atrophy of both frontal and temporal lobes and severely dilated ventricles were present in the brain of Patient IV:10 (1170 g), whereas only frontal atrophy was seen in the brain of Patient IV:60 (930 g). The brains of the two patients showed similar pathology: severe neuronal loss and gliosis in the second and third cortical layers of the frontal and temporal cortex (Fig. 4A), although the temporal cortex of brain IV:60 was relatively spared compared with the frontal cortex. Furthermore, neuronal loss was seen in the cornu ammonis of the hippocampus and the entorhinal cortex of both brains. A few neurofibrillary tangles in the pyramidal cells of the entorhinal cortex (no more than five per section) were present in the brain of Patient IV:60, as can be expected

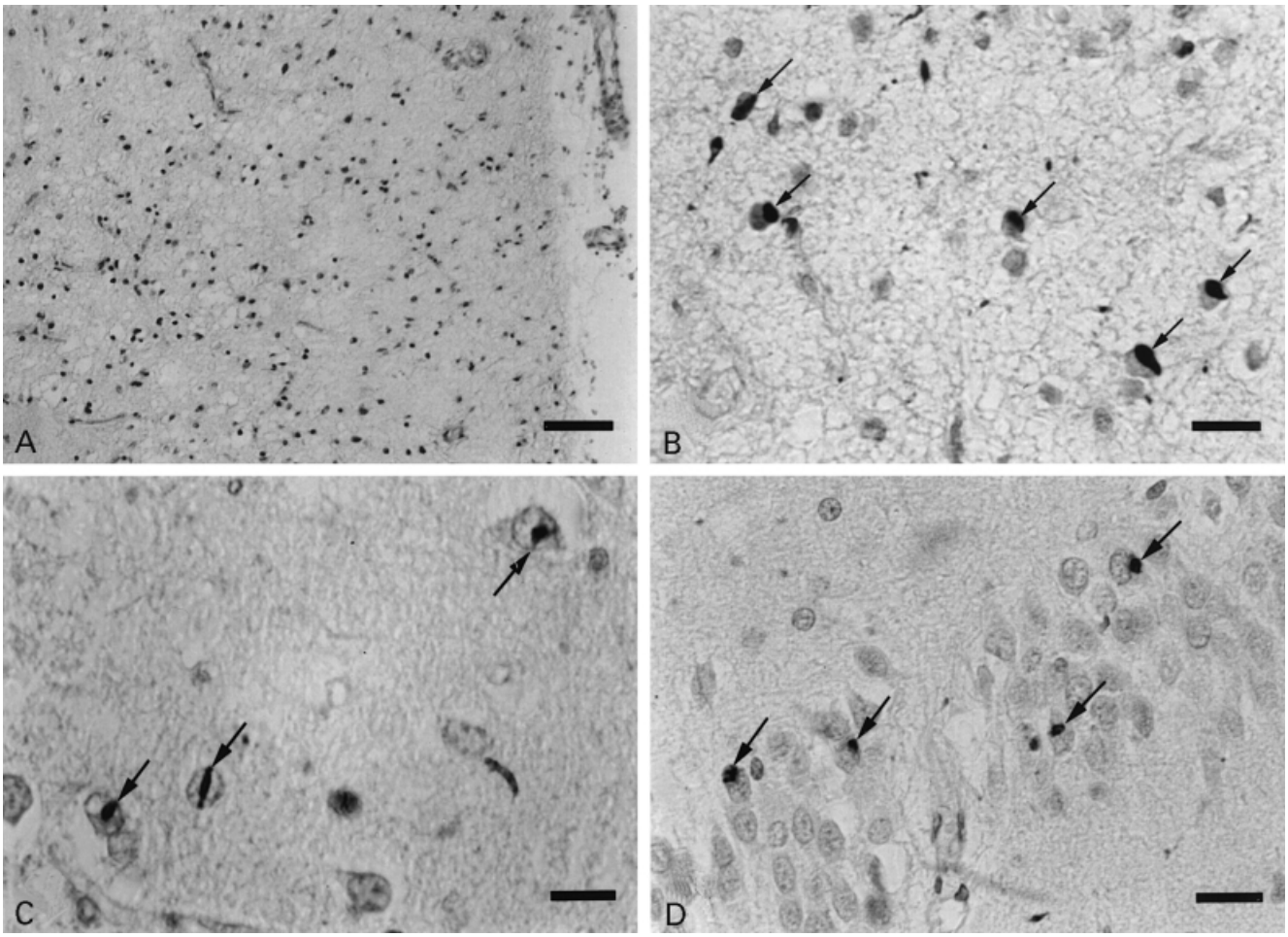

Fig. 4 Microscopic findings in Patient VI:60. (**A**) Haematoxylin and eosin staining of the frontal cortex, showing severe neuronal loss and microvacuolation. (**B**) Ubiquitin immunostaining of the frontal cortex, showing cytoplasmic inclusions in neurones of layer 2 (arrows). (**C**) Same region and staining as **B**, showing a small field of inclusions that appears to be located inside the nucleus (arrows). (**D**) Dentate gyrus of hippocampus, showing ubiquitin-positive cytoplasmic inclusions (arrows). Scale bars 240 μm in **A**; 100 μm in **B**–**D**.

at the age of 76 years. Both brains showed a normal occipital cortex and cerebellum. Severe loss of pigmented neurones was seen in the substantia nigra, and the thalamus, caudate nuclei and putamen were also affected. The nucleus hypoglossus of both patients and the cervical spinal cord (only available for brain IV:60) were normal. Neuritic or diffuse plaques, ballooned cells, Pick bodies and Lewy bodies were absent in both brains.

Staining with anti-ubiquitin antibody showed small, dense intracytoplasmic inclusions in neurones of the second layer of the frontal and temporal cortex of both brains, and also to a lesser extent in the parietal cortex of brain IV:10. The highest density of inclusions was present in the cingulate gyrus of both brains. The inclusions were not visible with conventional haematoxylin–eosin, Bodian or methenamine–silver staining, and were located preferentially in the perikaryal space directly next to the nucleus. The inclusions were sharply circumscribed and usually round- or crescent-shaped (Fig. 4B). A few ubiquitin inclusions, apparently located within the nucleus, were identified in a thorough examination. These inclusions had a cat's eye or target shape and were occasionally grouped together in small fields (Fig. 4C). Furthermore, some granular cells of the dentate gyrus contained cytoplasmic ubiquitin-positive inclusions, which were round and less dense than the inclusions found in the cortical regions (Fig. 4D). Ubiquitin-positive neurites were also present in the affected cortical layers in both patients. The subcortical regions did not contain any inclusions. The nucleus hypoglossus of the midbrain of both patients, as well as the spinal cord (available only for Patient IV:60) did not show any ubiquitin inclusions.

Phosphorylation-dependent and independent antibodies against tau protein and antibodies against α-synuclein, neurofilament (SMI-32), β-tubulin, MAP2 and polyglutamine (1C2) did not stain the inclusions. No amyloid plaques or ballooned cells were detected with βA4 antibody or αB-crystallin, respectively. In brain IV:10, staining with GFAP antibody showed very severe reactive astrocytosis in all

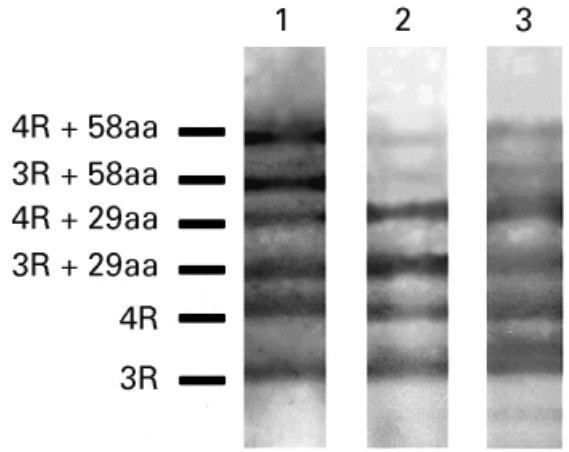

Fig. 5 Immunoblot of dephosphorylated soluble tau protein (BR134) from the frontal cortex of Patient IV:60 (lane 3) and a control patient with Alzheimer's disease (lane 2). All six tau isoforms are present and align with the six tau recombinant human brain tau isoforms (lane 1).

frontal and temporal cortical layers, whereas in brain IV:60 astrocytosis was moderate and restricted to the frontal and temporal subcortical white matter, with relatively mild changes in the cortex.

Biochemical studies

Analysis of soluble tau showed the presence of all six tau isoforms in affected regions of brain IV:60 (Fig. 5). The distribution of isoforms appeared normal, with similar amounts of three- and four-repeat tau isoforms. No sarkosyl-insoluble filaments could be detected by electron microscopy, in agreement with the fact that no sarkosyl-insoluble tau was found by immunoblotting. RT–PCR revealed the presence of both three- and four-repeat tau transcripts in the frontal cortex of Patient IV:60 (data not shown).

Discussion

The present study describes the clinicopathological features of a large family with autosomal dominantly inherited FTD. Ubiquitin-positive, tau-negative neuronal inclusions were the most characteristic neuropathological finding. The significant linkage to chromosome 17q21–22 in this family was not associated with either mutations in the *tau* gene or tau deposition in the brain, as can be found in a subset of families with FTD.

The clinical symptomatology of the present family is consistent with the diagnosis of FTD according to the Lund and Manchester criteria, and fits within the wide spectrum of the clinical phenotype in hereditary tau-related FTD (Bird *et al.*, 1999; van Swieten *et al.*, 1999; Heutink, 2000; Spillantini *et al.*, 2000). In the absence of disinhibition, the diagnosis in our family was based on the presence of initiative loss, reduced spontaneous speech and frontal deficits on neuropsychological testing (reduced concept-shifting and word fluency, mental inflexibility and impaired abstract thinking) and was supported by selective atrophy of the frontal and temporal cortex. However, there was great variation in age at onset—from 53 to 71 years. This differs from that seen in most families with tau-related FTD (Heutink, 2000). An age at onset of >65 years (which was found in two of the affected family members) is also uncommon in other hereditary forms of FTD. Other symptoms typical of FTD, such as disinhibition, obsessive–compulsive behaviour and signs of motor neurone disease, were not observed, although they are found in other FTD families with ubiquitin inclusions (Kertesz *et al.*, 2000; Savioz *et al.*, 2000). The reduction in striatal dopamine D_2 receptors in one of our patients with early parkinsonism indicated a similar pathophysiological mechanism of a postsynaptic defect, as found in an FTD patient with the P301S mutation in the *tau* gene (Sperfeld *et al.*, 1999).

The presence of cytoplasmic ubiquitin-positive inclusions in neurones of the frontal and temporal cortices in the present family may be a first clue in elucidating the aetiology of this form of FTD. These tau- and α-synuclein-negative inclusions are similar in appearance and distribution to those described in FTD with motor neurone disease, some cases of semantic dementia and in a few other families with FTD (Jackson *et al.*, 1996; Kertesz *et al.*, 2000; Kovari *et al.*, 2000; Rossor *et al.*, 2000). A small fraction of inclusions appeared to be located inside the nucleus of neurones, but may be located within the inward invaginations of the nuclear membrane, giving the false impression of intranuclear localization. These intranuclear-like inclusions were seen in only a few microscopic fields after thorough inspection and were too rare to be considered as a main pathological substrate in our family. Their exact localization can only be determined by electron microscope studies, which were hampered by the scarcity of the lesions and the altered morphology of post-mortem tissues. It would be very interesting to look for their presence in other familial and sporadic FTD cases.

The intranuclear-like appearance of some of the inclusions was different from the consistently intranuclear localization of inclusions found in Huntington's disease and other triplet repeat diseases (Huntington's Disease Collaborative Research Group, 1993; DiFiglia *et al.*, 1997) and has not been observed in FTD with tau pathology. It is unlikely that FTD in the present family is a triplet repeat disorder, as the inclusions did not stain with a polyglutamine antibody and anticipation in consecutive generations was not observed. As in other neurodegenerative diseases, the protein in the inclusions had probably been ubiquitinated in an attempt at degradation. Purification of the proteins in the inclusions and subsequent amino acid analysis of the isolated peptides might help to identify the genetic defect responsible for the disease.

Our study confirms for the first time significant linkage to the chromosome 17q21–22 region in a family with FTD showing neuronal ubiquitin-positive, tau-negative inclusions, and lacking mutations in the coding regions and exon–intron

boundaries of the *tau* gene. Tau pathology was absent in both cases autopsied, except for a very few neurofibrillary tangles in the entire entorhinal cortex of Patient IV:60, consistent with the age of death of this patient (76 years). The presence of only a few neurofibrillary tangles in the entorhinal cortex of the patient was insufficient for the detection of sarkosyl-insoluble tau. Interestingly, Zhukareva and colleagues have found reduced levels of sarhosyl-soluble tau protein in sporadic FTD cases and in a family with hereditary dysphasic disinhibition dementia, which has shown linkage to the same chromosomal region and absence of tau mutations (Zhukareva *et al.*, 2001). However, we did not find a striking qualitative difference in the amount and ratio of three- and four-repeat isoforms compared with control brain. However, more patients are needed to determine the exact ratios.

Kertesz and colleagues also found positive lod scores for the chromosome 17q21–22 region in one of the two other FTD families with ubiquitin-positive, tau-negative inclusions, but no mutations in the *tau* gene. Although an obligatory recombinant with an intragenic marker in the *tau* gene was found in one patient, only the 3′ end of the *tau* gene can be definitely excluded in this family as the marker was located within intron 9 (Kertesz *et al.*, 2000). Linkage to chromosome 17q21–22 or another genetic locus has not yet been demonstrated in the other FTD family with ubiquitin-positive inclusions and no tau mutations (Kovari *et al.*, 2000; Savioz *et al.*, 2000). It is interesting that a similar distribution of neuronal degeneration, most prominent in the second and third layers of the frontal and temporal cortices, is associated with two different pathological phenotypes, characterized either by abnormal tau deposition or by ubiquitin-positive inclusions.

The critical region of linkage in this family contains a number of interesting candidate genes (Froelich *et al.*, 1997). *GFAP* is one of the genes within the critical region. The severe reactive astrocytosis seen upon GFAP antibody staining in the affected cortices of the brain IV:10 and in the underlying white matter of brain IV:60 is similar to that described in the other two ubiquitin–FTD families, but its severity is much more pronounced than that found in the brains of Dutch patients with the P301L, G272V and R406W mutations (van Swieten *et al.*, 1999). However, astrocytosis is a non-specific phenomenon and only reflects severe neuronal loss. Another candidate gene might be the nerve growth factor receptor gene (Froelich *et al.*, 1997), which may play a role in the induction of apoptotic cell death in the absence of nerve growth factor. However, evidence for apoptosis in FTD is inconsistent (Giannakopoulos *et al.*, 1999; Su *et al.*, 2000). We are currently investigating other candidate genes by sequence analysis.

In summary, the present study of a family with FTD (family HFTD3) showing linkage to chromosome 17q21–22 initiates a new search for an alternative pathway of neurodegeneration in FTD, since ubiquitin-positive inclusions in neurones were found in the absence of both pathological tau deposition and mutations in the *tau* gene. The prevalence of ubiquitin-positive, tau-negative inclusions in both sporadic and familial FTD cases is unclear at present, and should be investigated systematically as they are a distinguishing feature and may be the hallmark of a specific subgroup of FTD cases.

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