A family with tau-negative frontotemporal dementia and neuronal intranuclear inclusions linked to chromosome 17


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Over 30 different mutations have now been identified in MAPt that cause frontotemporal dementia (FTD). However, there are several families with FTD that show definite linkage to the region on chromosome 17 that contains MAPt, in which no mutation(s) has been identified. Although these families could have a complex mutation of the MAPt locus that has evaded detection it is also possible that another gene in this region is associated with FTD. This possibility is supported by neuropathological findings in these families, which consist of neuronal inclusions that are immunoreactive for ubiquitin (ub-ir) but not for tau. In addition to neuronal cytoplasmic inclusions, several chromosome 17-linked families are reported to have ub-ir neuronal intranuclear inclusions (NII); a finding which is uncommon in sporadic FTD. Here, we describe detailed clinical and neuropathological findings in a new large, multigenerational family with autosomal dominant FTD and autopsy proven tau-negative, ub-ir neuronal cytoplasmic and intranuclear inclusions. We have demonstrated that this family is linked to a 19.06 cM region of chromosome 17q21 with a maximum multipoint LOD score of 3.911 containing MAPt. By combining the results of our genetic analysis with those previously published for other families with similar pathology, we have further refined the minimal region to a 3.53 cM region of chromosome 17q21. We did not identify point mutations in MAPt by direct sequencing or any gross MAPt gene alterations using fluorescent in situ hybridization. In addition, tau protein extracted from members of this family was unremarkable in size and quantity as assessed by western blotting. Neuropathological characterization of the ub-ir NII in this family shows that they are positive for promyelocytic leukaemia protein (PML) and SUMO-1 that suggests that these inclusions form in the nuclear body and suggests a possible mechanism of neurodegeneration in tau-negative FTD linked to chromosome 17q21.

Keywords: frontotemporal dementia; ubiquitin; linkage analysis; neuronal intranuclear inclusions

Abbreviations: FTD = frontotemporal dementia; NII = neuronal intranuclear inclusions; NSF = N-ethylmaleimide-sensitive factor; PML = promyelocytic leukaemia protein; ub-ir = immunoreactive for ubiquitin

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Introduction

The clinical syndrome of frontotemporal dementia (FTD) is characterized by changes in behaviour, personality and language with relative preservation of memory and perception (Brun et al., 1994; Snowden et al., 1996; Neary et al., 1998).
FTD is the second most common form of dementia in individuals under the age of 65. Approximately, half of all patients with FTD present with a family history of a similar disease in at least one first-degree relative, indicating a significant genetic contribution to the aetiology of this disease (Brun et al., 1994; Snowden et al., 1996; Neary et al., 1998).

It has been demonstrated that a proportion of familial FTD results from mutations in the MAPT gene on chromosome 17q21, which encodes the microtubule-associated protein tau (Hutton et al., 1998; Poorkaj et al., 1998; Spillantini et al., 1998). There have now been over 30 MAPT mutations reported in exons 1, 9, 10, 11, 12 and 13 that account for ~15–20% of familial FTD cases (Rademakers et al., 2004). Of significance, is that all FTD cases associated with pathogenic MAPT mutations, in which autopsy findings are available, have demonstrated prominent fibrillar tau pathology in neurons, and sometimes in glia (Reed et al., 2001; Rademakers et al., 2004). However, significant tau pathology is seen only in ~40% of all FTD cases irrespective of family history (Mann et al., 2000; Josephs et al., 2004; Lipton et al., 2004; Taniguchi et al., 2004). The most common neuropathology associated with FTD is the presence of neuronal cytoplasmic inclusions (NCIs) and neurites that are immunoreactive for ubiquitin (ub-ir) but not for tau (FTD-U). This ub-ir pathology is characteristically found in the dentate fascia of the hippocampus and in layer 2 of the frontal and temporal neocortex (Mann et al., 2000; Lipton et al., 2004; Taniguchi et al., 2004; Mackenzie et al., 2005). Apart from being ubiquitinated, the precise molecular composition of these inclusions is yet to be determined. FTD-U was first described in patients with motor neuron disease and dementia but has subsequently been recognized as a common neuropathological feature of FTD in patients without motor symptoms (Jackson et al., 1996).

Interestingly, seven families with autosomal dominant FTD have been reported to show linkage to the same region on chromosome 17q21 that contains MAPT, but with no pathogenic MAPT mutation(s) identified, despite extensive analysis of this gene (Benissu et al., 2004; Bird et al., 1997; Froelich et al., 2003; Kertesz et al., 2000; Lendon et al., 1998; Rademakers et al., 2002; Rosso et al., 2001). These findings suggest that either there is a complex mutation in the MAPT gene that has eluded detection or that a different gene in this region is responsible for this form of familial FTD. Importantly, in contrast to families with known MAPT mutations, those with linkage to chromosome 17 that lack defined MAPT mutations also lack significant tau pathology but possess FTD-U type histology, similar to the majority of cases of idiopathic FTD (Mann et al., 2000). Unfortunately, there are few detailed clinical and pathological descriptions of these pedigrees.

Recently, we reported that a subset of patients with familial FTD-U have the additional postmortem finding of ub-ir neuronal intranuclear inclusions (NII) (Mackenzie and Feldman, 2003). These NII have an unusual lentiform shape and are present in small neurons in multiple neuroanatomical sites. We suggested that these NII may be a specific pathological marker for a subset of FTD with a common genetic basis. Interestingly, two families with FTD-U linked to chromosome 17q21 that lacked tau mutations have been reported that also develop similar NII suggesting that this lesion might be a characteristic neuropathological hallmark of FTD linked to this locus.

Here we report a large Canadian family of English descent with pathologically confirmed FTD-U, hereby named UBC-17. We provide detailed clinical and pathological descriptions of multiple affected family members. Genetic analysis shows that the disease in UBC-17 is linked to the long arm of chromosome 17 and is not associated with any point mutations in MAPT. We further demonstrate that the gross structure of the MAPT locus appears intact excluding the possibility that a large deletion or insertion in MAPT is present in this family. In addition, we show that affected individuals from this family have normal levels of soluble tau in their brain tissue indicating that selective loss of tau protein is not the functional consequence of the pathogenic mutation. Finally, we demonstrate that affected members of UBC-17 also develop ub-ir NII and for the first time present a detailed immunohistochemical characterization of these lesions.

Materials and methods

Clinical data

Family UBC-17 first came to our attention when a neurologically normal family member contacted the University of British Columbia dementia clinic following the death of her demented father (the proband, member 17-1). The individual was concerned because there was a strong family history of dementia and the autopsy report on her father described an unusual, non-Alzheimer type of neurodegenerative disease. Review of the autopsy findings confirmed the pathological diagnosis of FTD-U with NII. Clinical and family history information was subsequently obtained by interviewing relatives, through the retrospective review of the medical records of deceased patients and the neurological examination of living patients.

The pedigree currently includes >250 individuals spanning 5 generations (partial pedigree provided in Fig. 1). There are 17 members with dementia, with affected individuals in each of the first four generations, consistent with an autosomal dominant pattern of inheritance and a high degree of penetrance. There are a large number of individuals in the younger generations who are still below the average age of onset.

Neuropathological samples and methods

Postmortem neuropathological evaluation has been conducted on four affected members of family UBC-17 (17-1, 17-52, 17-60 and 17-68). Formalin-fixed, paraffin-embedded tissue sections from each were stained using haematoxylin and eosin (H&E), Gallyas and modified Bielschowsky silver methods. Initial immunohistochemistry was performed using the Ventana ES automated staining system. The primary antibodies employed recognized ubiquitin (DAKO anti-ubiquitin; 1:500, following microwave antigen retrieval), non-phosphorylated neurofilament (NF) (DAKO anti-NF...
Fig. 1 Five generations of the UBC-17 family pedigree with disease-associated haplotype. Proband marked with an arrow. Persons with autopsy examination are marked with a black spot. Disease haplotype is boxed and haplotype alleles inferred from spouse and children are in italics. The pedigree has been anonymized to protect the family.
protein; 1: 2000, following protease digestion), phosphorylated NF (pNF) (Sternberger SMI 31; 1: 8000, following protease digestion), hyperphosphorylated tau (Innogenetics AT-8, pS202/pS205; 1: 2000 following microwave antigen retrieval and Sigma TAU-2; 1: 1000 with 3 h initial incubation at room temperature), α-synuclein (Zymed anti-α-synuclein; 1: 10,000, following microwave antigen retrieval), Aβ peptide (DAKO anti-beta amyloid; 1: 100 with initial incubation for 3 h at room temperature) and glial fibrillary acidic protein (DAKO anti-glial fibrillary acidic protein; 1: 4000).

Additional immunohistochemistry was performed on sections from three of the autopsy cases (17-1, 17-52 and 17-68) to further investigate the nature of the NII. Immunohistochemistry employed antibodies that recognize proteins containing expanded polyglutamine repeat regions (Chemicon 1C2; 1: 100, 24 h at room temperature following formic acid pre-treatment), heat shock proteins (HSP) 40 (Santa Cruz, 1: 100, following microwave antigen retrieval) and HSP70 (Stressgen, 1: 1400, following microwave antigen retrieval), cysc AMP response element binding protein (CREB) (Chemicon International, 1: 1000, following microwave antigen retrieval), and CREB binding protein (CBP) (Santa Cruz, 1: 400, following microwave antigen retrieval). Double immunofluorescence labelling employed antibodies against ubiquitin (rabbit polyclonal, Santa Cruz, 1: 50 or mouse monoclonal Novocasta, 1: 100) combined with either a goat polyclonal antibody against promyelocytic leukaemia protein (PML, Santa Cruz, 1: 50) or a rabbit polyclonal antibody against small ubiquitin modifier-1 (SUMO-1, Santa Cruz, 1: 50).

Stained sections were then incubated in either a cocktail of Alexafluor 594 donkey anti-rabbit and Alexafluor 488 chicken anti-goat (for ubiquitin/PML) or Alexafluor 488 goat anti-mouse and Alexafluor 594 donkey anti-rabbit (for ubiquitin/SUMO-1) secondary antibodies (Molecular Probes, all 1: 150). Sections were co-silksapped using Vectashield Mounting Medium containing 4′,6-diamidino-2-phenylindole as a nuclear counterstain. Sections were examined using a Zeiss Axioplan II fluorescence microscope (Carl Zeiss Microscopy, Germany) mounted with an AxioCam digital camera.

Images were acquired with Zeiss Axiovision 4 software.

A limited number of paraffin sections were also obtained from one affected member of the FTD-U family reported by Kertesz et al. (2000) that showed evidence of linkage to chr17q21, and one affected member of the chr17q21-linked Dutch family with FTD-U (HFTD3) reported by Rosso et al. (2000). These sections were immunostained, as above, for ubiquitin and tau and screened by one of us (IRM) for the presence of NII.

Genetic linkage analysis
DNA was available from 49 family members, including four affected members (Fig. 1). Two additional affected individuals in the pedigree could be partially reconstructed from spouse and child genotypes. We genotyped 19 STRP markers (D17s219, D17s1294, D17s798, D17s1814, D17s1299, D17s951, D17s1860, D17s1861, D17s934, D17s950, MAPT-1 (between MAPT exons −1 and 1, primer sequences MAPT-1F ACACCGGGCCAGCATTACTCT, MAPT-1R ATCAAGGCACTCATATAATAAAG), D17s791, D17s931, D17s806, D17s958, D17s1827, D17s1868, D17s1795, D17s1820), spanning ∼30 cM of chr17q. Sex averaged genetic distances were obtained from the Rutgers Combined Linkage-Physical Map Version 2.0 (Kong et al., 2004) and physical distances from the NCBI Genome Build 35.1 (http://www.ncbi.nlm.nih.gov). Genotyping was performed using an ABI 3100 and Genotyper v3.7 software (Applied Biosystems). Two-point linkage analysis was carried out using AUTOSCAN and MLINK (Lathrop et al., 1984) assuming an autosomal dominant model with the disease allele frequency set at 0.001. Age-dependent disease penetrance classes were established such that ages up to 40 years were assigned 0% penetrance, 40–49 years assigned a 10% penetrance, 50–59 years 43% penetrance, and 60–69 years assigned 77% penetrance and >70 years was assigned 100% penetrance. MLINK analysis was performed twice, once assuming equal marker allele frequencies and once using marker allele frequencies obtained from non-affected family members. A frequency of 0.005 was used for alleles on the disease-associated haplotype not observed in unaffected members. Parametric multipoint linkage analysis was performed using SIMWALK2 (Sobel and Lange, 1996) using the same conditions as in the two-point analysis. This latter analysis was also performed twice, with equal and with family-estimated marker allele frequencies.

MAPT gene sequence analysis
All coding exons of the MAPT gene were sequenced in two affected members of UBC-17 and one unaffected member using previously published methodology (Hutton et al., 1998). Each exon was sequenced on both strands using BigDye Ver3.1 and an ABI3100 (Applied Biosystems). Sequence variations detected were then checked for disease segregation in all the family members for whom samples were available.

Tau western blot analysis
Frozen frontal lobe brain tissue from UBC-17 individuals 17-68 and 17-60 and cerebellum from 17-60 along with tissue from a neurologically normal control and an Alzheimer’s disease case was homogenized in ice-cold phosphate-buffered saline with protease inhibitors (Roche). Protein concentration was determined and 20 µg of total protein solubilized by boiling in SDS–PAGE sample buffer prior to gel loading. Western blots were performed using standard ECL methods. Blots were probed with human specific tau phospho-independent polyclonal antibody (E1), stripped and reprobed with mouse monoclonal antibody specific to the N-terminus of N-ethylmaleimide-sensitive factor (NSF). GAPDH was also probed (antibody from Biodesign International) to confirm equal protein loading.

Fluorescent in situ hybridization analysis (FISH)
Immortalized metaphasic lymphocytes from UBC individual 17-68 and control individuals were processed with a Vysis® VP2000™ Processor and mounted onto slides. BAC 413P22 (Invitrogen) containing all of MAPT was fluorescently labelled using Nick Translation (Vysis). Cells were then analysed with Vysis Iplab system.

Results
Clinical features (overview)
We were able to obtain detailed clinical information on eight affected members of family UBC-17. However, because most of this information was reviewed retrospectively, we recognize that our knowledge of the clinical course of some patients may be incomplete. Each of these individuals met clinical diagnostic criteria for FTD (Brun et al., 1994; Neary et al., 1998;
Table 1 Summary of major neurological findings in affected members of family UBC-17

<table>
<thead>
<tr>
<th>Sex</th>
<th>17-1</th>
<th>17-2</th>
<th>17-50</th>
<th>17-51</th>
<th>17-52</th>
<th>17-53</th>
<th>17-60</th>
<th>17-68</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset (years)</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
<td>Male</td>
<td>Female</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td>Disease duration (years)</td>
<td>4</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>7</td>
<td>4</td>
</tr>
</tbody>
</table>

Cognitive abnormalities
- Executive impairment
  - 17-1: +
  - 17-2: +
  - 17-50: +
  - 17-51: +
  - 17-52: +
  - 17-53: +
  - 17-60: +
  - 17-68: +

- Language dysfunction
  - 17-1: +
  - 17-2: +
  - 17-50: +
  - 17-51: +
  - 17-52: +
  - 17-53: +
  - 17-60: +
  - 17-68: +

- Behavioural abnormality
  - 17-1: –
  - 17-2: –
  - 17-50: +
  - 17-51: +
  - 17-52: +
  - 17-53: +
  - 17-60: –
  - 17-68: +

- Memory impairment
  - 17-1: +
  - 17-2: +
  - 17-50: +
  - 17-51: +
  - 17-52: +
  - 17-53: +
  - 17-60: +
  - 17-68: +

- Apraxia
  - 17-1: +
  - 17-2: –
  - 17-50: +
  - 17-51: +
  - 17-52: +
  - 17-53: +
  - 17-60: –
  - 17-68: +

- Pyramidal dysfunction
  - 17-1: –
  - 17-2: –
  - 17-50: +
  - 17-51: +
  - 17-52: +
  - 17-53: +
  - 17-60: –
  - 17-68: –

- Parkinsonism
  - 17-1: +
  - 17-2: –
  - 17-50: +
  - 17-51: +
  - 17-52: +
  - 17-53: +
  - 17-60: –
  - 17-68: –

- Incontinence
  - 17-1: +
  - 17-2: +
  - 17-50: +
  - 17-51: +
  - 17-52: +
  - 17-53: +
  - 17-60: –
  - 17-68: –

- Other hallucinations
  - 17-1: +
  - 17-2: +
  - 17-50: +
  - 17-51: +
  - 17-52: +
  - 17-53: +
  - 17-60: –
  - 17-68: –

- Somnolence
  - 17-1: +
  - 17-2: +
  - 17-50: +
  - 17-51: +
  - 17-52: +
  - 17-53: +
  - 17-60: –
  - 17-68: –

McKhann et al., 2001) having early and progressive changes in personality, behaviour and/or language. The mean age of onset of neurological symptoms was 60 years (range = 53–70 years) with average disease duration of 6 years (range = 4–8 years). There is no evidence of genetic anticipation in this family. All affected individuals also had prominent early frontal executive impairment as well as some deficits of memory retrieval. The language disorder was characterized by word finding difficulties, non-fluent verbal output and perseverative speech, which usually progressed to mutism. Behavioural abnormalities were prominent early features in 6 out of 8 individuals with irritability, restlessness, poor judgement and inattention. Three affected family members developed prominent dressing apraxia. Pyramidal motor signs occurred in three individuals, however, only one (17-60) fulfilled clinical diagnostic criteria for motor neuron disease (Brooks, 1994). Extrapyramidal features, including rigidity and bradykinesia, occurred in three individuals. One of these had suffered from a severe tremor for many years prior to the onset of neurobehavioural symptoms. Three individuals developed incontinence of urine and stool during their course of illness. A summary of the major neurological features of these eight affected individuals is presented in Table 1.

Several family members who are currently in their late 30s or early 40s and who are considered to be ‘at-risk’ because they have affected parents and are younger than the average age of disease onset, report symptoms including depression, anxiety, behavioural abnormalities, subjective memory impairment and tremor. At this point, it is uncertain whether or not these individuals are in the early symptomatic stage of illness.

Neuropathology (overview)

Four affected members of UBC-17 underwent postmortem examination. Neuropathological changes were similar in morphology and anatomical distribution and varied only in severity. In all cases, cerebral atrophy was moderate to severe and was largely restricted to the frontal lobes. There was often mild atrophy of the head of the caudate nucleus and some loss of pigmentation of the substantia nigra.

Microscopic examination of the neocortex showed nonspecific chronic degenerative changes including neuronal loss and gliosis with an anterior-to-posterior anatomical gradient of severity (frontal > temporal > insula, cingulate, parietal > occipital). Superficial spongiosis was prominent in the frontal neocortex and mild in the temporal. The hippocampus had a normal population of pyramidal neurons and dentate granule cells. Chronic degenerative changes also affected certain subcortical structures including the caudate nucleus, putamen, dorsomedial nucleus of the thalamus and substantia nigra. Lower motor neurons (LMN) in the brainstem were normal in number. The entire spinal cord was available in only one case (17-60) and showed some loss of anterior horn cells.

More specific pathological changes were seen with special stains and immunohistochemistry. Apart from a small number of neurofibrillary tangles identified in one family member (17-60), no other pathology was identified with silver stains, or immunohistochemistry for tau, α-synuclein, NF or pNF; specifically, there were no senile plaques, Pick bodies, Lewy bodies, glial inclusions or achromatic neurons. In contrast, ub-ir neurites and NCIs were present in the superficial laminae of the frontal and temporal neocortex in all cases (Fig. 2A). The inclusions were present in small neurons, appeared dense and had an oval or arcuate shape (Fig. 2B). Similar inclusions and neurites were also present in large numbers in the caudate and putamen (Fig. 2C) and less frequently in the globus pallidus, dorsal medial nucleus of the thalamus and the periaqueductal grey matter of the midbrain. Small numbers of dense or granular ub-ir NCI were present in dentate granule cells of the hippocampus (Fig. 2D). Rare filamentous skein-like NCI were found in pigmented neurons of the substantia nigra (Fig. 2E). In one case (17-60) a single motor neuron in the hypoglossal nucleus contained a filamentous NCI (Fig. 2F). There were no filamentous skeins or Lewy body-like inclusions in the anterior horn cells.
in the one case where the spinal cord was available for examination.

Discrete, dense, ub-ir NII were identified in all four cases and had a similar anatomical distribution to the dense NCI, being most numerous in the frontal neocortex and striatum and less common in the dentate granule cells, globus pallidus and thalamus (Fig. 3A and B). The number of NII varied between anatomical regions and they were always much less numerous than NCI. In the most severely affected regions, the average frequency of NII was approximately one per five \( \times 20 \) microscopic fields and it was rare to find more than two neurons with NII in a single \( \times 20 \) field (0.25 mm\(^2\)). Most NII had a striking lentiform (cat’s-eye) shape (Fig. 3C and D) while a few appeared as slightly curved, thick rods (Fig. 3E). In some cases, the intranuclear localization of the inclusion was confirmed by the way in which it distended the nuclear membrane (Fig. 3D). Small round NII likely represented inclusions cut in cross-section.

In addition to ubiquitin, all NII were reactive for SUMO-1 (Fig. 4) and a proportion was positive for PML. There was no

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**Fig. 2** Ubiquitin-immunoreactive neuronal cytoplasmic inclusions (NCI) and neurites. (A and B) Superficial layers of frontal neocortex with numerous neurites (A) and dense cytoplasmic inclusions in small neurons (B) (Patient 17-1). (C) Neurites and NCI in striatum (Patient 17-68). (D) Dense and granular cytoplasmic inclusions in hippocampal dentate granule cells (Patient 7-68). (E) Pigmented neuron in the substantia nigra with a filamentous skein-like NCI (Patient 17-1). (F) Single hypoglossal motor neuron with filamentous NCI (Patient 17-60). Ubiquitin immunohistochemistry. Scale bar: A, 50 \( \mu \)m; B, 20 \( \mu \)m; C, 50 \( \mu \)m; D, 50 \( \mu \)m; E, 15 \( \mu \)m; F, 15 \( \mu \)m.
reactivity for antibodies against expanded polyglutamine repeats, HSP40, HSP70, CREB or CBP.

A summary of the major neuropathological findings in these cases, including semiquantitative assessment of the ub-ir pathology is presented in Table 2.

Illustrative cases
Patient 17-1

At age 65, this patient presented with progressive memory difficulties, decreased verbal output and impaired concentration. He became unable to perform complex, multistep tasks and had marked anosognosia and personal neglect. He developed urinary incontinence early in his disease and had a prominent gait apraxia. He also developed parkinsonian features with rigidity and diminution of facial expression. Neuroimaging demonstrated hydrocephalus and diffuse cerebral atrophy with no focal or asymmetric tissue loss. His initial clinical diagnosis was normal pressure hydrocephalus and he was treated with a ventriculoperitoneal shunt, without improvement. His language deficit evolved into a progressive non-fluent aphasia that culminated in mutism. He was

Fig. 3 Lentiform ubiquitin-immunoreactive neuronal intranuclear inclusions (NII) in (A) frontal neocortex and (B) striatum (arrows) (Patient 17-1). Intranuclear localization indicated by the surrounding nuclear membrane (C) that was sometimes deformed by the inclusion (D) (Patient 17-1). (E) Occasional NII had a slightly curved bar morphology (Patient 17-1). Ubiquitin immunohistochemistry. Scale bar: A, 25 μm; B, 35 μm; C, 15 μm; D, 15 μm; E, 15 μm.

Fig. 4 NII showing co-localization of ubiquitin and SUMO-1. (A) Ubiquitin immunofluorescence (green). (B) SUMO-1 immunofluorescence (red). (C) Merged image, ubiquitin and SUMO-1 (co-localization of immunoreactivity, yellow; nucleus blue). Scale bar: 8 μm.
eventually bed bound, unable to feed himself and incontinent of urine and stool. His duration of disease was 4 years.

The postmortem brain weight was not recorded. However there was severe symmetric atrophy of both frontal lobes with milder atrophy of the temporal lobes and anterior parietal lobes. The head of the caudate nucleus was slightly shrunken while all other subcortical structures appeared grossly normal. Histological examination revealed severe neuronal loss and gliosis affecting the frontal lobe neocortex with marked superficial spongiosis. Chronic degenerative changes were also present in the temporal and parietal neocortex, but to a milder degree. The hippocampus was intact, with normal numbers of pyramidal neurons and dentate granule cells. There was a moderate degree of neuronal loss and gliosis affecting the caudate nucleus, putamen and dorsomedial nucleus of the thalamus and mild loss of pigmented neurons from the substantia nigra. LMN in the brainstem and upper cervical spinal cord were normal in number and morphology. Ub-ir NCI and dystrophic neurites were numerous in the frontal neocortex, sparse in the temporal cortex and rare in other neocortical regions. Moderate numbers of NCI were also present in dentate granule cells of the hippocampus. In subcortical regions, NCI and neurites were numerous in the caudate and putamen, and moderate in number in the globus pallidus, medial thalamus and periaqueductal grey matter. A few pigmented neurons in the substantia nigra contained filamentous skein-like cytoplasmic inclusions. There was no ub-ir NCI in LMN. NII were frequently seen in small neurons of superficial frontal and temporal neocortex and the striatum, and occasionally present in dentate granule cells, globus pallidus and dorsomedial nucleus of the thalamus. The only other neuropathological finding was a moderate degree of arteriolosclerosis.

**Patient 17-2**

At age 58, he experienced progressive difficulties with problem solving and reasoning that affected his vocational abilities. He also presented with word finding difficulty, memory impairment and significant anosognosia. Neurological findings included prominent parkinsonism with cogwheel rigidity, bradykinesia and tremor. He experienced early urinary incontinence. Neuroimaging demonstrated diffuse cerebral atrophy with no focal or asymmetric tissue loss. His course was progressive with non-fluent aphasia becoming mutism. Towards the end of his disease, he was unable to climb stairs, however, it was unclear whether or not this was due to motor neuron disease. His disease course to death was 6 years. Autopsy was not performed.

**Patient 17-50**

At age 70, this patient developed episodic memory retrieval deficits and executive impairment including poor judgement and difficulty driving. Early behavioural changes included apathy, asopontaneity, irritability and anosognosia. He experienced difficulty in swallowing and he had occasional urinary incontinence. A formal swallowing assessment identified a bulbar apraxia. He had normal tone, power and bulk of his extremities but had evidence of upper motor neuron disease with very brisk reflexes. Neuroimaging demonstrated moderate generalized cerebral atrophy with a probable right frontal lobe infarct. His dysphagia continued to worsen and he developed marked emotional lability. Death occurred 7 years after the onset of initial symptoms. Autopsy was not performed.

**Patient 17-51**

This patient had suffered for many years from a resting tremor of both hands and head titubation. At age 61, he developed a non-fluent aphasia with progressive word finding difficulty.

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Table 2 Major neuropathological findings in affected members of UBC Family 17

<table>
<thead>
<tr>
<th>UBC family 17 member</th>
<th>17-1</th>
<th>17-52</th>
<th>17-60</th>
<th>17-68</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain weight (grams)</td>
<td>n.a.</td>
<td>n.a.</td>
<td>1138</td>
<td>1250</td>
</tr>
<tr>
<td>Atrophy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal lobes</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Temporal lobes</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Caudate</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Substantia nigra</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Histopathology/immunohistochemistry</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Frontotemporal neocortex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spongiosis</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Neuronal loss/gliosis</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Neurites</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>NCI</td>
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Neurites, ubiquitin immunoreactive (ub-ir) dystrophic neurites; NCI, ub-ir neuronal cytoplasmic inclusions; NII, ub-ir neuronal intranuclear inclusions. n.a., not available. Semiquantitative grading: –, none; +, mild/rare; ++, moderate/occasional; ++++, severe/numerous.
Other cognitive impairments included a striking dressing apraxia and memory impairment. Behavioural changes included irritability and restlessness. Both his parkinsonism and cognitive impairments progressed and he developed hallucinations late in the course of his disease. He died 8 years after the onset of his cognitive symptoms. Autopsy was not performed.

**Patient 17-52**

At the age of 60, this patient developed personality changes with paranoia, irritability, impulsivity and restlessness. He was reported to have difficulties with his finances, with the performance of complex multistep tasks and with driving. He demonstrated a non-fluent aphasia with anomia and memory retrieval deficits. There were no associated extrapyramidal or motor neuron features noted and he did not have significant apraxia. Disease duration to death was 6 years.

The postmortem brain specimen showed a moderate degree of symmetric frontal atrophy. There was a small acute-on-chronic subdural haematoma overlying the left frontal lobe, without significant herniation. There was also a small chronic infarct within the right occipital cortex. The subcortical structures appeared grossly normal. Histological examination showed severe chronic degenerative changes with neuronal loss, gliosis and superficial spongiosis, affecting the frontal and temporal neocortex. There was chronic loss of pyramidal neurons from the CA1 sector of the hippocampus and mild chronic degeneration of the striatum and substantia nigra. Ub-ir NCI and neurites were extremely numerous in the superficial layers of the frontal and temporal neocortex and present in small numbers in the dentate fascia of the hippocampus, striatum and periaqueductal grey matter. Rare ub-ir filamentous skein-like inclusions were present in the substantia nigra. Brainstem LMN were normal with no inclusions. NII were numerous in the frontal and temporal neocortex and rare in the striatum.

**Patient 17-53**

This 62-year-old woman presented with executive impairment where she was reported to have difficulty with her household chores. She was also noted to be neglecting her personal hygiene. Cognitive assessment revealed a non-fluent aphasia and behavioural changes including apathy, asponaety and irritability. She became more somnolent, sleeping for >10 h per day. Later in her course, she developed parkinsonism with focal, asymmetric upper extremity cogwheel rigidity. She also had weakness of her left arm and symmetrically brisk reflexes. Neuroimaging demonstrated generalized cerebral atrophy and a lesion in the right frontal lobe, suggestive of an old infarct. Her disease duration was 6 years. Autopsy was not performed.

**Patient 17-60**

At the age 53, this farmwife developed difficulties operating familiar machinery and difficulty completing her chores. She had poor attention, impaired memory and anosognosia. Her behavioural changes included euphoria and motor restlessness. Associated neurological features, consistent with motor neuron disease, included wasting of the tongue, fasciculations of her hands and thighs, and brisk reflexes. She also demonstrated severe dysgraphaesthesia. Cranial MRI showed profound asymmetric frontal atrophy, greater on the right side, and hydrocephalus. SPECT scan demonstrated greatly reduced perfusion in both frontal regions and right temporoparietal area. Her disease duration to death was 7 years.

The postmortem brain specimen weighed 1138 g and showed moderate, symmetric frontal lobe atrophy and mild pallor of substantia nigra. Chronic degeneration with superficial spongiosis was severe in the frontal lobes and mild in the temporal regions. The hippocampus had a normal neuronal population. There was severe chronic degeneration of the caudate nucleus with moderate involvement of the putamen, medial thalamus and substantia nigra. There was moderate arteriolosclerosis. There were numerous ub-ir NCI and neurites in the superficial frontal neocortex, moderate numbers in the temporal neocortex and striatum, and rare NCI in dentate granule cells of the hippocampus. NII were moderately frequent in the frontal neocortex, rare in the striatum and present in a single neuron in the hypoglossal nucleus. There were rare filamentous skein-like inclusions in nigral neurons. Throughout the brainstem and spinal cord, the corticospinal tracts appeared normally myelinated. There was mild-to-moderate loss of LMN from the brainstem and spinal cord with some of the remaining LMN appearing shrunken. A single motor neuron in the hypoglossal nucleus contained a filamentous cytoplasmic inclusion (Fig. 2F), however no ub-ir NCI were identified in spinal cord anterior horn cells. The only other pathology noted was rare argyrophilic, tau-positive, neurofibrillary tangles in the entorhinal cortex.

**Patient 17-68**

This 57 year-old man presented with word finding difficulty and stuttering. At that time, cognitive abnormalities included difficulty performing multistep tasks, and maintaining attention. He had prominent behavioural changes, including perseveration, impulsivity and frustration. He had difficulties with calculations, right left orientation and had finger agnosia. He later developed a dressing apraxia. His neuroimaging showed frontal atrophy and bilateral subcortical white matter changes. SPECT scan demonstrated decreased activity in both frontal lobes and in the left superior posterior temporoparietal region. He died following a 4-year disease course.

The formalin-fixed postmortem brain specimen weighed 1250 g and showed moderate atrophy of the frontal lobes and the head of the caudate nucleus. There was moderate loss of pigmentation of the substantia nigra. The neocortex of the frontal and inferior temporal lobes demonstrated severe neuronal loss and gliosis. There was rarefaction of the subcortical
white matter with decreased myelin staining and moderate arteriolosclerosis. There was a dense network of ub-ir NCI, NII and dystrophic neurites in neocortical layers II and III. In the hippocampus, moderate numbers of NCI and occasional NII were present in dentate granule cells. The striatum showed chronic degenerative changes, numerous ub-ir neurites and NCI and occasional NII. There was a moderate loss of pigmented neurons from substantia nigra with rare ub-ir filamentous skein-like cytoplasmic inclusions. Other brainstem regions and cerebellum were normal. A section from the upper cervical spinal cord showed well-myelinated corticospinal tracts and a normal population of LMN with no inclusions.

Genetic analysis
The pattern of inheritance within UBC-17 is consistent with an autosomal dominant mode of transmission. Two-point linkage analysis produced a maximum LOD score of 3.65 (nominal $P$-value $P = 0.00002$) with marker MAPT-1 (Table 3) with allele frequencies estimated from the family. Analysis using equal marker allele frequencies gave a maximum LOD score of 3.11 (nominal $P$-value $P = 0.0008$) at marker D17s950 and 2.91 (nominal $P$-value $P = 0.0001$) at D17s791; both these markers flank MAPT-1. These data represent significant evidence of linkage according to Lander and Kruglyak criteria for a replication study (Lander and Kruglyak, 1995). Power calculations using SIMLINK revealed that the theoretical maximal LOD score in UBC-17, modelled with DNA available from available affected individuals, for a linked marker, was 4.3, with an average max LOD score of 1.82, assuming equal allele frequencies. Moreover, multipoint analysis using SIMWALK2 produced a maximum LOD score of 3.87 from D17s950 to D17s791 when equal marker allele frequencies were used and 3.91 from D17s1861 to D17s791 with marker allele frequencies obtained from the family. All known affected individuals shared a common haplotype from D17s1294 to D17s806, a region of 19.06 cM (sex averaged) that corresponds to a physical size of 17.76 Mb. Collectively, these data provide strong evidence of linkage to this region in this family. The shared disease-associated haplotype is defined at the centromeric end by a recombination within individual 17-60 between D17s1294 and D17s798, and at the telomeric end by a recombination in individual 17-68 between D17s931 and D17s806 (Fig. 1).

MAPT is the obvious candidate gene within the critical region in UBC-17 (D17s1294 to D17s806). However, sequence analysis of the MAPT gene failed to identify potential pathogenic mutations that would alter the protein sequence of tau, and no exonic or intronic variants were found that would be expected to disrupt splicing. Several polymorphisms were detected deep within intronic sequences, however, none of these variants segregated with disease in UBC-17 (data not shown) and are therefore unlikely to be of aetiological relevance. Taken together, these data indicate that a simple point mutation within the coding exons and flanking intronic sequence of tau is unlikely to be the cause of FTD-U in UBC-17.

FISH analysis
FISH analysis of immortalized lymphocytes from the UBC-17 affected individual 17-68 using a BAC probe that contains all

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Genetic distances are sex averaged and from the Rutgers Combined Linkage-Physical Map Version 2.0 (Build 35).
of MAPt (Fig. 5) demonstrated a single copy of this locus on each chromosome. These data argue against the possibility that a large duplication or deletion of this locus is the mutational event causing disease in this family.

Western blot analysis
In order to establish whether there are changes in the levels or size of tau protein in UBC-17 we performed western blot analysis of total protein homogenate from brain tissue from two affected individuals (Fig. 6). Levels of total tau in frontal lobe from 17-68 (lane 4) were comparable to that of control brain (lane 1). Tau levels were reduced in both frontal lobe (lane 5) and cerebellum (lane 6) of individual 17-60 compared to control (lanes 1 + 2). However, lower molecular weight tau degradation products were present in these samples, indicating that tau had been present. Furthermore, the levels of another neuronal protein, NSF, in 17-68 were comparable to control but were also reduced, similarly to tau, in 17-60. This indicates the reduction of protein was not specific to tau and suggests the reduction in tau and NSF reflects the extensive neurodegeneration observed in the brains of patients from UBC-17.

Neuropathology in other chr17-linked families
Previous reports of two other FTD-U families, linked to chromosome 17, have made brief mention of ub-iir NII found on postmortem examination (Rosso et al., 2001; Rademakers et al., 2002). We had the opportunity to review the pathological material from one affected member of Dutch family HFTD3 and confirmed the presence of lentiform ub-iir NII, as previously reported (Rosso et al., 2001). The NII in this case had a similar lentiform morphology and anatomical distribution. In addition, we obtained tissue sections from an affected member of the family reported by Kertesz et al.
(2000) and found that it also showed rare ub-ir NII in the cerebral neocortex.

Discussion

The presence of ub-ir, tau-negative NCIIs is becoming increasingly recognized as the most common neuropathological subtype of FTD (FTD-U) (Jackson et al., 1996; Kovair et al., 2000; Mann et al., 2000; Josephs et al., 2004; Lipton et al., 2004; Taniguchi et al., 2004). In contrast, ub-ir NII have only recently been identified as an additional pathological feature of a subset of FTD-U cases (Rosso et al., 2001; Woulfe et al., 2001; Rademakers et al., 2002; Froelich et al., 2003; Mackenzie and Feldman, 2003; Benussi et al., 2004; Bigio et al., 2004). Woulfe et al. (2001) were the first to provide a detailed description of ub-ir NII in three patients with FTD-U. They reported that these inclusions have a unique lentiform morphology and characteristic anatomical distribution (small neurons of the superficial neocortex, dentate granule cells of the hippocampus and striatum). They found no NII in a small sample of other neurodegenerative conditions, including other cases of FTD and motor neuron disease. All three cases with NII had at least one relative with dementia. In a subsequent review of postmortem material at our centre, we found NII to be a specific pathological marker for a subset of patients with autosomal dominant FTD-U and we hypothesized a common genetic defect (Mackenzie and Feldman, 2003).

Crucial for this present study is that NII, similar to those found in UBC-17, have also been reported in several other FTD-U families with linkage to chromosome 17q21 and no demonstrable tau mutation. Rosso et al. (2001) described a Dutch family with autosomal dominant FTD. Some family members had parkinsonism but no motor neuron disease. Postmortem examination of two affected members showed motor neuron disease-type ub-ir NCIIs and no tau pathology. The authors also noted 'a few ubiquitin inclusions, apparently located within the nucleus, (which) had a cat’ s-eye or target shape’ and stated that anticipation was not present in the family, and that the NII were not immunoreactive to an antibody specific for polyglutamine repeats, arguing against a polyglutamine expansion being the cause of disease in this family (Rosso et al., 2001). Rademakers et al. (2002) also reported linkage to 17q21 in a family with autosomal dominant FTD but no clinical motor neuron disease features. Autopsy on a single affected member again showed no tau pathology, but motor neuron disease-type NCIs, and a few ub-ir ‘cat-eye’ shaped NII. Similar to the report of Rosso et al. these inclusions were not immunoreactive to an antibody reactive to polyglutamine. Kertesz et al. (2000) described a family with autosomal dominant FTD with possible linkage to 17q21 with a maximum LOD score of +1.68 at D17s800. Although they described only NCIs, personal review of the pathological material (IRM) identified a small number of ub-r NII. Indeed, as described in the Results section, comparative neuropathological study of tissue sections from each of these families for the first time (Kertesz et al. and Rosso et al., and UBC-17) revealed ub-ir NII with a similar morphology and regional distribution.

There are additional reports of other FTD families that are linked to the same region on chromosome 17 without MAPt mutations, however, details of ubiquitin immunohistochemistry were not provided (Bird et al., 1997; Lendon et al., 1998).

We employed additional immunochemical techniques to further characterize the nature of the NII in familial FTD-U. Although NII are a relatively uncommon feature of neurodegenerative conditions, they are a characteristic finding in all the currently recognized human polyglutamine diseases, in which the genetic basis is abnormal expansion of a CAG trinucleotide repeat region within the protein-coding exons of the gene (such as Huntington’s disease and several types of spinocerebellar ataxia) (Davies et al., 1998). However, the NII in our cases did not stain using an antibody against expanded polyglutamine repeats (1C2), which labels the NII in polyglutamine diseases. Furthermore, NII were not reactive for a variety of other proteins that have been shown to localize to the NII of polyglutamine diseases, including HSP40, HSP70, CREB and CBP (Chai et al., 1999; Terashima et al., 2002). These findings suggest that the NII in FTD-U are not composed of polyglutamines and are consistent with our previous genetic analysis of cases of familial FTD-U with NII, in which we evaluated all human genes containing a CAG repeat region and failed to identify any abnormal expansion (Mackenzie et al., 2004).

The positive reaction of some NII in our cases for PML suggests that the inclusions may form within nuclear bodies (Takahashi et al., 2003). Nuclear bodies are small regions of the nucleus involved in a variety of nuclear processes such as transcription, growth suppression, apoptosis and proteosomal degradation of unwanted proteins. PML is a normal component of nuclear bodies and labels small (possibly early) NII in a number of neurodegenerative conditions including polyglutamine disorders and so-called NII disease (I. R. Mackenzie and J. Woulfe, unpublished data, Haltia et al., 1984). NII in our cases were also consistently reactive for SUMO-1, a ubiquitin-like protein that covalently binds to and regulates the localization, function and stability of various nuclear proteins. SUMO-1 has been shown to recruit proteins into nuclear bodies and to stabilize these proteins and antagonize their proteosomal degradation. Studies in other neurodegenerative conditions in which SUMO-1 co-localizes with NII have suggested that SUMO-1 promotes the formation of NII, causes proteosomal dysfunction, disrupts the normal function of nuclear bodies and contributes to cell death (Terashima et al., 2002; Pountney et al., 2003). Additional investigations are needed, however, these findings do provide evidence for a similar mechanism of neurodegeneration in FTD-U with NIIIs. Furthermore, NIIIs are somewhat reminiscent in shape of the mitotic spindle at metaphase and it is possible that unknown ubiquitinated proteins accumulate on this structure following a failed attempt to re-enter the cell cycle. Nevertheless, the exact nature of ub-ir NIIIs and their
relationship to neurodegeneration, if any, in FTD-U remains to be determined.

In UBC-17, a maximum two-point LOD of 3.65 was obtained with marker MAPT-1 and multipoint analysis produced a maximum LOD score of 3.91 from D17s1861 to D17s791. These results meet criteria for significant linkage and are wholly consistent with other reported families with ub-ir NII and linkage to chromosome 17 (Rosso et al., 2001; Radmakers et al., 2002; Froelich et al., 2003; Benussi et al., 2004). Clear recombination events at D17s1294 (centromeric) and D17ss806 (telomeric) define the boundary of the shared haplotype among the affected individuals within UBC-17 and thus indicate a critical region for the location of the mutated gene of 19.06 cM, corresponding to a physical size of 17.76 Mb in this family. Radmakers et al. (2002) identified a centromeric recombination event for the critical region in their large Dutch family with FTD-U and NII (Dutch family, 1083) at D17s1787. Therefore, combining these results allows us to further refine the minimal region for familial FTD-U with NII linked to chr17q21 to a 3.53 cM region between D17s1787 and D17s806. This corresponds to a physical region of 6.19 Mb with ~165 genes and contains many attractive candidates, in addition to MAPT, that could conceivably harbour mutations that are responsible for familial FTD-U with NII.

Although the MAPT gene lies within the critical region and is an obvious candidate, simple point mutations in the coding exons and flanking intronic sequence of MAPT have been excluded as a cause of FTD-U in UBC-17 and all the above-mentioned families. In addition, we have ruled out the presence of a large duplication or deletion of MAPT in UBC-17 using metaphase FISH. However, it is still possible that a complex change involving a small duplication, deletion or rearrangement of MAPT could be responsible for disease. Our analyses and those of other groups would have missed this type of mutation. TaqMan real-time PCR quantification of MAPT exons 2 and 14 in a Swedish tau-negative FTD-U family linked to chromosome 17q21 also ruled out duplication or deletion of the entire MAPT gene in that family (Johnson et al., 2004). Although these results are in agreement with our UBC-17 FISH data, neither of this or our FISH analysis excludes the possibility of pathogenic deletions or duplications restricted to single exons or to regulatory regions of MAPT. Nevertheless, western blot analysis of soluble tau in UBC-17 (Fig. 6) and in the family reported by Rosso et al. (2001) did not reveal any evidence of abnormally sized tau protein species further arguing against a pathogenic abnormality of MAPT.

Although the precise role of MAPT in the aetiology of FTD in this series of families is uncertain, it is interesting to note that certain members of the chromosome 17-linked FTD family referred to as HDDD2 have been reported to lack soluble tau protein while tau mRNA levels remain normal (Zhukareva et al., 2001). However, it has also been reported that two members of this family possess extensive tau pathology (Zhukareva et al., 2003). Nevertheless, Zhukareva et al. (2001) suggested that loss of soluble tau in HDDD2 and the pathological FTD subtype described as ‘dementia lacking distinctive histopathology’ could be pathogenic. In contrast, tau and NSF western blot data from UBC-17 (Fig. 6) argue against this hypothesis. These results show no reduction in tau and NSF levels (individual 17-68) relative to controls and show an apparent reduction in both tau and NSF (individual 17-60). These data indicate that there is no disease specific loss of tau protein in UBC-17 and the observed reduction in tau and NSF levels in individual 17-60 is likely a reflection of extensive neurodegeneration. Our findings are therefore in agreement with the data of Taniguchi et al. (2004) who also showed that loss of tau in FTD was not a specific event and that other neuronal proteins were also decreased (e.g. NeuN).

An alternative explanation for the disease in UBC-17 and other FTD-U families linked to chromosome 17q21 is that there is another mutated gene in the critical region. While this may seem unlikely, it is important to note that the currently defined critical region is extremely gene rich, contains many potential candidates and that mutations in one such protein (GFAP) are already known to cause another neurodegenerative condition, Alexander’s disease (Brenner et al., 2001).

In summary, we have described detailed clinical and pathological features of multiple members of a new family with FTD-U and NII linked to chromosome 17. We found no obvious mutations of MAPT, excluding both point mutations and gross duplications/deletions. In addition, no specific abnormality in tau protein levels was observed further arguing against the selective loss of tau protein as the pathogenic mechanism in this family. Our findings suggest that a mutation affecting a gene on chromosome 17q21, other than MAPT, is associated with a specific subset of autosomal dominant FTD in which the pathology is characterized by motor neuron disease-type ub-ir NCIIs and lentiform NII. Alternatively, there is a novel mutation of MAPT, or the surrounding region, that lies undetected using the methods of our and other groups. The neuropathological characterization of NII would further suggest that they form within the nuclear body via a sumoylation-dependent mechanism. The results of our genetic analysis of Family UBC-17 combined with the published data from Radmakers et al. (2002) further refines the reported candidate region for this disease to a 3.53 cM region on chromosome 17q21. Identifying the gene associated with familial FTD-U with NII is vital in order to understand the molecular processes which cause neurodegeneration in this condition and to develop therapeutic strategies. Defining the biochemical defect should also help to determine the pathogenesis of sporadic FTD-U and to clarify the relationship between FTD-U and FTD with tau pathology.

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References


