Frontotemporal Dementia

in the Netherlands

Sonia Rosso

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in the Netherlands

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in Nederland

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Chapter 1.3
Rosso SM, Van Swieten JC. New developments in frontotemporal dementia and parkinsonism linked to chromosome 17. Curr Opin Neurol, 2002;15:423-8.

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Chapter 2.2
Rosso SM, Landweer EJ, Houterman M, Donker Kaat L, van Duijn CM, van Swieten JC. Medical and environmental risk factors for sporadic frontotemporal dementia; a retrospective case-control study. (Submitted)

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Chapter 4.4
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Chapter 5.1
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1 General introduction

Chapter
1.1

Introduction to the thesis

In 1892, Arnold Pick described the first patients with a clinical syndrome that is currently named frontotemporal dementia (FTD).[1] He emphasised the focal aspect of cortical atrophy in his patients, to this day the hallmark of this disorder. Following a detailed description of the neuropathological changes by Alois Alzheimer in 1911,[2] including the argyrophilic neuronal inclusions later known as Pick bodies, the term Pick's disease was introduced in 1926.[3] Over the years, many different names have been used to describe this clinical and pathological entity: frontal lobe dementia, dementia of non-Alzheimer type, dementia of frontal lobe type, Pick's disease, and others. In 1994, the term FTD was introduced to describe the clinical syndrome associated with focal frontotemporal degeneration,[4] with semantic dementia and primary progressive aphasia being recognised as clinical variants of FTD.[5] Typical features of this syndrome, which often presents with a presenile onset, are progressive behavioural changes and language disturbances. FTD can be caused by a number of neuropathological substrates, including Pick's disease, which is currently defined by the presence of argyrophilic Pick bodies.[6]

In 1994, a genetic-epidemiological study on FTD was started at the Erasmus MC of Rotterdam. The main research questions addressed in this study are the estimation of the prevalence of FTD in the Netherlands, the clinical aspects of the temporal variant of FTD, and further elucidation of the genetic and other risk factors involved in the aetiology of FTD.

After an introduction to FTD in general (chapter 1.2) and familial FTD in particular (chapter 1.3), epidemiological findings are presented in chapter 2. Results of the genetic-epidemiological study are given in chapter 2.1, concerning a total of 245 FTD patients identified between January 1994 and June 2002. Emphasis is placed on the prevalence of FTD in the province Zuid-Holland, results of genetic analyses, and pathological findings in a subgroup of 40 autopsied patients. In chapter 2.2, a case-control study is reported regarding medical and environmental risk factors of sporadic FTD, in which 80 patients with sporadic FTD were compared with 124 age, sex, and surrogate informant matched controls. In chapter 3 characteristics of patients with the temporal variant of FTD are explored. Chapter 3.1 reports on the presence of complex compulsive behaviour in patients with the temporal variant of FTD, while in chapter 3.2 the apolipoprotein E genotype is related to the distribution of atrophy in a group of 111 FTD patients. The role of the *tau* gene in familial FTD is investigated in chapter 4. Two novel *tau* mutations are reported (chapters 4.1 and 4.2), with detailed description of the clinical features, neuropathology, biochemical analyses and functional studies. In chapter 4.3 we investigated the co-occurrence of amyloid pathology in a subgroup of FTD patients with *tau* mutations, while in chapter 4.4 we measured the levels of tau and amyloid protein in cerebrospinal fluid of nine FTD patients with *tau* mutations. Finally, in chapter 5.1 a large pedigree of FTD patients is presented which shows genetic linkage to the *tau* containing region on chromosome 17, but no mutations in the *tau* gene. Neuropathologically, this family is characterised by tau-negative, ubiquitin-positive inclusions, suggesting a different aetiology of FTD in this family compared with families with *tau* mutations. In the last chapter the main findings of this study are presented and the possible implications are discussed.

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Chapter
1.2

Frontotemporal dementia

Abstract

Frontotemporal dementia (FTD) is a neurodegenerative disorder characterised by progressive behavioural disturbance, aphasia, and a decline in frontal cognitive functions. Frontotemporal atrophy on CT and MRI, as well as hypoperfusion of the frontal brain regions on single-photon emission computed tomography (SPECT), are characteristic findings. Neuropathological examination reveals deposition of abnormally phosphorylated tau protein in neurons and glialcells in a number of the sporadic and familial cases, while aspecific changes with neuronal loss, spongiosis and gliosis are found in the remaining cases. A familial form with an autosomal dominant pattern of inheritance is seen in about 20 percent of patients. Mutations in the *tau* gene have been identified in a number of these FTD families, with deposition of abnormal tau protein in affected brain regions. Presymptomatic DNA-testing is now available for relatives of patients with *tau* mutations, and must only be considered after extensive genetic counselling.

Frontotemporal dementia (FTD) is a neurodegenerative disorder, characterised by progressive behavioural changes and disturbance of language and frontal functions. Memory problems are not prominent at the initial stage. Clinical discrimination of FTD from the much more common Alzheimer's disease has been facilitated due to the introduction of clinical criteria according to the Lund-Manchester Groups.[1] A correct diagnosis is important, as the clinical presentation and course of the disease require a different approach to both patient and partner than in Alzheimer's disease.[2]

The prevalence, aetiology, and clinical and pathological heterogeneity of FTD have been the object of extensive research over the past few years. Primary progressive aphasia (PPA) and semantic dementia (SD) can be differentiated from FTD clinically, but show similar neuropathological characteristics, suggesting a common cause. Therefore, in this chapter we will use the term FTD to describe all clinical variants of this neuropathological spectrum. The identification of the responsible genetic defect for the hereditary form of FTD has led to more insight into the pathophysiological background of a whole group of neurodegenerative disorders, the tauopathies. Furthermore, presymptomatic testing is now available, and it is important that extensive genetic counselling is offered to all at-risk individuals.

Prevalence and familial clustering of FTD

The prevalence of FTD is estimated to be 10 to 20 percent of all presenile dementias.[3] In 1998, a preliminary epidemiological study of FTD in the Netherlands estimated the prevalence of FTD to be 1 per 100.000 at the age 50 to 60 years, and 3 per 100.000 at age 60 to 70 years.[4] Subsequent research has shown that the prevalence of FTD is probably 2 to 3 timer higher than previously suspected (chapter 2.1). It probably concerns 25 to 50 new patients every year. PPA and SD are substantially less common, although exact figures are not known. A familiar form of FTD with an autosomal dominant pattern of inheritance is seen in about 20% of patients, with dementia also being present in affected relatives at a similar age and with a similar clinical manifestation.[5]

Clinical symptoms

The disease usually starts between age 40 to 60 years, with a clear peak in incidence between ages 50 to 60. The disease duration varies between 5 to 15 years.[1,6] Typical is the onset with disinhibited behaviour. Roaming, impatience and gluttony are also important symptoms.

Table 1. Clinical criteria for FTD, PPA en SD[1]

Features common to all clinical syndromes

- Insidious onset and gradual progression*
- Onset before 65 years
- Positive family history of similar disorder in first degree relatives
- Motor neurone disease

Core diagnostic features per clinical syndrome:*

- Frontotemporal dementia
- Early decline in social interpersonal conduct
- Early decline in regulation of personal conduct
- Early emotional blunting
- Early loss of insight

- Primary progressive aphasia

Non-fluent spontaneous speech with at least one of the following:
- Agrammatism
- Phonemic paraphasias
- Anomia

- Semantic dementia
- Language disorder characterised by:
  - progressive, fluent, empty sponaneous speech
  - loss of word meaning (impaired naming *and* comprehension)
  - semantic paraphasias, *and/or*
- Perceptual disorder charachterised by:
  - prosopagnosia (impaired recognition of faces)
  - associative agnosia (impaired recognition of objects)

Diagnostic exclusion criteria

- Abrupt onset with ictal events
- Head trauma related to onset
- Early, severe amnesia
- Spatial disorientation
- Logoclonic, festinant speech with loss of train of thought
- Myoclonus
- Corticospinal weakness
- Cerebellar ataxia
- Choreoathetosis

* Criteria necessary for diagnosis

However, other executive and behavioural modalities that may not be regarded as suspect for FTD, can be affected.[7-10] Initiative loss with self-neglect and diminished attention for surroundings are often the only initial symptoms, and may be unjustly interpreted as a depression. Other patients show obsessive-compulsive behaviour with continuous specific activities (making jig-saw puzzles, counting, collecting) or fixation on certain ideas.[7] Schizophrenia may be considered as an initial diagnosis, until cognitive decline becomes evident and the correct diagnosis is made.[8,9] Artistic talent, such as remarkable drawing or painting efforts, may arise in some patients with bitemporal atrophy.[10] Diminished emotional response to major life-events (death of a close relative, birth of grandchild) may sometimes lead to painful situations for spouses and unjustly lead to a referral for psychotherapy.
Within the recently defined clinical consensus criteria (Table 1), the decline in social and personal conduct, emotional bluntness, and loss of insight are obligatory features for the diagnosis FTD.[1] PPA is characterised by non-fluent spontaneous speech, with agrammatism, phonemic paraphasias and naming difficulties,[1,11] while a fluent, but empty spontaneous speech with loss of comprehension, semantic paraphasias and perceptual agnosia is seen in patients with SD.[1,12]

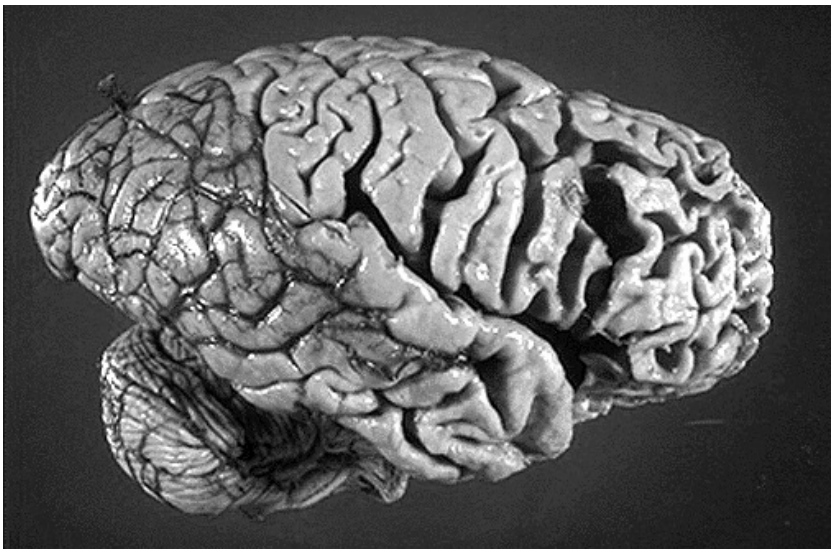
Psychometric evaluation

The pattern of cognitive disturbances characteristic for FTD has become clear over de past decade.[6] Insufficient attention, lack of self-control and impulsive behaviour upon formal testing are typical features of frontal dysfunction. A decreased wordfluency (naming of animals etc.), diminished abstract thinking (concrete thinking or inability to explain proverbs), as well as mental inflexibility are prominent in the early stages of the disease. The relatively intact orientation and memory functions, and the spared visuo-spatial abilities help differentiate FTD from Alzheimer's disease.

Imaging

Focal atrophy of the frontal and temporal lobes is characteristic for this disorder (Figure 1),[1] and is usually most severe frontally. The atrophy is asymmetric in about 30% of patients and increases dramatically within a few years. Temporal atrophy predominates in patients with SD and PPA, and is more often asymmetric (up to 70%).[11,12] Structural imaging (CT or MRI) is still normal at presentation in about 10% of patients. Clinical experience has taught us that hypoperfusion of the frontotemporal cortex using Single Photon Emission Computed Tomography (SPECT) is seen in FTD patients at the earliest stage.[13]

Figure 1. Brain of a FTD patient with focal frontal atrophy



Neuropathological classification of FTD

Neuronal loss, gliosis and spongiosis of the superficial layers of the frontotemporal cortex are characteristic for FTD, as well as PPA and SD. These changes may vary in distribution and severity over the frontal or temporal cortices. Neuropathologically, FTD may be divided into three subtypes regarding the presence and type of deposition of abnormally phosphorylated tau protein:[14-17]

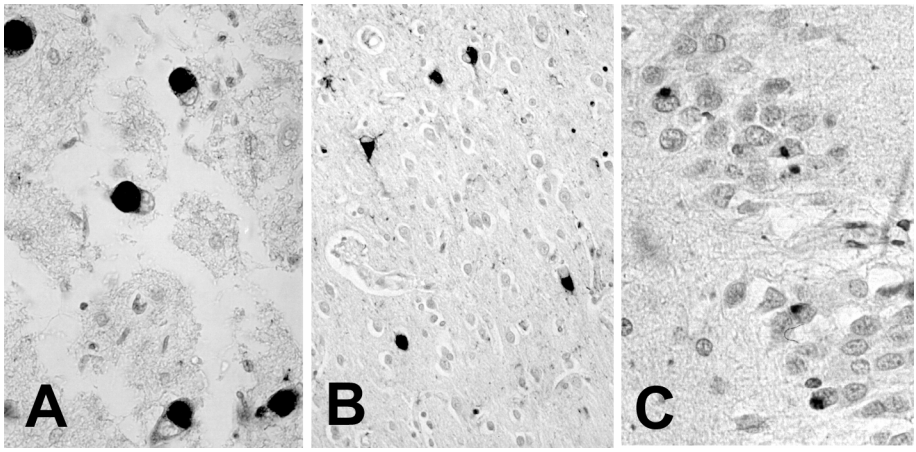
- Pick's disease: is a histopathological diagnosis that may only be made in the presence of numerous tau protein-containing Pick bodies in the cortex, hippocampus and different subcortical nuclei. This group also comprises about 20% of the total FTD group (Figure 2A)[14]

- Familiar FTD with tau pathology: present in about 15-20% of cases and characterised by deposition of abnormally hyperphosphorylated tau protein in neurones (ranging from pretangles, to neurofibrillary tangles and Pick-like bodies) and occasionally in the glial cells (Figure 2B).[15-17]

- FTD without tau deposition: is seen in about 60% of cases. It often concerns sporadic cases, but familial cases without detectable *tau* mutations have been described. In some cases ubiquitin-positive inclusions are found in the second layer of the frontotemporal cortex and dentate gyrus of hippocampus (Figure 2C).[14,16]

The interrelation between the clinical, neuropathological and genetic findings is schematically represented in Figure 3.

Figure 2. Neuropathology in FTD



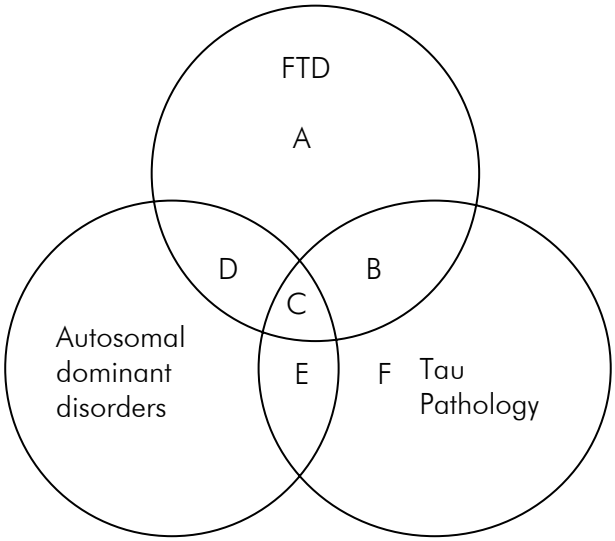
A. Classical Pick's disease with Pick bodies. B. Tau pathology in a FTDP-17 brain (S320F mutation). C. FTD with ubiquitin-positive inclusions (Immunohistochemistry with AT8 tau antibody in A and B, ubiquitin antibody in C).

Genetic factors

Since 1998, numerous different mutations have been found in the gene for the *microtubule-associated protein tau* in patients with familial FTD;[18-24] this protein is of importance to the stability of neuronal cells. Genotype-phenotype studies have shown that the onset age in most *tau* mutations is between 40 and 60 years, although specific mutations may lead to a more severe phenotype with onset ages of around 30 years.[21,22] The presence of early parkinsonism, epileptic seizures, and corticobasal degeneration is also associated with specific mutations.[21-23] The genetic cause for a number of FTD families without *tau* mutations still remains unclear, as tau-negative, ubiquitin-positive inclusions are the only characteristic neuropathological feature.[5,23,25] A single family of Danish descent has shown genetic linkage to a locus on chromosome 3.[26] Future research will be focussed on the identification of different genetic loci and the responsible genetic defect on chromosome 3.

Because the tau deposits are considered to play a central role in the aetiology, the term "tauopathy" was introduced. Abnormal deposition of tau protein is also found in brains of patients with other neurodegenerative disorders, such as Alzheimer's disease, Progressive Supranuclear Palsy (PSP) and Corticobasal Degeneration. These disorders differ neuropathologically by the distribution and type of tau deposition. The responsible genetic factors and pathophysiological mechanisms behind these disorders are not yet fully understood, although genetic studies have shown that the *tau* gene is probably also implicated in the pathogenesis of PSP.[27]

Figure 3. Relationship between clinical manifestation of FTD, autosomal dominant inheritance, and tau deposition at neuropathology



A. Sporadic forms of FTD without tau pathology. B. Sporadic FTD with tau-positive Pick bodies (Pick's disease) C. Hereditary forms of FTD with tau pathology (FTDP-17 patients with mutations in the *tau* gene). D. Hereditary forms of FTD without tau pathology (familiar FTD with or without ubiquitin positive inclusions). E. Other tauopathies with autosomal dominant inheritance, such as rare cases of familial PSP. F. Other sporadic neurodegenerative disorders with tau pathology, such as Alzheimer's disease and PSP.

Genetic counselling and presymptomatic testing

The identification of the responsible genetic defect in a number of families with FTD has enabled presymptomatic testing in at-risk relatives. Many family members have been aware of the hereditary character of the disease and their personal risks for many years. Fear of developing the disease and preoccupation with onset of the first symptoms may become a constant preoccupation for these at-risk individuals.[28] Sometimes relatives of FTD patients want to know the risk of an hereditary form of FTD in order to make important life decisions. When asked as to the hereditary nature of the disorder, a well-informed family history is essential. Information on diseases, cause of and age at death of all first and, if possible, second degree family members must be obtained. If a familiar form of FTD is suspected, it is important to refer both patient and family to a centre for clinical genetics or to a neurological centre with expertise in neurogenetic disorders.

Patient management

There are currently no therapeutic options for patients with FTD, only pharmaceutical intervention for disturbing behavioural problems is possible. Over the past years, the burden for caregivers has been the focus of much research.[29,30] The severity of behavioural problems of FTD patients appears to lead to a considerable increase of the burden for caregivers (usually the spouse), with education, ability to express emotions, social structure and the premorbid relationship with the patient as important determinants. Family members of FTD patients often have an exceptional need for accurate information on this relatively rare disorder, as well as advice regarding the best way to approach the behavioural problems of the patient. A common problem is the total lack of insight and refusal of care by the patient, to the despair of the family. Hopefully, future research will characterise the specific problems of caregivers, making interventions and practical solutions possible to diminish the caregiver burden.

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Chapter
1.3

Familial frontotemporal dementia

Abstract

The identification of *tau* mutations in frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) has revealed invaluable information regarding the role of the tau protein in neurodegenerative disease. Over the past year, several new mutations have been identified, and experimental studies have provided more insight into the mechanism of neurodegeneration due to *tau* mutations and possible interactions with amyloid pathology. Extensive clinical and pathological variation is seen in patients with different types of mutations, as well as in patients with the same mutation. Mutations may be found in patients with frontotemporal dementia (FTD), parkinsonism, progressive supranuclear palsy (PSP) , and corticobasal degeneration, justifying mutation analysis in familial cases of these disorders. Genetic heterogeneity exists in FTD, because a number of FTDP-17 families have neither *tau* mutations nor tau pathology. Genetic linkage has been found in familial FTD (chromosome 3), FTD with amyotrophic lateral sclerosis (9q21-q22), and FTD with inclusion body myopathy (9p13.3-p12). Tau deposits may consist of mainly mutated protein, or of mutated and wild-type protein in equal amounts, depending on the mutation. Recent animal studies show that amyloid-β deposition may accelerate formation of neurofibrillary tangles. Hopefully, the identification of responsible genetic defects and associated proteins will be helpful in improving our understanding of the role of the tau protein in the common neurodegenerative process of frontotemporal degeneration.

In less than one decade, familial frontotemporal dementia (FTD) has emerged as a distinct clinical disease entity, with pathological and genetic heterogeneity. Although the common pathological substrate consists of neuronal loss, spongiosis, and gliosis in the frontal and temporal cortices, further classification of FTD into different pathological subtypes can be made by means of immunohistochemical (anti-tau and anti-ubiquitin antibodies) and biochemical techniques.[1]

The term 'frontotemporal dementia and parkinsonism linked to chromosome 17' (FTDP-17) was proposed at a consensus meeting in Ann Arbor in 1996 in order to include the clinical and pathological spectrum of 13 large families with significant linkage to chromosome 17q21-22.[2] After the identification of mutations in the *tau* gene in some of these families in 1998,[3-5] many novel *tau* mutations have been found in predominantly smaller families over recent years.[6-9] All of these families have in common the accumulation of hyperphosphorylated tau protein in neurones or glial cells, or both, in frontal and temporal cortices, as well as subcortical structures. However, genetic heterogeneity still exists in FTD families with linkage to chromosome 17q21-22, because a number of these families show neither *tau* mutations nor tau deposition on neuropathological assessment, suggesting that there may be a second gene involved that is located close to the *tau* gene.[10] Genetic heterogeneity is further emphasised by the identification of three other genetic loci by linkage analysis in FTD families with variable phenotypes but without tau pathology: one on the pericentromeric region of chromosome 3[11] and another two on chromosome 9.[12,13]

Tau mutations in FTDP-17

The tau protein is considered to play an important role in assembly and stabilisation of microtubuli in axons. Six different isoforms are produced from the single *tau* gene by alternative splicing of exons 2, 3 and 10. Three isoforms contain three amino acid repeats, encoded by exons 9, 11, and 12, whereas the inclusion of the amino acid repeat encoded by exon 10 gives rise to the other three isoforms, which have four repeats. *Tau* gene mutations can be distinguished into two distinct types. In the first type, missense mutations in exon 9, 10, 11, 12, and 13 reduce the ability of the tau protein to bind to microtubuli and some also enhance the rate of heparin-induced assembly of tau into filaments. In the second type of mutation, the effect of the intronic and some coding mutations in exon 10 is at the messenger RNA level, resulting into a change of the ratio of tau isoforms of three amino acid repeats to those of four amino acid repeats. From November 2000 until Februari 2002, eight new *tau* mutations have been identified; the most important pathological and biochemical findings in these mutations are summarised in Table 1.[14-22]

Table 1. Novel tau mutations identified since November 2000

Mutation	Clinical syndrome	Tau pathology	Western Blot	Filaments	Additional studies	Ref.
K257T	FTD	Pick-like inclusions	3R>4R	Narrow twisted	↓ microtubule binding	14, 15
ΔN296	Atypical PSP in homozygotes, PD in heterozygotes	NA	NA	NA	NA	18
N296N	FTD + supranuclear gaze palsy	Corticobasal inclusion bodies	NA	NA	↑ 4R with exon trapping	17
N296H	FTD + parkinsonism	Glial tauopathy	↑ 4R	Variable	NA	20
Intron10+11	FTD, parkinsonism + mental retardation	Fibrillary changes neurons + glia	NA	NA	↑ 4R with exon trapping	19
S320F	FTD	Pick-like inclusions	↓ 3R0N + 4R2N	Straight and twisted	↓ microtubule binding	22
E342V	FTD	Pick-like inclusions with NFT's	↑ 4R0N	Paired helical filaments	↑ mRNA with 4R	16
K369I	FTD	Pick-like inclusions	All 6 isoforms	Irregularly twisted ribbons	↓ microtubule binding	21

PD= Parkinson's disease, 3R= tau isoforms with 3 amino acid repeats, 4R = tau isoforms with 3 amino acid repeats, N = amino terminal inserts.

The frequency of *tau* gene mutations has been studied in different populations of FTD patients, with diagnoses made according to more or less strict criteria. A genetic-epidemiological study conducted in the Netherlands revealed *tau* mutations in 17.6% of patients,[23] whereas the prevalence was 13.6% in an autopsy-verified series of FTD patients,[24] and only 5.9% in a referral series from the USA.[25] The P301L mutation is the most common missense mutation.[23,25,26] Some investigators have suggested that a selection bias might have skewed the results of the Dutch study, resulting in overrepresentation of familial cases. However, in a follow-up study (chapter 2.1), the percentage remained above 10% after the identification of novel mutations (S320F and L315R), as well as known mutations (G272V, P301L, and R406W) in patients identified since the previous publication. *Tau* mutations are mostly found in patients with a positive family history for dementia, being present in 10 to 50% of such patients.[1,23,25,27] The presence of tau pathology in familial FTD cases increases this prevalence to nearly 100%. Although most authors agree that nearly all cases of familial FTD with tau pathology exhibit *tau* mutations, rare forms of familial FTD with tauopathy, but no *tau* mutations have been described.[28]

As a result of the identification of *tau* mutations, genetic testing in individuals who are at-risk for FTD has become available. Preliminary studies indicate that only a minority of such individuals request genetic testing, which is not surprising in view of the fact that preventive interventions and treatments are still lacking.[29,30] Unfavourable test results have had considerable impact on life, marriage, and family planning; however, they may also lead to relief of uncertainty on the other hand. An important observation by Geschwind *et al.* is that asymptomatic carriers of the P301L mutation already showed alterations in frontal executive and attentional tasks decades before the predicted onset of dementia.[31] Because those alterations did not correlate with age, this frontal executive dysfunction is probably already determined at the developmental stage.

Phenotypic variation in *tau* mutations

Deposition of hyperphosphorylated tau is the pathological hallmark of FTDP-17 with *tau* mutations. Several novel mutations in the *tau* gene have extended the clinical and pathological phenotype of FTDP-17. Phenotypic variation is large between families with different mutations, but also within families with the same mutation.

Although mutations are mainly found in patients with classical FTD, the identification of *tau* mutations in patients with Progressive Supranuclear Palsy (PSP),[18,19,32] Corticobasal Degeneration (CBD),[17,33] and dementia with epilepsy[34] justifies *tau* mutation analysis in hereditary forms of related disorders. Clinical features of PSP were observed in patients with the silent S305S mutation, the intron mutation at the +11 position, and in two

siblings homozygous for the ΔN296 mutation.[18,19,32] Interestingly, two family members, both of whom were heterozygous for the ΔN296 mutation, suffered from mild, late-onset Parkinson's disease (PD).[18] The co-occurrence of mental retardation in patients with +11 intron mutation suggests an additional effect of this *tau* mutation or of the concomitant silent P301P polymorphism.[19] Resemblance to CBD in the P301S mutation is based on clinical features,[33] but also on neuropathological features in the novel N296N mutation.[17] The latter mutation is characterised by degeneration of pyramidal tracts, tau-positive coiled bodies in oligodendroglia, and threads with pretangles in subcortical nuclei, and functional studies have shown that the primary effect of this silent mutation in exon 10 is probably overproduction of four amino acid repeat isoforms of tau.

The genetic background of Pick's disease, which is characterised by Pick bodies that consist mainly of tau isoforms with three amino acid repeats, has also been extended by the identification of several novel *tau* mutations (K257T, S320F, E342V, K369I, and G389R).[14-16,21,22,35] The similarity to sporadic Pick's disease is supported by similar staining patterns with tau-antibody 12E8 in K257T, S320F, and K369I mutations, as well as increased levels of three amino acid repeat tau isoforms in K257T brain.[14] A few of these mutations also exhibit altered levels of specific tau isoforms in the sarkosyl-soluble fraction (a decrease in 3R0N and 4R2N in S320F[22] and an increase of 4R0N in E342V[16]) suggesting a possible effect of these mutations on either the alternative splicing of amino terminal inserts or selective degradation of specific isoforms.

Related sporadic tauopathies

In order to appreciate the role of tau protein in the pathophysiology of neurodegeneration in FTDP-17, it is worthwhile studying other related sporadic disorders with tau pathology, such as PSP, CBD, and Pick's disease. Homozygosity of the extended H1 haplotype of the *tau* gene has been significantly associated with both PSP and CBD.[36-38] Although a recent study showed that tau deposition in PSP consists mainly of tau isoforms with four amino acid repeats, the ratio of four-repeat to three repeat tau did not appear to be influenced by the H1 haplotype.[39] A novel tauopathy, which is also characterised by selective deposition of four repeat tau, has been described in a sporadic case of FTD.[40] The tau pathology in this case differs from PSP and CBD by the presence of severe cortical pathology, which consisted of dense globular tau-positive inclusions in neurons and glia cells, and the absence of neuropil threads and abundant astrocytic plaques. Although the pathology is similar, it can be distinguished from FTD due to intron mutations by the negative family history, a very late age at onset, and the absence of both parkinsonism and *tau* mutations.[40]

Another type of tauopathy is suggested by the observation of profound and selective reduction in all six soluble tau isoforms in different cortical regions in some cases of Dementia Lacking Distinctive Histology (DLDH) and in a FTDP-17 family (HDDD2) that lacked mutations in the *tau* gene.[41] The normal expression of tau messenger RNA in these cases indicates that there is either a defect in post-transcriptional regulation of tau messenger RNA or increased degradation of the tau protein. In our opinion the loss of tau in DLDH still requires confirmation in other studies.[42]

Biochemical experiments and animal models

In-vitro studies have shown that several *tau* mutations result in reduced binding and polymerisation of the tau protein with microtubuli, which is supposedly important for the normal function of the neuronal cytoskeleton.[43] Studies in living cells transfected with mutant complementary DNA and expressing mutant P301L or V337M tau protein[44] confirmed those findings by showing reduced bundling of microtubuli and higher proportion of tau protein in the soluble fraction as compared with wild-type protein.[44]

Studies with site-specific antibodies against mutant P301L tau have shown that tau deposits in P301L brains consist mainly of mutant P301L tau protein.[45,46] Furthermore, the mutant P301L tau is strikingly decreased in the soluble fraction, and levels appear to be inversely correlated with the extent of tau pathology. In contrast to this, tau deposits in brains of patients with the R406W mutation consist of equal amounts of highly phosphorylated wild-type and R406W mutant tau.[47] Although the underlying pathophysiological mechanism in these mutations is not yet understood, a change in the conformation or in the degradation of mutant tau protein may explain the different observations in those studies.

Expression of human tau with the P301L mutation in transgenic mouse lines leads to tau filament formation, neuronal loss, and gliosis in brain stem, cerebellar nuclei and spinal cord.[48-50] These mice show limb weakness and behavioural changes. Amyotrophy reflects loss of motor neurones in the anterior horns. Tau filaments are selectively formed in oligodendrocytes throughout the brain and spinal cord in the transgenic mouse line expressing mutant G272V tau.[51] Neuronal loss with filamentous tau aggregation in hippocampus, as is observed in the transgenic mouse expressing mutant V337M human tau, appears to be correlated with impaired recognition of location on standard behavioural tests.[52] Although transgenic mouse studies have major limitations in the choice of a specific promotor, different levels of expression, and the expression of only a single tau isoform, which explains some of the observed differences, they provide

invaluable information regarding the pathophysiological mechanisms of neuro-degeneration.

The possible interaction between amyloid-β protein (the major constituent of diffuse and neuritic plaques) and tau pathology in Alzheimer's disease, and in a few FTDP-17 cases is also very interesting.[53,54] The absence of high numbers of plaques in a series of 54 FTD patients (six of whom had *tau* mutations) by Mann *et al.*[55] does not support the idea that tau pathology leads to amyloid-β deposition.[55] However, inversely, amyloid-β deposition promotes neurofibrillary tangle formation in double mutant (tau/amyloid precursor protein) mice,[56] as well as in P301L mice injected with amyloid-β-42 fibrils.[57] Interestingly, the neurofibrillary tangles in P301L mice did not appear in the cortex at the site of amyloid-β-42 injection, but rather in the amygdala from which neurones project to the injection site. Gotz *et al.*[57] propose that this anatomical separation is consistent with amyloid-β-42 induced axonal damage, and possibly with impaired axonal transport of tau.

Other types of hereditary FTD

There are at least three types of pathology in familial FTD.[1] The first is characterised by tau deposition in neurons or glialcells, or both, in patients with *tau* mutations. The second is characterised by the presence of cytoplasmic ubiquitin-positive, tau-negative inclusions and dystrophic neurites in frontal and temporal cortices, and hippocampus.[10,58,59] One large Dutch family presented with a clinical presentation similar to that observed in patients with FTDP-17 due to *tau* mutations, except for a older and broader range of age at onset.[10] Genetic analysis showed strong evidence for linkage to chromosome 17q21-22, in the absence of identified mutations in the *tau* gene. The presence of a few intranuclear ubiquitin-positive inclusions, which have also been observed in some sporadic cases of FTD and motor neurone disease,[60] may be a neuropathological marker, although the role of ubiquitin in the aetiology of this FTD subtype is not evident. Familial ubiquitin-positive FTD constitutes at least 20% of the total group of hereditary FTD,[1] but most families are too small for significant linkage and further genetic analyses are awaited.[59] In the third type of pathology, a group of families show neither tau-positive or ubiquitin-positive neuronal inclusions, and are collectively called dementia lacking distinctive histology (DLDH).[1,61,62]

Hereditary FTD in some families is associated with amyotrophic lateral sclerosis, and this form has shown linkage to a genetic locus on chromosome 9q21-q22.[12] On the basis of available pathological findings in these families, tau pathology is absent or only sparse. Hereditary FTD associated with inclusion body myopathy and Paget's disease is linked to another locus on chromosome 9 (i.e. 9p13.3-p12).[13]

Finally, linkage to the pericentromeric region on chromosome 3 has been found in a single large FTD family of Danish descent.[11] We expect that the identification of the responsible genetic defects and the associated proteins in familial FTD forms may prove to be helpful in improving our understanding of the role of tau protein in the common neurodegenerative process of frontotemporal degeneration.

Conclusion

Increasing numbers of *tau* gene mutations are being found in FTD families, as well as in some patients with clinical features of PSP and CBD, justifying genetic analysis of the *tau* gene in familial forms these disorders. The diversity of *tau* mutations has revealed invaluable information regarding the role of tau protein in FTDP-17 in particular, but also in neurodegeneration in general. Although the site of the mutation correlates to some extend with the morphological and biochemical characteristics of the tau deposits, it is still unclear which other genetic or environmental factors determine the phenotypic variation. Hopefully, the identification of novel mutations, both in the *tau* gene and in yet to be discovered genes, will be helpful in improving our understanding of the process of frontotemporal neurodegeneration.

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2

Epidemiological aspects of FTD

Chapter

2.1

Frontotemporal dementia in the Netherlands; patient characteristics and prevalence estimates from a population-based study

Abstract

Since 1994, a population-based study of frontotemporal dementia (FTD) in the Netherlands aims to ascertain all patients with FTD and first prevalence estimates based on 74 patients were reported in 1998. Here, we present new prevalence estimates after expansion of our FTD population to 245 patients, with emphasis on the prevalence in the province Zuid-Holland where the main study centre is located. All neurologists and physicians in nursing homes received a yearly postal enquiry about suspected FTD cases. FTD was diagnosed in 245 patients according to the Lund-Manchester criteria, supported by neuroimaging and neuropsychology. *Tau* mutation analysis was performed in a subgroup of 154 patients (63%), and 40 out of 98 patients (41%) who died during follow-up came to autopsy during the course of the study. The prevalence of FTD in the province Zuid-Holland was 3.6 per 100.000 at age 50 to 59 years, 9.4 per 100.000 at age 60 to 69, and 3.8 per 100.000 at age 70 to 79. The average age at onset of the 245 patients (51% female) was 57.6±9.0 years. Dementia in one or more first-degree family members was found in 43% of patients and mutation analysis of the *tau* gene showed mutations in 34 patients (19 P301L, five L315R, four G272V, four R406W, one ΔK280, one S320F), all with a positive family history (14% of the total population, 32% of patients with a positive family history). Pathological findings in the 40 autopsied patients consisted of dementia lacking distinctive histology in 22%, FTD with ubiquitin-positive inclusions in 33%, Pick's disease in 15%, and tauopathy in the remaining 30% of patients, with *tau* mutations identified in more than half of the latter patients. We

conclude that the prevalence of FTD in the Netherlands is higher than previously reported, confirming that FTD is more common than expected. The finding of *tau* mutations in 32% of patients with a positive family history for dementia justifies mutation screening in FTD patients with a positive family history, while *tau* mutations in non-familiar cases are rare.

Frontotemporal dementia (FTD) has become increasingly recognised by clinicians and pathologists as a major cause of dementia over the last decade. Reliable clinical criteria for the diagnosis FTD have been established,[1,2] and several pathological subtypes are now distinguished using immuno-histochemical techniques.[3,4] The identification of mutations in the *Microtubule-associated protein tau* gene has led to a new classification of familial FTD, in which subclasses show specific pathological features.[5] The frequency of *tau* mutations varies considerably in different FTD populations, ranging from zero to 18%.[6-10] Although the number of different *tau* mutations is still increasing, some FTD families show neither tau pathology nor *tau* mutations, and may be distinguished by typical ubiquitin-positive inclusions, emphasising genetic heterogeneity.[5,11-13]

Since 1994, a population-based study aims to ascertain all patients with FTD in the Netherlands. Preliminary results indicated a maximal prevalence of 2 per 100.000 inhabitants at age 60 to 69.[14] However, a recent study in the Cambridge area of the United Kingdom (UK) found a substantially higher prevalence of 15 per 100.000 inhabitants aged 45 to 64 years.[15] Although FTD is known to occur at older age, the prevalence of FTD in patients older than 65 has not yet been investigated. In the current study, we describe a large cohort of FTD patients ascertained between January 1994 and June 2002 in the Netherlands, and present estimates of the prevalence of FTD at different ages, the frequency of *tau* mutations, and the distribution of different pathological subtypes. We also attempted to evaluate the proportion of misdiagnosis and non-referral by looking at all patients with a neuropathological diagnosis compatible with FTD, autopsied during the same period at the Netherlands Brain Bank (NBB).

Patients and Methods

Study design and diagnosis

A complete ascertainment of patients with FTD in the Netherlands was attempted from January 1994 until June 2002.[14] All hospital-based neurologic and psychiatric practices (n=183) and physicians in psychogeriatric hospitals or nursing homes (n=269) received

a yearly postal enquiry about all suspected FTD cases, irrespective of their family history. Additionally, we visited four university medical centres specialised in dementia in June 2002 (P.S., W.v.G, H.P.K, F.V.) and reviewed medical records (including neuropsychological evaluation and hard copies of neuroimaging) of all suspected FTD patients examined after 1994 to assess whether they fulfilled the criteria for probable FTD. The diagnosis FTD was based on the criteria of Lund and Manchester,[1,2] and included (1) a progressive behavioural disorder with insidious onset; (2) affective symptoms; (3) speech disorder; (4) preserved spatial orientation and praxis and (5) selective fronto-temporal atrophy (computed tomography [CT] or magnetic resonance imaging [MRI]) or selective fronto-temporal hypoperfusion (single photon emission computed tomography [SPECT] with 99mTc-hexamethyl propyleneamine oxime [HMPAO]). Consensus about the clinical diagnosis between research physician, neurologist, and neuropsychologist was obtained, and in case of uncertain diagnosis, the final decision was made based on supplementary clinical, neuropsychological, and neuroimaging data later in the course of the disease.

Spouses and first-degree relatives assisted in obtaining detailed clinical history on the onset and evolution of the disease, with emphasis on frontal symptoms, speech and spatial functions, as well as memory problems. The age at onset was defined as the age at which the first symptom compatible with the diagnosis FTD was observed by a close relative or caretaker. The duration of the disease was determined in all patients who died during the course of the study. A positive family history was defined as at least one first-degree relative with dementia before the age of 80. An autosomal dominant pattern of inheritance was considered present if at least three affected individuals (including the proband) over two or more generations were identified, and through genealogical research we attempted to link these familial cases in order to determine the number of individual families. The pattern of cerebral atrophy on CT or MRI was evaluated, and patients were classified according to the predominance of either frontal or temporal atrophy as described previously.[16] Left-right asymmetry was considered to be present if there was at least one grade difference.

Tau mutation analysis was performed in a subgroup of 154 patients (63%). Mutation analysis for 90 patients was previously described.[7] For another 50 cases all 11 coding exons of the *tau* gene, including flanking intronic sequences, were amplified from genomic DNA. Sequence analysis was performed on exons 1, 2, 3, 4, 5, 7, 9, 11, and 13. For the remaining 14 patients only exons 9 to 13 of the *tau* gene were sequenced by our clinical testing laboratory. The possibility of post-mortem examination was discussed with relatives of patients who died during follow-up. In collaboration with the NBB, all brains that became available for autopsy were processed for routine staining,

including Bodian silver staining, as well as immunohistochemistry with tau (AT8, 1:40, Innogenetics, Ghent, Belgium) and ubiquitin (1:500, Dako, Glostrup, Denmark) antibodies as described previously.[12] The NBB complies with all relevant ethical and legal guidelines regarding obtaining consent from autopsy, anonymity of the donors and the collection use and transport of tissue samples as well as the safety procedures of working with human post-mortem tissues.[17] The study was approved by the Medical Ethics Committee of the Erasmus Medical Centre of Rotterdam. Informed consent for participation (including DNA-studies) was obtained from the spouse or a first-degree relative of each patient.

Prevalence estimates

Point prevalence estimates for the Netherlands were calculated for the 1st of January of each year between 1995 and 2000 to evaluate consistency of ascertainment over the years. Subsequently, age-specific prevalences were calculated for 10 year periods for the chosen census day, 1st of January 1998, for both the Netherlands and the province of Zuid-Holland, in which the main study centre is located. The number of patients with FTD resident in the appropriate target area and alive on the census day, was divided by the total number of inhabitants in the same area. Information about population size was derived from the Central Bureau for Statistics in the Netherlands. The 95% confidence intervals (CI) were calculated using a recommended method for small numbers with Confidence Interval Analysis (CIA) software.[18] Differences across groups were compared using Student's *t*-test and Chi-square test when appropriate. Differences in prevalence ratios between different target areas, as well as between the present study and published studies, were compared using the recommended method for unpaired samples (Newcombe) with CIA software.[18]

Evaluation of accuracy of prevalence estimates

In order to assess the accuracy of our prevalence estimates, we looked at all patients autopsied at the NBB during the course of the study, who had a neuropathological diagnosis compatible with FTD. Firstly, we evaluated the proportion of autopsied patients with a clinical diagnosis other than FTD, in order to assess the proportion of misdiagnosis of FTD by primary physicians. Secondly, we looked for autopsied patients with a clinical diagnosis of FTD, to determine if non-referral by the primary physician occurred in our study. Subsequently, we evaluated what effect correction for the proportion of non-referral would have on our prevalence estimates.

Results

Demographic and clinical features

During the entire study period a total of 245 patients (125 women, 120 men) with probable FTD were identified according to the Lund-Manchester criteria. FTD patients referred by neurologists and psychiatrists (n=135) were examined at the out-patients clinic, the remaining patients were seen at nursing-homes (n=110). The mean duration of symptoms at ascertainment was 3.6±2.5 years in the former group, and 6.0±3.2 years in the latter group. The average age at onset was 57.6±9.0 years, with a broad range from 33 to 80 years. Fifty-four patients (22%) were older than 65 years at onset of symptoms. Ninety-eight patients died during the course of the study, after a mean duration of symptoms of 7.9±3.6 years (range 2 to 19 years). The family history was positive for dementia in 105 of patients (43%). Forty-nine of these patients (20%) had an autosomal dominant pattern of inheritance, and genealogical research showed that they came from 18 independent families. The information on affected family members was too limited to determine whether there was a true familiar form of FTD in the remaining 56 patients (23%). Symptoms of motor neurone disease (MND) developed during the course of the illness in 11 patients (4%), six of whom had a positive family history for either dementia or amyotrophic lateral sclerosis. Parkinsonism early in the course of the disease, was present in 34 patients (14%), whereas most patients developed a hypo-kinetic-rigid syndrome later in the course of the disease. Predominantly temporal atrophy on neuroimaging was seen in 97 (40%), which was asymmetric in 70 patients (72%). In patients with frontotemporal atrophy, asymmetry was seen in only 27% of patients.

Prevalence estimates

The estimated overall prevalence of FTD in the Netherlands climbed from 0.9 per 100.000 in 1995 to 1.1 per 100.000 in 1997, and remained stable thereafter (Figure 1). A total of 174 patients were alive and suffering from FTD in the Netherlands on the 1st of January 1998, our census day, resulting in an overall prevalence of 1.1 per 100.000 inhabitants (95% confidence interval (CI): 1.0 to 1.3). The overall prevalence in the province Zuid-Holland was at least twice as high, namely 2.7 per 100.000 inhabitants (95% CI: 2.1 to 3.5), based on 55 patients. Because there is no evidence that familial aggregation in Zuid-Holland is more common, this difference is most likely due to underascertainment in regions further away from the main study centre. The age-specific prevalence in Zuid-Holland, summarised in Table 1, was highest in patients aged 60 to 69 (9.4 per 100.000 inhabitants). The prevalence of FTD at age 45 to 64 was 4.0 (95% CI: 2.8 - 5.7) per 100.000 inhabitants based on 31 patients.

Figure 1. Overall prevalence estimates in the Netherlands on the 1st of January for subsequent years during the course of the study*

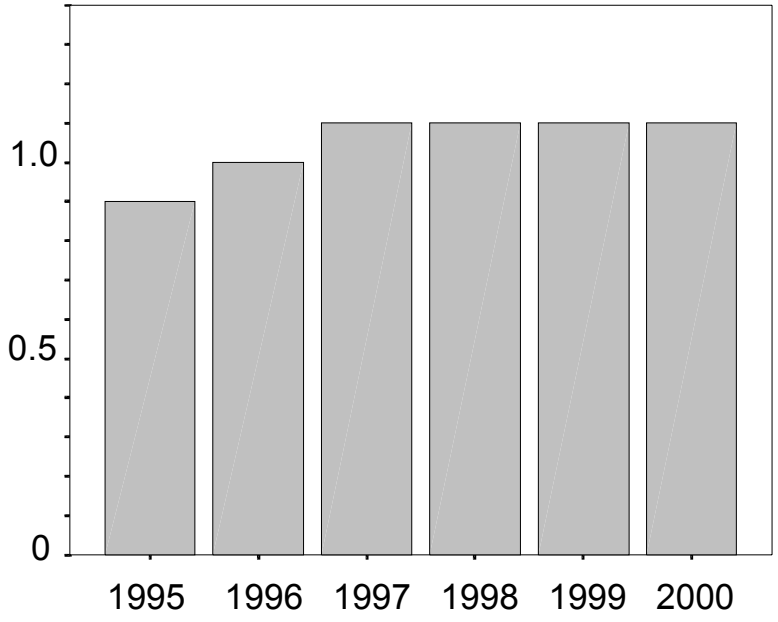

Table 1. Age-specific prevalence estimates of FTD on 1st January 1998

	Patients	Population	Prevalence* (95% CI)
30 to 39 years	1	556.346	0.2 (0.03 – 1.0)
40 to 49 years	6	488.269	1.2 (0.6 – 2.7)
50 to 59 years	14	391.154	3.6 (2.1 – 6.0)
60 to 69 years	26	276.581	9.4 (6.4 – 13.8)
70 to 79 years	8	212.754	3.8 (1.9 – 7.4)
Total population	55	2.043.949	2.7 (2.1 – 3.5)

* per 100.000 inhabitants

At the NBB we identified 50 patients autopsied after January 1st 1994, with a pathological diagnosis compatible with FTD. Thirty-three (66%) patients had been ascertained in the current study, whereas 17 (34%) had not been not referred. Although the diagnosis FTD had been considered during life in nine of these 17 patients (18%), including two from Zuid Holland which had not been referred to our centre. The remaining eight patients (16%) had been diagnosed with other disorders, most frequently Alzheimer's disease. Assuming that the proportion of misdiagnoses is similar throughout the Netherlands, correction for a non-referral of 34% would increase the prevalence of FTD at ages 45 to 64 years in Zuid-Holland (based on 31 and 52 patients respectively) from 4.0 to 6.7 per 100.000 inhabitants (95% CI: 5.1 to 8.8).

Genetic studies

Tau mutation analysis was carried out in a subgroup of 154 (63%) patients (Table 2). Missense mutations in the *tau* gene were identified in 34 patients (19 P301L, five L315R, four G272V, four R406W, one ΔK280, one S320F). Through genealogical research we were able to link a large number of these 34 patients, and reduce the number of independent families to 10 (three P301L, two L315R, one G72V, two R406W, one ΔK28, and one S320F). The frequency of *tau* mutations was 14% of the total FTD population, 32% in patients with a positive family history for dementia, and 56% of the independent autosomal dominant families (10 out of 18). Mutation analysis in the remaining 120 patients did not reveal any *tau* mutations. Of the 48 patients with a positive family history for dementia but no *tau* mutations upon sequencing, nine came from two large FTD pedigrees with ubiquitin-positive, tau-negative inclusions and significant linkage to chromosome 17q21-22.[12,19] Another six patients were from unrelated FTD families with a clear autosomal dominant pattern of inheritance, but too small for informative linkage analysis. *Tau* screening was also negative in the remaining 72 patients, all of whom had a negative family history of dementia.

Pathological findings

Brain autopsy was performed in 40 out of 98 (41%) patients, who died during the course of the study (16% of the total population). This group consisted of nine (22%) patients with the pathological diagnosis Dementia Lacking Distinctive Histology (DLDH), 13 (33%) patients had ubiquitin-positive inclusions in neurons of the second layer of the frontotemporal cortex and dentate gyrus of the hippocampus (FTD-MND type), and 18 (45%) patients showed tauopathy (*tau* mutations in seven, sporadic Pick's disease in six, other tauopathy without *tau* mutations in three, other tauopathy without *tau* mutation screening in two patients).

Table 2. Family history, autosomal dominant forms of FTD and tau mutation analysis in 245 FTD patients

	Number*	Analysis	Mutations
Total patient group	245	156 (64%)	34 (14%)
Sporadic form	140	72 (515)	0
Total positive family history	105	82 (78%)	34 (32%)
- autosomal dominant	49 (18)	49 (100%)	34 (69%)
- P301L	19 (3)		
- L315R	5 (2)		
- G272V	4 (1)		
- R406W	4 (2)		
- ΔK280	1 (1)		
- S320F	1 (1)		
- No mutation**	15 (8)		
- possible familial FTD	56	33 (59%)	0

* Number between brackets is number of independent families.

** Nine patients from two FTDP-17 pedigrees with ubiquitin-positive inclusions[12,19]

Discussion

The present population-based study, consisting of the largest series (n=245) of FTD patients reported so far, showed a maximum prevalence of 9.4 per 100.000 at age 60 to 69 years in the province Zuid-Holland of the Netherlands. Prevalence estimates for the Netherlands as a whole were a factor two lower, probably due to underascertainment in regions further away from the study centre. An age at onset higher than 65 years was found in 22% of patients. The family history was positive for dementia in 43% of patients (n=105), and *tau* mutation screening showed mutations in 32% of these patients(n=34), 14% of the total population. Pathology in 40 patients who died during follow-up, showed DLDH in 22%, FTD with ubiquitin-positive inclusions in 33%, Pick's disease in 15%, and tauopathy in the remaining 30% of patients.

The highest prevalence of 9.4 per 100.000 emphasises that FTD is much more common than previously considered. This was also apparent in the only other study on the prevalence of FTD in the UK, where a prevalence of 15 per 100.000 was found at ages 45 to 64.[15] In the current study, the maximum prevalence estimate in patients aged 45 to 64 was 4.0 per 100.000, significantly lower than in the UK study. The lower prevalence estimate in the Netherlands may be explained by methodological differences between the two studies. One limitation of the present study in this respect is the possibility of underascertainment due to misdiagnosis by the primary specialist, which was not a problem in the UK study as all patients with dementia were clinically evaluated. In the autopsy series of the NBB, 34% of patients had not come to our attention, either due to misdiagnosis or non-referral by the primary specialist. A correction for a non-referral proportion of 40% would increase the prevalence of FTD at ages 45 to 64 in Zuid-Holland from 4.0 to 6.7 per 100.000 inhabitants, still considerably lower than in the UK study. Other factors which may contribute are the ethnic background of the population. The population of Zuid-Holland consists for a considerable part (10%) of non-Caucasian ethnic groups, which do not have the same risk of FTD as Caucasians do. In the current study, over 99% of patients were indeed of Caucasian origin. In contrast, Ratnavalli explicitly mentioned that ethnic minorities were underrepresented in the Cambridge study population.[15]

An important observation of the present study was that 22% of our patients had an age at onset higher than 65 years (63% between ages 65 and 70, and 37% after the age of 70). Interestingly, two of the four original patients described by Arnold Pick had an age at onset of 69 and 73 years.[20] However, van Mansvelt in his large literature review of Pick's disease emphasised its presenile onset.[21] The older age at onset in a subgroup of patients has not been given much attention in more recent literature, although the frequency in several studies varied from 10% up to 44% in a pathologically confirmed series by Giannakopoulos.[22-27]

A positive family history was present in 43% of Dutch FTD patients, similar as reported previously.[14] These patients have proven (*tau* mutation) or convincing autosomal dominant inheritance of FTD in 20%, whereas the pattern of inheritance could not be determined in the remaining patients due to small pedigrees or limited information on affected family members. *Tau* mutations were seen in 14% of Dutch patients, similar to the 18% percent reported in our previous study of 90 FTD patients[7] and to 14% in the stringently diagnosed Manchester series described by Houlden.[6] However, most other studies show fewer *tau* mutations, with percentages in Sweden, U.S.A. and Japan of zero, 6% and 8% respectively.[8-10] As also observed in series from France and Northern America,[9,28] the most common *tau* mutation in the Netherlands was P301L (19 patients),

which may be due to a founder effect in these populations. The absence of intronic splice-site mutations appears to be common on the European Continent , in contrast to the U.K. where mainly intronic mutations downstream of exon 10 are found.[6,9,28]
More than half of the pathologically confirmed cases did not have deposition of hyperphosphorylated tau in affected brain regions, as also reported in other pathological series.[4,29] DLDH was the diagnosis in 22% and FTD with ubiquitin-positive inclusions in 33% of these cases. MND was present in only three of 13 cases with ubiquitin-positive inclusions. Tauopathy was found in 45% of our patients, which is quite a high percentage compared with other series,[3,4,30] although the frequency of classical Pick's disease, diagnosed in 15% of our patients, varies considerably in different studies, ranging from 8 to 35%.[3,4,30-32] The cause of tauopathy in five other patients (two with a positive family history) has to be elucidated in the future. Boeve *et al.* recently presented a similar family characterised by tauopathy, but extensive sequencing of the *tau* gene also did not reveal a *tau* mutation.[33] It may be that a gene other than the *tau* gene is responsible for the extensive tauopathy in these familial cases.
In conclusion, frontotemporal dementia is more common in the Netherlands than we previously reported, with a maximum prevalence at ages 60 to 69. As nearly a quarter of patients had an age at onset of higher than 65, more attention should be paid to FTD at older ages in epidemiological studies. The presence of *tau* mutations in 32% of patients with a positive family history justifies *tau* mutation analysis in familial cases of FTD.

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Chapter

2.2

Medical and environmental risk factors for sporadic frontotemporal dementia; a retrospective case-control study

Abstract

We performed a retrospective case-control study with 80 sporadic FTD patients and 124 age, sex, and surrogate informant matched control subjects, regarding various medical and environmental risk factors. Firstly, head trauma was associated with an odds ratio of 3.3 (95% confidence interval: 1.3 to 8.1). Although we cannot exclude that recall bias accounts for part of the observed association, the frontal lobes are known to be especially vulnerable to head trauma. Secondly, thyroid disease was associated with a 2.5 times increased risk of FTD (95% CI: 0.9 to 7.9), unfortunately not statistically significant due to limited power. As altered thyroid hormone levels have also previously been observed in FTD, future studies will be important to confirm this observation.

Frontotemporal dementia (FTD) is a neurodegenerative disorder with a predominantly presenile onset of behavioural changes and cognitive decline.[1] Since the identification of mutations in the *tau* gene in familial forms of FTD, there is increasing interest in genetic factors which may predispose to the disease. About sixty percent of FTD patients do not have a family history for dementia and are considered to be sporadic cases. Genetic factors, such as apolipoprotein E genotype and the H1-haplotype of the *tau* gene, have been inconsistently associated with the sporadic form of FTD.[2-4] To our knowledge, no studies have been reported to date addressing non-genetic risk factors for FTD, in contrast to other neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease, and progressive supranuclear palsy. The identification of new risk factors for sporadic FTD may help us understand which mechanisms underlie the aetiology of sporadic forms of FTD. Therefore, we performed a retrospective case-control study with 80 patients with sporadic FTD and 124 age, gender, and surrogate informant matched controls, regarding possible medical and environmental risk factors.

Methods

Cases

Cases were identified through our nation-wide study on FTD in the Netherlands between January 1994 and January 2002. The diagnosis FTD was based on the criteria of Lund and Manchester,[1] and included (1) a progressive behavioural disorder with insidious onset; (2) affective symptoms; (3) speech disorder; (4) preserved spatial orientation and praxis and (5) selective fronto-temporal atrophy (CT/MRI) or selective fronto-temporal hypoperfusion (SPECT) on neuroimaging. The method of case-ascertainment has been described previously.[5] The age at onset was defined as the age at which the first symptom compatible with the diagnosis FTD was observed by the surrogate informant. Cases were considered to have a sporadic form of FTD if there were no first-degree relatives with dementia with an onset before the age of 70, nor a *tau* mutation identified upon mutational analysis.

Controls

For each case, one or two controls were identified through either the nursing-home where the patient resided or the general practitioner. In order to avoid selection-bias, co-operating physicians were asked to recruit persons who would be willing to answer a verbatim questionnaire as a surrogate respondent regarding a family member (spouse, parent, or sibling) with current age (± 5 years) and sex matched to that of the patient.

These family members then served as surrogate informant matched controls. An exclusion criterion was that the controls were not suffering from clinically diagnosed dementia. Furthermore, as all patients were of Caucasian origin, only Caucasian controls were included.

Data collection

Because many of our FTD patients were in late stages of the disease and unreliable as historians due to language difficulties, the information was collected from a close relative of the patient, the surrogate informant. Only spouses, offspring, and siblings were questioned, as information from more distant relatives is unreliable. A structured verbal interview was obtained by one of the investigators from the surrogate informant of both cases and controls. A risk factor was considered positive only if it preceded the date of onset of FTD in the matched case. The investigators were blinded to the information available from the medical records of the FTD patients to avoid information bias. The level of education (divided into three categories: (1) primary school only, (2) secondary school or lower/intermediate professional education, and (3) higher professional education or university) was registered, as was employment or hobbies associated with exposure to chemicals, pesticides or insecticides. Questions regarded history of the medical problems (Table 1), and smoking and alcohol consumption (Table 2). Head trauma was studied extensively and was positive if it was followed by severe headache, nausea, blurred or double vision, dizziness, memory loss or loss of consciousness. Severe head trauma was present only if the trauma was followed by loss of consciousness. We also collected information on the practice of sports associated with chronic head trauma (i.e. boxing, football). Thyroid problems were positive if confirmed by a specialist or prescription of thyroid-related medication by a general practitioner. Smoking was measured in pack-years, i.e. the average number of cigarette packs smoked per day times the number of years the individual smoked. Alcohol consumption was measured in drinks per day and considered mild-to-moderate if individuals used between one to three drinks per day, and severe if more than three drinks per day, for a period of at least 10 years.

Statistical analysis

Statistical procedures were performed using Statistical Package of Social Sciences (SPSS) software. Differences between cases and controls were analysed using Chi-square test for categorical variables and *T*-test for continuous data. Variables with a p-value lower than 0.05 were evaluated in a multivariate analysis in which these variables were studied simultaneously. Conditional logistic regression was used to determine odds ratio's

adjusted for age, sex, and surrogate respondent relationship, with each case with it's control(s) classified as a stratum.

Results

The 80 patients had a mean age of 64.3±9.4 years, while the 124 controls were on average 62.5±10.2 years (p=0.2). All cases and controls were Caucasian. The surrogate respondent was a spouse for 54 cases (67%), an off-spring for 22 cases (28%), and a sibling for four cases (5%). The level of education in both cases and controls was similar. Cases did not practice (contact) sports associated with chronic head trauma, or had employment/hobbies associated with exposure to chemicals, pesticides or insecticides more regularly than control subjects. Regarding history of medical problems, both head trauma and thyroid disease showed a p-value lower than 0.05.

Table 1. History of medical problems of cases and controls

	Sporadic FTD n=80	Controls n=124	P-value
Hypertension	11 (14%)	18 (15%)	n.s.*
Diabetes Mellitus	5 (6%)	3 (2%)	n.s.
High cholesterol	6 (8%)	7 (6%)	n.s.
Myocardial infarction	0	3 (2%)	n.s.
Stroke	0	0	n.s.
Meningitis/encephalitis	0	0	n.s.
Seizures	0	1 (1%)	n.s.
Head trauma	19 (24%)	10 (8%)	p=0.002
- with loss of consciousness	5 (6%)	3 (2%)	n.s..
Thyroid disease	11 (14%)	6 (5%)	p=0.03
Headache, > once a month	20 (25%)	25 (20%)	n.s.
Migraine	12 (15%)	9 (7%)	n.s.
Herpes Zoster	4 (5%)	7 (6%)	n.s.
Cold sores	30 (38%)	3 (26%)	n.s
Severe influenza	5 (6%)	9 (7%)	n.s.

* n.s. = not significant (p<0.05)

With conditional logistic regression, only head trauma remained a significant independent risk factor for FTD and was associated with a odds ratio of 3.3 (95% confidence interval: 1.3 to 8.1). The time between head trauma and onset of dementia spanned several decades in some FTD patients. Head trauma followed by loss of consciousness was also more frequent in FTD patients, but was too infrequent to be a significant risk factor. Thyroid disease was associated with an odds ratio of 2.5 (95% CI: 0.9 to 7.9) using conditional logistic regression (p=0.09). Smoking and alcohol consumption were not associated with higher or lower risks of FTD.

Table 2. Smoking and alcohol use in cases and controls

	Sporadic FTD n=80	Controls n=124	P-value
Smoking:			n.s.*
Never	32 (40%)	53 (43%)	
< 20 pack years	27 (34%)	45 (36%)	
≥ 20 pack years	21 (26%)	26 (21%)	
Alcohol consumption:			n.s.
< 1 drink per day	28 (35%)	55 (44%)	
1 to 3 drinks per day	46 (58%)	60 (48%)	
> 3 drinks a day	6 (8%)	9 (7%)	

* n.s. = not significant (p<0.05)

Discussion

To our knowledge, this is the first case-control study regarding medical and environmental risk factors in patients with sporadic FTD. Head trauma was associated with a 3.3 fold increased risk of FTD in cases compared to controls and thyroid disease with a 2.5 fold increased risk (not statistically significant using conditional regression analysis). Neither smoking or alcohol use was associated with FTD, nor exposure to chemicals, pesticides or insecticides, which have been implicated in related disorders such as Parkinson's disease and progressive supranuclear palsy.

Numerous difficulties arise in identifying risk factors for disorders such as FTD. Prospective studies, needed to establish a causal relationship between an exposure and a disease, are unfortunately not viable for rare disorders such as FTD. The problem with case-control studies, however, is that relevant exposure takes place before the onset of the disease, and unless the information is documented for both patients and controls in a standardised way (i.e. registers, medical records), we are dependent on retrospective information, which is subject to recall bias and may lead to false positive results. We opted for a design with surrogate informants, because the demented patients were unable to give accurate information, and the information we wished to collect was not completely available from registers or medical records. Unfortunately, information is often lost with this design, resulting in reduced statistical power. However, if information regarding exposure is collected uniformly in cases and controls, information bias will not be major problem.

Head trauma, associated with a 3.3 times increased risk of FTD in the current study, has also been associated with Alzheimer's disease in several case-control studies, although this has not been confirmed in prospective studies.[6;7] Head trauma followed by loss of consciousness, a measure for more severe traumatic brain injury, also appeared to be more frequent in FTD patients, although this difference was not significant due to the fact that it was a very uncommon occurrence. The frontal lobes are especially sensitive to head trauma and experimental animal models and post-mortem studies have shown axonal damage and dysfunction, mostly localised in the frontal lobes, following even mild traumatic brain injury.[8] However, we cannot exclude that recall bias plays a role in the observed association, as surrogate informants may be more prone to remember head trauma in patients than controls, as a possible cause of their dementia.

Thyroid disease was associated with a 2.5 fold increased risk of FTD, although the association did not reach significance using conditional logistic regression. It is well known that thyroid problems can lead to cognitive disturbance and even dementia, but thyroid disease may also be associated with FTD specifically. First of all, experimental studies have shown that splicing of juvenile and adult tau mRNA variants is regulated by thyroid hormone[9] and Pick's disease, one of the tauopathies, is a common cause of the clinical syndrome of FTD. Secondly, a study regarding thyroid hormone levels in different dementia syndromes showed that abnormalities were rather common in FTD (38%).[10] Therefore, thyroid problems may play a role in the aetiology of sporadic FTD, and further studies will be important to evaluate this association.

Smoking and alcohol consumption have also been extensively studied in different neurodegenerative disorders. Smoking has been found to be protective regarding Parkinson's disease[11] and harmful regarding risk the of Alzheimer's disease,[12] but in the

current study neither effect was found. Also in contrast to the current study, light to moderate alcohol consumption (one to three drinks per day) has been shown to have a protective effect in Alzheimer's disease,[13] but not in Parkinson's disease.[14] A possible explanation why these risk factors were not identified in the current study, may be that the clinical syndrome of FTD is caused by number of different neuropathological substrates, such as Pick's disease, Dementia Lacking Distinctive Histology, and FTD with ubiquitin inclusions.[15] It is probable that these different neuropathological subtypes arise through different pathophysiological mechanisms and contrasting effects of risk factors may be diluted in a mixed group of patients. Because it is currently impossible to predict the neuropathological subtype of FTD based on clinical grounds, it will be important to conduct similar retrospective studies in patients with pathological verification of the disease.

In conclusion, the search for risk factors for sporadic FTD will be difficult, but may nevertheless be of importance in both the elucidation of the pathophysiological mechanisms leading to disease, as in the search for therapeutic opportunities and preventive interventions. Therefore, we hope that future research will place more emphasis on the resolution of this problem.

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3

Temporal variant of FTD

Chapter

3.1

Complex compulsive behaviour in the temporal variant of frontotemporal dementia

Abstract

As metabolic and structural changes in frontotemporal-subcortical pathways have been reported in patients with obsessive-compulsive disorders, we investigated the correlation between complex compulsive behaviour (CCB) and the distribution of atrophy in a group of 90 patients with frontotemporal dementia (FTD). CCB was defined as complex, intentional, and time consuming repetitive behaviour, which was distinguished from simple compulsive behaviour (SCB), such as verbal and motor repetitions and utilisation behaviour. Cortical atrophy on CT and/or MRI was semiquantitatively assessed in frontal, temporal, parietal and occipital regions, and the pattern of atrophy was compared between patients with and without CCB or SCB. Linear measures were used to establish the presence of caudate atrophy (bicaudate ratio) and ventricular enlargement (bifrontal ratio). CCB was reported in 18 (21%) and SCB in 53 (61%) FTD patients. Frontotemporal atrophy was present in 64 patients (74%), and predominant temporal atrophy in 23 (26%). The pattern of atrophy was asymmetric in 25 patients (29%). Logistic regression analysis showed that temporal lobe atrophy ($p<0.005$), as well as asymmetry of atrophy ($p<0.05$) were independently associated with CCB, after adjusting for age at onset, gender, duration of symptoms at the time of imaging, severity of atrophy, and bicaudate and bifrontal ratio. No relationship was found between the presence of SCB and the distribution of atrophy, although patients with SCB tended to have more caudate atrophy ($p<0.1$). Temporal lobe atrophy appears to mediate CCB in patients with FTD, especially if asymmetry of atrophy is present. Future studies with quantitative and volumetric measurements of the cortical and subcortical structures may further clarify the aetiology of CCB in FTD.

Frontotemporal dementia (FTD) is a neurodegenerative disorder of predominantly presenile onset, characterised by progressive behavioural changes and cognitive decline. Several investigators have paid attention to specific behavioural symptoms or cognitive changes and have tried to correlate them to the distribution of atrophy or hypoperfusion.[1-3] Temporal lobe pathology in FTD has been associated with language difficulties[4,5] and facilitation of visual and musical talents[6,7] when the left lobe is affected, and psychiatric disturbances when the right lobe is affected.[2,4] Snowden *et al.* divided FTD patients into three clinical subtypes and found distinct patterns of hypoperfusion in each subtype: the disinhibited subtype was associated with orbitofrontal hypoperfusion on SPECT, the apathetic subtype with dorsolateral hypoperfusion of the frontal lobe, and the stereotypic subtype, which is characterised by the presence of complex compulsive behaviour, with both striatal and temporal hypoperfusion.[8]

Repetitive behaviour shows a spectrum of complexity, with complex compulsive behaviour (CCB) at one end of the spectrum, and simple motor and verbal repetitions at the other end. CCB is frequently reported in patients with FTD, and consists of a time-consuming preoccupation with certain ideas or activities. It often occurs at an early stage of the disease, and is useful in distinguishing FTD from Alzheimer's disease.[9,10] As metabolic and structural changes in orbitofrontal-subcortical pathways have been reported in patients with both idiopathic and acquired obsessive-compulsive disorders,[11-16] the occurrence of CCB in FTD is pathophysiologically interesting.

Here, we address the correlation between CCB and the distribution of atrophy in a group of 90 patients with FTD, ascertained from a genetic-epidemiological study in the Netherlands.

Methods

Patients were recruited in the Netherlands between January 1994 and June 1998; the method of ascertainment has been reported previously.[17] The mean age at onset of the disease was 54.8 ± 8.4 (± standard deviation) years; the mean duration of illness 4.9 ± 2.9 years. A positive family history for dementia was present in 34 (39%) patients. The clinical diagnosis was established according to the criteria of the Lund and Manchester groups[18,19] by two independent neurologists blinded to family history. The core diagnostic features of FTD are (1) an insidious onset and gradual progression, (2) an early decline in social interpersonal conduct, (3) an early impairment in regulation of personal conduct, (4) early emotional blunting, and (5) early loss of insight.[19] The diagnosis was supported by neuroimaging and neuropsychological evaluation at

ascertainment and/or by review of previous neuropsychological reports when testing was no longer possible at time of ascertainment. Fifteen patients had *tau* gene mutations (G272V, P301L, R406W, and ΔK280),[20] and the clinical diagnosis was confirmed by neuropathological examination in an additional 12 patients who died during follow-up. The Medical Ethics Committee of the University Hospital of Rotterdam approved the study and the spouse or a first-degree relative of each patient gave informed consent for participation.

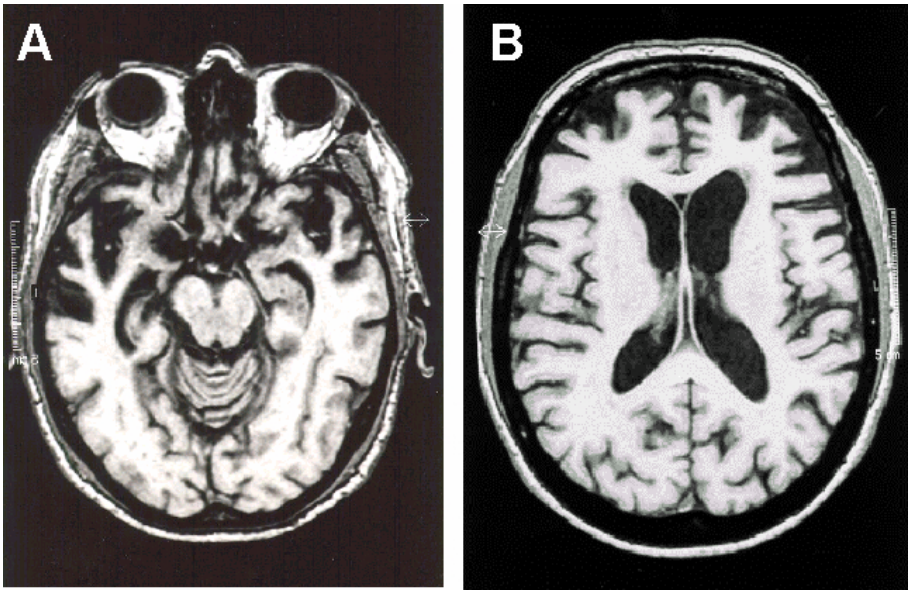
The investigators asked for the presence of repetitive behaviour in all patients in an interview with a primary caregiver or close family member. Repetitive behaviour was divided into simple compulsive behaviour (SCB; repetitive motor or verbal actions and environmentally dependant behaviour which is not internally generated) and complex compulsive behaviour (CCB; complex, intentional, and time-consuming (more than 1 hour a day) behaviour, resulting in an interference with regular daily routines). In the absence of clear clinical criteria for CCB, a descriptive characterisation of the type of CCB was attempted. The anxiety component, which is characteristic of patients with obsessive-compulsive disorder as defined by the DSM-IV,[21] is difficult to assess in patients with FTD as they show limited insight and do not perceive their behaviour as irrational or excessive, especially after the early stage of their disease.

Computed tomography (CT) and magnetic resonance imaging (MRI) scans were evaluated by two reviewers blinded to clinical symptoms and family history. Frontotemporal atrophy on CT and/or MRI was present in 87 patients (31 men and 56 women). The remaining three patients did not have any atrophy on structural imaging, but showed anterior hypoperfusion on single-photon emission computed tomography (SPECT) scanning and were not included in further analyses. The extent of regional cortical atrophy in frontal, temporal, parietal, and occipital lobes of both hemispheres was semiquantitatively rated according to the four-point scale of Scheltens[22] into no, mild, moderate, and severe atrophy. The severity of atrophy was defined as the score of the region which was most severely affected (no sum-score). A distinction was made between patients with predominance of temporal atrophy (temporal atrophy more severe than frontal atrophy), and patients with frontal or frontotemporal atrophy. Left-right asymmetry was considered to be present if there was at least one grade difference between analogous regions of the left and right hemisphere (Figure 1). Caudate atrophy and ventricular enlargement were estimated by means of linear ratios: the bicaudate ratio (BCR), defined as the intercaudate distance divided by the distance between the inner tables of the skull at the intercaudate line, and the bifrontal ratio (BFR), defined as the bifrontal distance divided by the distance between the inner tables of the skull at the

bifrontal line.[23] Consecutive scans (with an interval of at least 6 months) were available in 34 patients.

The neuroradiological features of patients with and without CCB were compared using the Student's *t* or chi-square test, and Fisher's exact test was used when appropriate. The determinants of CCB were analysed by multiple logistic regression. The critical level for statistical significance was set at $p<0.05$ for all tests. In 20 patients both CT and MRI scans, made within a six month interval, were available. There was good agreement for assessment of the distribution of atrophy, as established by both CT and MRI (kappa value: 64%, $p<0.001$). The correlation between the linear ratios measured in CT and MRI was higher than +0.95 ($p<0.001$) for both measures, and a paired t-test revealed no statistically significant differences between the means. Therefore, both CT and MRI scans were used to determine the distribution of atrophy and to measure the linear ratios in this study, and no further distinction was made.

Figure 1. Neuroimaging examples.



A. T1-weighted Magnetic Resonance Imaging (MRI) of FTD patient with CCB showing asymmetric temporal atrophy: severe atrophy of the right temporal lobe (including the amygdala) and moderate atrophy of the left temporal lobe. B. MRI of a patient without CCB showing mild symmetric atrophy of the frontal lobes.

Table 1. Demographic data, compulsive features, and distribution of atrophy in patients with CCB

No and sex	Onset age	Interval onset-CT/MRI	Quality of complex compulsive behaviour*	Distribution of atrophy on neuroimaging
10M/8F	50.9±9.4 (33-65)	2.9±1.7 (0.7-7.5)	Preoccupation with certain ideas Health (5) Hyperreligiousity (1) Environment (2) Safety (2) Others (2) Preoccupation with single activities Jigsaws (2) Drawings (2) Others (7) Adherence to fixed time schedule (8) Parsimony (6) Arranging of belongings in particular order (4) Cleaning rituals (1)	Frontal ≥ temporal, symmetric (5) Frontal ≥ temporal, L>R (1) Temporal>frontal, symmetric (2) Temporal > frontal, L>R (5) Temporal>frontal, R>L (5)

* Most patients had more than one manifestation of complex compulsive behaviour. M=male, F= female, L=left, R=right.

Results

Complex compulsive behaviour

CCB was present in 18 (21%) patients with FTD. It often presented at an early stage of the disease and diminished in complexity with progression of the illness. CCB consisted of visual compulsive preoccupation in four patients: ritualistic completion of jigsaw puzzles in two patients, copying embroidery patterns in detail by one patient, and production of hundreds of identical drawings in another patient. Other manifestations of CCB were a preoccupation with certain ideas (health, religion, financial status and environment), and coercive behaviour directed at family members who did not adhere to these ideas. Extreme fixation to daily routines was also a common feature, as was repetitive checking behaviour (time, locks, clothing) and arranging of objects in a particular order. The demographic data, compulsive features, and distribution of atrophy of patients with CCB are listed in table 1. SBC, including both verbal and motor repetitions and utilisation behaviour, was present in 53 (61%) of patients.

The age at onset was significantly lower in patients with CCB: 50.9 ± 9.4 compared to 56.1 ± 7.7 years ($p=0.02$). Duration of illness, gender distribution, and the percentage of patients with a positive family history were similar in both groups. There was no difference in duration of symptoms at time of imaging between patients with and without CCB (2.9 ± 1.7 years for patients with CCB, and 3.2 ± 1.8 years for patients without CCB). The demographic and radiological features of patients with and without CCB are shown in Table 2.

Type of atrophy

Frontotemporal atrophy was seen in 64 patients (74%): mild in 23 (36%), moderate in 32 (50%), and severe in 9 (14%) patients. Temporal atrophy was seen in 23 patients (26%): mild in 9 (39%), moderate in 12 (52%), and severe in 2 (9%). The severity of atrophy did not differ between patients with frontotemporal and temporal atrophy ($p=0.8$). Patients with temporal atrophy never showed more than mild atrophy of the frontal lobes. Asymmetry of atrophy was present in 25 (29%) of patients, and was more frequent in patients with temporal atrophy ($p=0.004$). Patients with asymmetric atrophy showed more severe atrophy than patients with symmetric atrophy ($p=0.02$). The distribution of atrophy did not change over time in 31 out of 34 patients (91%) of whom consecutive scans were available. Both ventricular enlargement (BFR) and bicaudate atrophy (BCR) were most prominent in patients with frontotemporal atrophy ($p=0.014$ and $p=0.009$ respectively). The average duration of symptoms at time of neuroimaging was 3.1 ± 1.8 years. The distribution and severity of atrophy are shown in Table 3.

Correlation between atrophy and compulsive behaviour

CCB was associated with atrophy of the temporal lobe: 12 out of 18 patients (67%) with CCB had temporal atrophy, compared to 11 of 69 patients (16%) without CCB ($p<0.001$). Asymmetry of atrophy was also associated with CCB: 11 of 18 patients (61%) with CCB had asymmetric atrophy, compared to 14 of 69 patients (20%) without CCB ($p=0.001$). The left-right distribution of atrophy was not associated with the occurrence of CCB, and there were no differences between the two groups in severity of atrophy, caudate atrophy (as estimated by the BCR), or ventricular enlargement (BFR). SCB was present in 53 (61%) patients: 9 (17%) with predominant temporal atrophy, and 44 (83%) with frontotemporal atrophy, which did not differ from patients without SCB. Although patients with SCB tended to have a more caudate atrophy (mean BCR: 0.25) than patients without (mean BCR: 0.23), this difference did not reach significance ($p=0.1$).

Table 2. Demographic data and imaging features of patients with and without CCB*

	CCB (n=18)	no CCB (n=69)
Demographic data:		
Age at onset (years ± SD)**	50.9±9.4	56.1±7.7
Duration of illness (years)	4.3±2.3	5.2±3.0
Men/women (number)	10/8	21/48
Positive family history	6 (33%)	28 (41%)
Neuroimaging features:		
Frontotemporal distribution:**		
- Frontotemporal atrophy	6 (33%)	58 (84%)
- Temporal atrophy	12 (67%)	11 (16%)
Asymmetry*	11 (61%)	14 (20%)
Left/right sided atrophy	6/5	6/8
Moderate to severe atrophy	12 (67%)	43 (62%)
BCR (mean)	0.23	0.25

* The remaining three patients had normal structural imaging.

** significant difference ($p<0.05$).

Table 3. Distribution and severity of atrophy in total patient population

	Mild	Moderate	Severe
Temporal:			
- asymmetric	2 (17%)	8 (66%)	2 (17%)
- symmetric	7 (64%)	4 (36%)	0
Frontotemporal:			
- asymmetric	2 (15%)	7 (54%)	4 (31%)
- symmetric	21 (41%)	25 (49%)	5 (10%)

Logistic regression analysis showed that both temporal atrophy (p=0.004), as well as asymmetric atrophy (p=0.02) were significantly and independently associated with CCB. Patients with temporal atrophy had a 7.1 (95% confidence interval (CI): 2.0 – 25.7) fold increased risk of developing CCB, and patients with asymmetry of atrophy a 5.4 (95% CI: 1.4 – 20.6) fold increased risk. Multivariate logistic regression analysis with age at onset, duration of the symptoms at the time of imaging, gender, severity of cortical atrophy, BCR, and BFR as covariates, showed that only age at onset had an additional significant contribution to the model (p=0.03, odds ratio 0.9, 95% CI: 0.8 - 1.0). However, since age at onset did not have an impact on the odds ratios for temporal and asymmetric atrophy, it was not a confounder.

Discussion

This study describes the occurrence of complex compulsive behaviour (CCB) in 21% of patients from a large population-based study of 90 patients with FTD. Visual compulsive behaviour, preoccupation with certain ideas, repetitive checking, counting, and extreme fixation to specific activities and times are manifestations of this behaviour. CCB was independently associated with both temporal atrophy and asymmetric atrophy, after adjusting for age at onset, duration of symptoms at time of imaging, and severity of atrophy. The caudate nuclei did not appear to be involved in the development of CCB in our patients.

This study confirms that CCB is a common feature in patients with FTD, which differs from observations in Alzheimer's disease patients.[9] CCB often occurs at the initial stage

of the disease and is followed by more elementary motor or verbal repetitions at a later phase. The higher frequencies of repetitive behaviour of up to 80% reported by some other investigators are probably due to the inclusion of these simple repetitions and utilisation behaviour in their studies.[24,25] It will be interesting to investigate whether the manifestations of CCB in FTD differ qualitatively from compulsive behaviour observed in idiopathic and acquired obsessive-compulsive disorders. It has been suggested that serotonin may play a role in the aetiology of idiopathic obsessive-compulsive disorders, because of the anti-obsessional effect of selective serotonin reuptake inhibitors (SSRIs).[26] That serotonin may also be involved in the pathogenesis of behavioural disturbances in patients with FTD is supported by the finding of reduced levels of post-synaptic serotonin in post-mortem studies of FTD patients.[27,28] Furthermore, a partial treatment response to SSRIs was found in a small sample of FTD patients with behavioural symptoms which included compulsions,[29] although further studies are needed to verify this effect.

Isolated atrophy of the left temporal lobe was described for the first time by Arnold Pick in a patient with a progressive language disorder and dementia.[30] The profile of language deficits in FTD patients has more recently been denoted as primary progressive aphasia and semantic dementia.[31,32] The frequency of temporal atrophy in the present study (26%) is similar to that in a large review of cases of Pick's disease (17%) from literature.[33] The asymmetric pattern of temporal atrophy, in the present as well as in other studies, is a distinctive feature of FTD compared to Alzheimer's disease and other neurodegenerative conditions, and may determine the clinical picture of FTD.[25,33-35] Right-sided temporal atrophy has been associated with characteristic psychiatric symptoms, such as psychosis, mania, and bizarre affect,[2,4] whereas emergence of artistic (musical) talent can be a clinical manifestation of left temporal lobe involvement.[6,7]

Temporal atrophy on neuroimaging is significantly correlated with CCB in the present study, and has also been mentioned in association with striatal atrophy in a number of FTD cases with compulsions by Snowden *et al.*[8] It is well known that structural and functional changes of striatum (caudate nucleus and globus pallidus) are associated with repetitive behaviour,[11,13,15,36-38] in analogy to findings in Gilles de la Tourette's disease and Huntington's disease.[39] However, several case reports have suggested that frontal and temporal regions may contribute independently to the development of compulsive behaviour, based on the presence of isolated frontal or temporal lesions (infarctions, arachnoid cysts) or epilepsy in patients with acquired obsessive-compulsive disorders.[12,14,36,40,41] A possible mechanism might be that these lesions disrupt the frontotemporal-limbic-subcortical nuclei circuitry involved in the suppression of

compulsive thoughts and behaviour in normal subjects.[12,14] However, we do not have an explanation for the association between asymmetric temporal atrophy and CCB. Both left and right temporal lobes were equally often affected, and no difference in the quality of CCB was observed in patients with left and right sided pathology.

In the present study, CCB was not associated with caudate atrophy. Our findings are consistent with observations of Snowden *et al.* of a small number of FTD patients that temporal lobe disease appears to be associated with more complex compulsive behaviour, whereas severe striatal disease is linked to more elementary compulsive behaviour.[8] It is possible that the amygdala, located in the medial temporal lobe, is involved in the development of CCB in our patients with temporal atrophy. Recent findings of reduced volumes and increased metabolism of limbic structures, including the amygdala, in patients with idiopathic obsessive-compulsive disorder, support this hypothesis.[12,42]

One limitation of the present study is the lack of a formal and quantitative assessment of CCB by means of questionnaires.[43,44] However, lack of insight of patients with FTD prevents them from describing distress and anxiety associated with their compulsions. Therefore, conventional questionnaires are not useful in assessment of severity of CCB in FTD. Another limitation of this study is that quantitative volumetry of temporal lobes and adjacent limbic structures could not be carried out owing to unavailability of coronal MRI in most patients. However, a recent study showed that visual assessment of medial temporal lobe atrophy is both quicker and more accurate than volumetry in differentiating Alzheimer's disease patients from controls.[45,46] In analogy, we suggest that visual assessment of cortical atrophy, if performed by a single rater,[22] is both reliable and as accurate as volumetry. Furthermore, it has been shown that the BCR is a good indicator of caudate atrophy,[23] although volumetric measurements will be needed in future studies of FTD patients with CCB to exclude involvement of the caudate nuclei.

In summary, we have observed CCB in 21% of our FTD patients. Most patients with CCB have asymmetric temporal atrophy, and logistic regression analysis confirmed that both temporal atrophy and (to a lesser extent) asymmetric atrophy were independently associated with the risk of developing CCB. Limbic structures of the temporal lobe, for example the amygdala, might be involved in the inhibition of compulsive impulses, leading to compulsive behaviour when damaged. Elementary motor and verbal compulsions (SCB) were not associated with temporal atrophy.

Future quantitative neuroimaging and functional MRI studies in FTD patients will help clarify the distinct contributions of the different anatomical regions to the development of CCB.

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Chapter
3.2

Apolipoprotein E4 in the temporal variant of frontotemporal dementia

A letter

Although the apolipoprotein E4 (apoE4) allele has consistently been associated with Alzheimer's disease and other types of dementia in many studies,[1] its association with frontotemporal dementia (FTD) is controversial. After our report in 1997 of increased apoE4 allele frequencies in sporadic FTD and its effect on the age at onset,[2] other studies of cases of FTD with pathological confirmation or *tau* mutations did not confirm this effect.[3-5] However, recently it has been shown that semantic dementia, the temporal variant of FTD, may be associated with higher frequencies of the apoE4 allele.[6] Therefore, we have genotyped apoE in our expanded FTD patient population and have assessed whether patients with predominance of temporal atrophy have higher frequencies of the apoE4 allele.

Methods

Patients were ascertained through a clinico-epidemiological survey of patients with FTD in the Netherlands.[2] We identified 111 patients with the diagnosis probable FTD, established according to the Lund and Manchester criteria. Thirteen of the patients had an autosomal dominant form (defined as at least three affected family members in two generations) of FTD, with *tau* mutations identified in 10 (P301L, G272V, R406W and ΔK280), and were excluded from further analyses. Predominant temporal atrophy, semiquantitatively assessed on CT and/or MRI, was found in 31 (32%) patients, whereas frontal atrophy with or without temporal atrophy was present in 67 (68%) patients. Nine of the 31 patients (29%) with temporal atrophy fulfilled the criteria for semantic dementia, and four patients (13%) showed severe problems in language comprehension, although the diagnosis semantic dementia could not be definitely established due to incomplete or inconclusive neuropsychological testing. The remaining 18 patients (58%) showed mainly decreased spontaneous speech and wordfinding difficulties. The clinical diagnosis of FTD was pathologically confirmed in all 17 patients who came to autopsy (five of whom had predominant temporal atrophy). Non-demented control subjects (n=561) were taken from the Rotterdam study.[7] All patients and controls were genotyped for the apoE allele as described by Slooter *et al.*[1] Both genotype frequencies and apoE4 allele frequencies were calculated for each group and compared with non-demented controls using a chi-square test.

Results

Six percent of the 98 patients with sporadic FTD had the apoE4/E4 genotype, compared with 2.3% of non-demented controls (p=0.04). This genotype was present in 9.7% of patients with the temporal variant of FTD (p=0.01, compared with non-demented controls), compared with only 4.5% in patients with frontotemporal atrophy (p=0.5). Genotype frequencies of heterozygote E4 (E4/*) and homozygote E4 (E4/E4) carriers are summarised in table 1. The frequency of the apoE4 allele in all patients with sporadic FTD was 21.9%, compared to 15.3% in the non-demented controls (p=0.02). In patients with temporal atrophy the apoE4 allele frequency was as high as 29.0% (p=0.004), whereas in the patients with frontotemporal atrophy only 18.7% (p=0.3) of alleles was apoE4. No association between ApoE4 and the age at onset, nor the duration of symptoms, was found in the overall group, nor in the subgroups.

Table 1. Frequency of apoE genotypes and E4 alleles in different groups

Group	Patients	Genotype (%)†			Alleles	
		E4/E4	E4/*	No E4	%E4	P-value
Non-demented controls	561	2.3	26.0	71.7	15.3	ref.
Sporadic FTD	98	6.1	31.6	62.3	21.9	0.02
- Temporal atrophy	31	9.7	38.7	51.6	29.0	0.004
- Frontotemporal atrophy	67	4.5	28.4	67.1	18.7	0.3

†: E4/E4 = E4 homozygotes, E4/* = E4 heterozygotes, No E4 = all other genotypes.

Conclusion

Our results show that the apoE4 allele frequency is increased in patients with the temporal variant of FTD compared with non-demented controls. Although a biological hypothesis justifying such an association is still lacking, the effect of the apoE4 allele on the predominance of temporal atrophy compared with frontal atrophy has also been observed in patients with Alzheimer's disease.[8] To verify the association between the

apoE4 allele and the temporal variant of FTD, a large study with pathological confirmation of the clinical diagnosis of FTD is required to exclude admixture of patients with Alzheimer's disease. However, in all 17 patients who were autopsied in our series, including five patients with temporal atrophy, the clinical diagnosis was neuropathologically confirmed. This shows that the clinical criteria according to the Lund and Manchester groups, when combined with neuroimaging and psychometric evaluation, are highly accurate. We conclude that the association we previously found between the apoE4 allele and sporadic FTD may be due to a selective increase of this allele in patients with the temporal variant of FTD.

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4

The *tau* gene in hereditary FTD

Chapter
4.1

A novel *tau* mutation (S320F) causes a tauopathy with inclusions similar to those in Pick's disease

Abstract

Mutations in the *tau* gene cause familial frontotemporal dementia and parkinsonism linked to chromosome17. In this article, we describe a novel missense mutation, S320F, in the *tau* gene in a family with presenile dementia. To our knowledge, it is the first mutation to be described in exon 11 of *tau*. The proband died at age 53, after a disease duration of 15 years, and autopsy revealed a neuropathological picture similar to Pick's disease. Recombinant tau protein with the S320F mutation showed a greatly reduced ability to promote microtubule assembly, suggesting that this may be the primary effect of the mutation.

The identification of different types of mutations in the *tau* gene in familial frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17), and the association of these mutations with a spectrum of filamentous tau pathology, has established the important role of the *tau* gene in causing neurodegeneration.[1-3] The primary effect of intronic and some coding region mutations in exon 10 is at the mRNA level, resulting in a change in ratio of 3- to 4-repeat tau isoforms. By contrast, most missense mutations reduce the ability of mutant tau to interact with microtubules and other molecules, and some also stimulate the *in vitro* assembly of tau into filaments.

In this article, we report a novel missense mutation (S320F) in *tau* in a family with presenile dementia. It constitutes the first known mutation in exon 11 of *tau*. Experimentally, the S320F mutation resulted in a markedly reduced ability of tau to promote microtubule assembly.

Subject and Methods

Clinical history of the proband

The proband, a travelling salesman, presented at age 38 with complaints of mild memory problems and spatial disorientation. Neuropsychological examination, computerised tomography and electroencephalography, were normal at this time. Nine years later, at age 47, memory problems and naming difficulties had evidently worsened. Furthermore, he had become introverted, mentally inflexible, and disinterested. Psychometric evaluation revealed fluent aphasia, word finding difficulties, impairment of comprehension, and abstract thinking. Extrapyramidal signs and motor neurone disease were absent. Magnetic resonance imaging of the brain showed moderate bilateral temporal atrophy. The patient died at age 53 years. The proband's mother also died with a similar dementing illness at age 53 years. Neither her parents (ages at death, 57 and 90 years), nor any of her seven siblings were reported to have developed dementia.

Immunohistochemistry

Immunohistochemistry with phosphorylation-dependent (AT8, AT180, AT270, PHF1, MC1, and 12E8 [1:500, donated by P. Seubert, Elan Pharmaceuticals, San Francisco, Ca]), and phosphorylation-independent tau antibodies (BR01, Tau 2) was performed, as well as with antibodies directed against ubiquitin, β-amyloid, α-synuclein, and αB-crystallin, as described previously.[4]

DNA extraction and mutational analysis

Genomic DNA of the proband was extracted, and exons 9 to 13 of *tau* were amplified and sequenced as described.[5] Exon 11 of *tau* was also sequenced from the genomic DNA of a healthy maternal uncle of the proband (aged 84), as well as 50 control individuals.

Tau extraction, immunoblotting and electron microscopy

Sarkosyl-soluble and -insoluble tau was extracted, dephosphorylated, and analysed as described previously,[6] and incubated with BR01 tau antibody (1:2,000). The ratio of soluble 3- to 4-repeat tau was assessed using Image Master 1D elite software (Amersham Pharmacia Biotech, United Kingdom). Dispersed filaments from the sarkosyl-insoluble fraction were processed for electron microscopy and immunolabelled with tau-antibodies BR01 and AT8, as described previously.[6]

Microtubule assembly

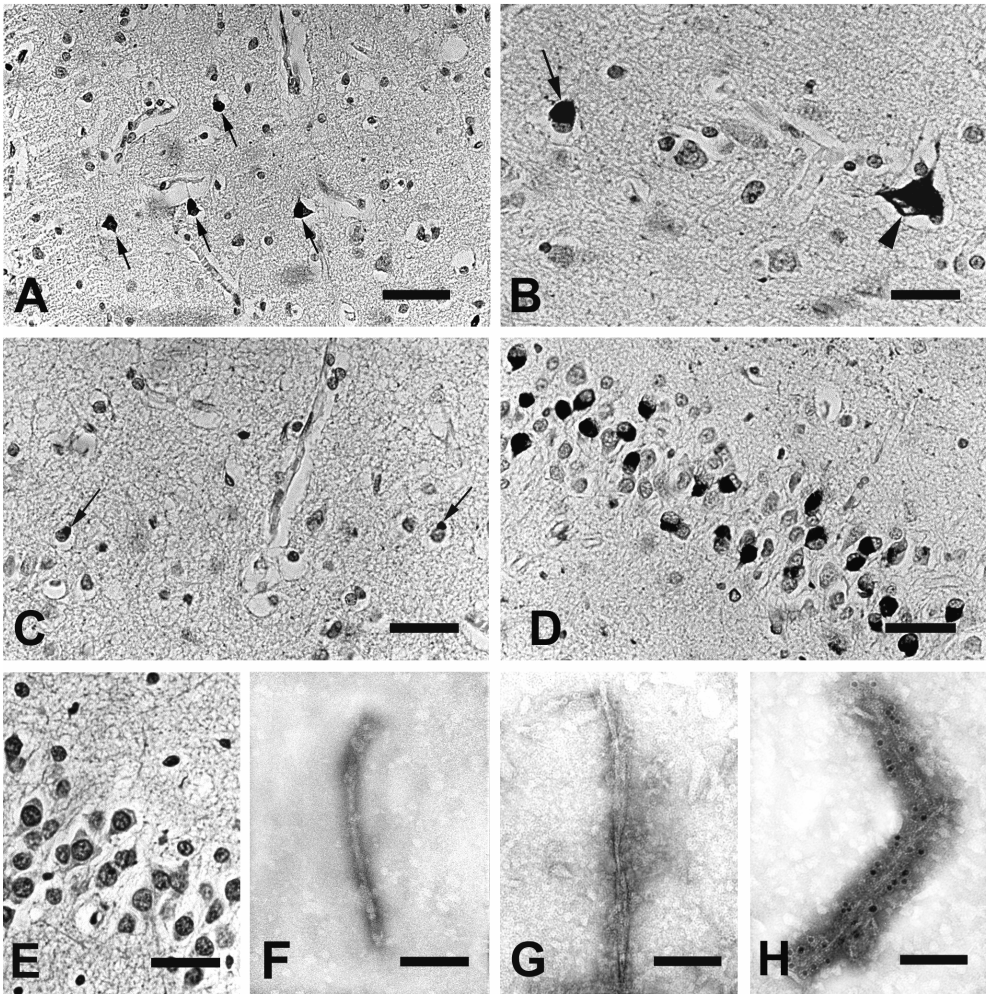
Site-directed mutagenesis was used to change S320 to phenylalanine in the 3-repeat 381 and 4-repeat 412 amino acid isoforms of human tau (numbering of 441 amino acid isoform of human tau), expressed from cDNA clones htau37 and htau46, respectively. Wild-type and mutant tau proteins were expressed in *Escherichia coli* BL21(DE3), purified, and incubated with bovine brain tubulin as described previously.[7] Assembly into microtubules was monitored over time by change in turbidity at 350 nm.

Results

Sequencing of the proband's genomic DNA showed a C to T transition in exon 11 at the second base position of codon 320 (TCC to TTC), which results in the substitution of serine by phenylalanine (S320F). This change was not observed in the healthy maternal uncle of the proband or in 100 control chromosomes.

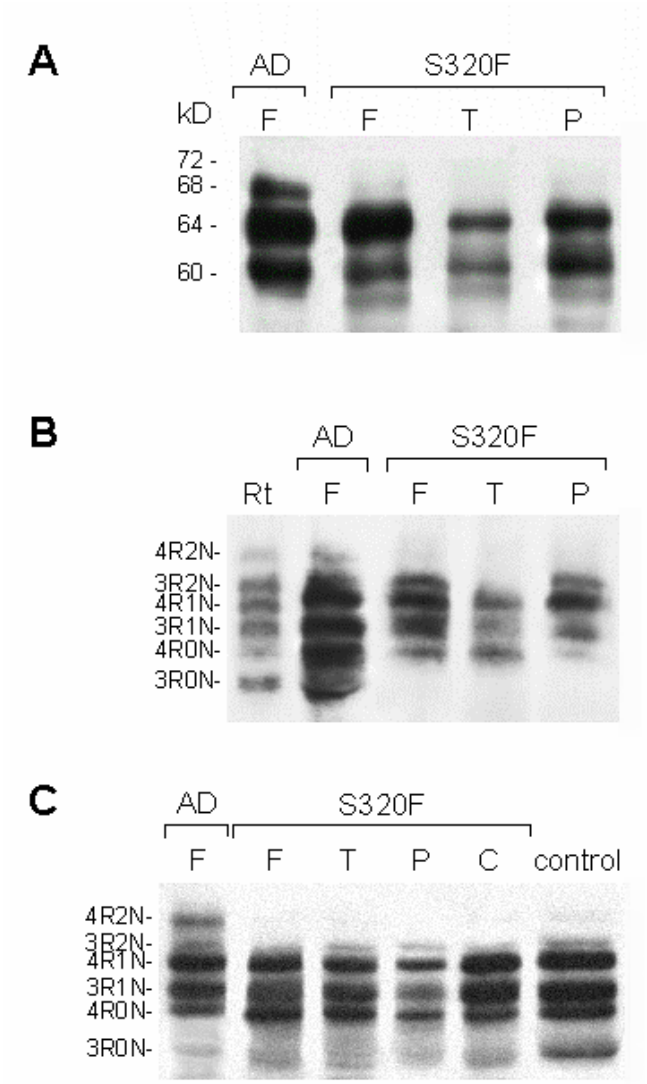
At autopsy, the proband's brain (weight 1,200 g) showed focal bilateral atrophy of the anterior temporal lobes, with only very mild frontal atrophy. Severe neuronal loss and gliosis were present in the temporal cortex, cingulate gyrus, entorhinal cortex, and hippocampus. The substantia nigra was not affected. A few Pick cells were seen in the temporal cortex. Bodian silver staining did not show any Pick bodies or neurofibrillary tangles. Immunohistochemical staining showed extensive tau pathology in the form of Pick-like bodies and more diffuse cytoplasmic staining in neurons of the frontal, temporal, and parietal cortices; the dentate gyrus of the hippocampus; the amygdala; and the ventral striatum (Fig. 1A-E).

Figure 1. Neuropathological findings in the proband's brain



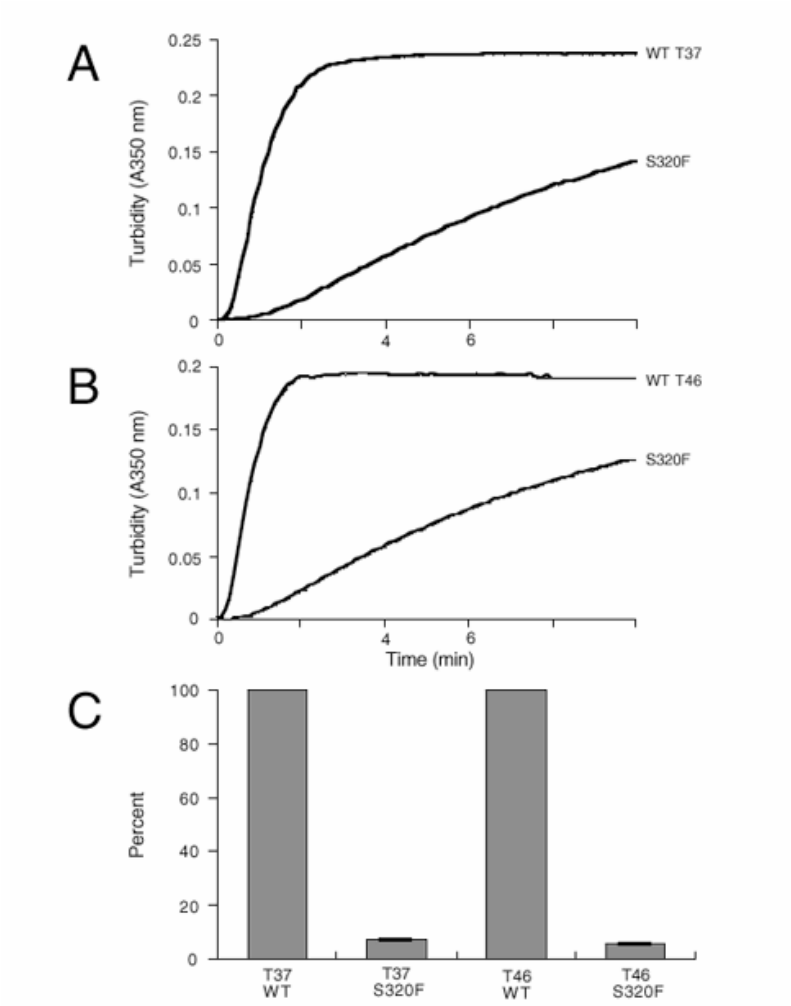
(A,B) Immunostaining of frontal cortex with the phosphorylation-dependent anti-tau antibody AT8 shows multiple tau-positive inclusions (A), some of which resemble Pick bodies (arrow in B), while others show a more diffuse staining of the cytoplasm (arrowhead in B). (C) A small number of AT8-positive glial cells is seen in the frontal cortex. (D,E) The granule cells of the dentate gyrus of the hippocampus contain numerous inclusions resembling Pick bodies that are immunoreactive with AT8 (D), but not with the phosphorylation-dependent anti-tau antibody 12E8 (E). (F-H) Electron micrographs of tau filaments isolated from the proband's brain show unlabelled straight (F) and twisted (G) filaments, as well as a twisted filament labelled by AT8 (H). Scale bars: 150 μm (A), 100 μm (B-E) and 80 nm (F-H).

Figure 2. Sarkosyl-insoluble tau and soluble tau from the proband's brain.



(A,B) Immunoblot of sarkosyl-insoluble tau before (A) and after (B) alkaline phosphatase treatment. (C) Immunoblot of soluble tau after alkaline phosphatase treatment. Immunoblotting was done using the phosphorylation-independent anti-tau antibody BR01. F = Frontal cortex; T = Temporal cortex; P = Parietal cortex; C = Cerebellum; AD = Alzheimer's disease, Rt = recombinant tau.

Figure 3. Effects of the S320F mutation on the ability of three-repeat htau37 (381 amino acid isoform of human tau) and four-repeat htau46 (412 amino acid isoform of human tau) to promote microtubule assembly



A. Polymerisation of tubulin induced by wild-type htau37 and htau37S320F. B. Polymerisation of tubulin induced by wild-type htau46 and htau46S320F. Microtubule assembly was monitored over time by turbidimetry. C. Optical densities for wild-type and mutant htau37 and htau46 at 2 minutes (expressed as percentages of wild-type htau37 and htau46 taken as 100%). Each results is expressed as the mean ± the standard error of the mean (n = 5).

The Pick-like bodies were immunoreactive with all anti-tau antibodies tested, with the exception of antibody 12E8. A few glial cells, probably oligodendrocytes, in affected regions also contained tau-positive inclusions. Staining with β-amyloid and α-synuclein was negative.

By immunoblotting, sarkosyl-insoluble tau ran as two major bands of 60 and 64 kDa (Fig. 2A). Following dephosphorylation, these bands resolved into four bands that aligned with human tau isoforms 4R0N, 3R1N, 4R1N, and 3R2N, except in the temporal cortex, where the 3R2N band was not observed (Fig. 2B). Following dephosphorylation, soluble tau gave a pattern similar to that seen in Alzheimer's disease (AD; Fig. 2C), with a ratio of 3- to 4-repeat tau isoforms of 0.92 versus 1.01 in the control brain.

Electron microscopy of preparations of sarkosyl-insoluble filaments showed filaments with two distinct morphologies (Fig. 1F,G). The major species (approximately 80% of filaments) was a straight filament, very similar to the filaments seen in AD brain. The minor species (approximately 20%) was an irregularly twisted filament with a cross-over spacing of 110-160 nm and a diameter of 6-8 nm in its narrow part. Both types of filament were decorated by BR01 and AT8 antibodies (Fig. 1H).

Recombinant 3-repeat htau37 and 4-repeat htau46 with the S320F mutation showed a markedly reduced ability to promote microtubule assembly when compared with the corresponding wild-type proteins (Fig. 3A, B). Thus, the S320F mutation led to a 90 to 95% reduction in the rates of microtubule assembly when expressed as the optical density at 2 minutes (Fig. 3C).

Discussion

This study describes a novel mutation in exon 11 of the *tau* gene in a patient with presenile dementia. S320F is the first mutation to be described in exon 11 of *tau*. The initial clinical diagnosis was AD, but neuropathological findings closely resembled Pick's disease (PiD). The inclusions in S320F brain were similar to those described in sporadic PiD[8] and in some other cases with *tau* mutations,[9-14] except that they were undetectable with Bodian silver staining. They were immunoreactive with all anti-tau antibodies used, with the exception of antibody 12E8, which recognises tau phosphorylated at S262, or S356, or both. This 12E8-negative staining of Pick bodies has also been described in sporadic PiD,[15] and in K257T, G272V, and K369I mutations,[9,11,14] indicating that these

epitopes are not substantially hyperphosphorylated in most cases with Pick-like pathology. However, the 12E8-positive staining in G389R mutation suggests that nonphosphorylation of these sites is not required for the formation of Pick bodies.[10]

Sarkosyl-insoluble tau extracted from S320F brain resolved into two major bands of 60 and 64 kDa, like the pattern seen in sporadic PiD,[8] and in K257T and G389R mutations.[10,11,13] However, following dephosphorylation, the normally abundant band corresponding to tau isoform 3R0N was missing. Four major bands aligning with isoforms 4R0N, 3R1N, 4R1N, and 3R2N were observed instead. As the 60 kDa band corresponds to the 3R0N tau isoform in AD brain,[6] the presence of a 60 kDa band in the absence of 3R0N tau in the case described herein implies that the isoform composition of sarkosyl-insoluble tau differed from that of AD. Previously, unexpected tau isoform patterns have been observed in the E342V mutation and in one of two families with a G389R mutation.[12,13] As in the present case, it remains to be seen whether these patterns are a direct and general result of the *tau* mutations, or whether they are limited to the individual cases within each family studied so far. Soluble tau from S320F brain consisted of all 6 isoforms, similar to that seen in AD and other missense mutations in *tau*. The two distinct filament morphologies, straight and twisted, have also been described in some cases of sporadic PiD and in some other cases with *tau* mutations and a Pick-like phenotype.[8,10]

The S320F mutation is located within the highly conserved third microtubule-binding domain of tau. A serine residue is found at this position in all known tau sequences, as well as in related proteins MAP2 and MAP4. Accordingly, recombinant tau with the S320F mutation showed a greatly reduced ability to promote microtubule assembly, suggesting that this may be its primary effect. It is conceivable that this mutation has additional effects. It is located within the core region of the paired helical filament of AD, two residues amino-terminal of C322, which is known to be required for the dimerisation of tau.[17,18] The S320F mutation removes a potential phosphorylation site in tau. *In vitro* studies have shown that microtubule-affinity regulating kinase, protein kinase N, and cyclic adenosine monophosphate-dependent protein kinase (in presence of heparin) can phosphorylate S320.[19,20] It has even been suggested that phosphorylation of this site may inhibit the assembly of tau protein into filaments.[19] However, at present, there is no evidence to suggest that S320 is phosphorylated in either normal or pathological tau *in vivo*.

In conclusion, the present study describes a novel *tau* mutation that causes a syndrome similar to Pick's disease. It further underlines the relevance of tau protein dysfunction in the aetiology and pathogenesis of frontotemporal dementia in general, and Pick's disease in particular.

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Chapter
4.2

Variable phenotypic expression and extensive tau pathology in two families with a novel *tau* mutation L315R

Abstract

Mutations in the *tau* gene cause familial frontotemporal dementia and parkinsonism linked to chromosome 17. Here we describe two Dutch families with frontotemporal dementia and the novel L315R mutation in exon 11 of *tau*. Both families showed a large variation in disease expression, which ranged from an age of onset of 25 years to no disease in a 82-year-old carrier. In affected individuals, extensive tau pathology was present in nerve cells (Pick-like inclusions) and astroglial cells, particularly in frontotemporal cortex and hippocampal formation. Sarkosyl-insoluble tau extracted from cerebral cortex showed the presence of straight and twisted tau filaments and a pattern of pathological tau bands similar to that of Pick's disease. Upon dephosphorylation, only five of the six brain tau isoforms were observed, with the shortest isoform being undetectable. All six tau isoforms were present in soluble brain tau. Recombinant tau proteins with the L315R mutation showed a reduced ability to promote microtubule assembly.

Frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17) is a neurodegenerative disease caused by mutations in *tau*.[1-3] Where examined, these mutations lead to the assembly of the normally soluble tau protein into abnormal filaments.[4-6] It follows that the pathway leading from soluble to filamentous tau protein is central to FTDP-17. Tau filaments are also characteristic of other neurodegenerative diseases, such as Alzheimer's disease (AD), progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), Pick's disease (PiD), argyrophilic grain disease and the amyotrophic lateral sclerosis-Parkinsonism-dementia complex. The study of FTDP-17 continues to throw light on these more common diseases, as exemplified by a clinical picture of PSP in cases with the recently identified R5L and ΔN296 mutations in *tau*.[7,8]

Six tau isoforms are produced in the adult human brain by alternative mRNA splicing from a single gene.[9] They differ from each other by the presence or absence of 29- or 58-amino acid inserts located in the amino-terminal half, and an additional 31-amino acid repeat located in the carboxy-terminal half. Inclusion of the latter, which is encoded by exon 10, gives rise to the three isoforms with 4 repeats (4R) each; the other three isoforms have 3 repeats (3R) each. Similar levels of 3R and 4R tau isoforms are found in normal cerebral cortex.[10] The repeats constitute the microtubule-binding domains of tau.[11,12]

Known mutations in *tau* are either missense, deletion or silent mutations in the coding region, or intronic mutations located close to the splice-donor site of the intron following exon 10. Most coding region mutations are located in the microtubule-binding repeat region or close to it and reduce the ability of mutant tau to promote microtubule assembly.[13,14] Some of these mutations also stimulate the assembly of tau into filaments *in vitro* and lead to a reduced ability of mutant tau to bind to protein phosphatase 2A.[15-17] The intronic mutations and some exon 10 mutations have their primary effect at the RNA level and change the ratio of 3R to 4R tau. Where examined, this leads to the relative overproduction of 4R tau in human brain.

Neuropathologically, filamentous tau deposits in nerve cells are characteristic of FTDP-17.[4-6] In addition, tau inclusions are also observed in glial cells in cases with mutations in exon 10 or in the intron following exon 10. The presence of both neuronal and glial tau inclusions thus correlates with either the expression of mutant 4R tau or the relative overproduction of 4R tau. By contrast, the presence of tau inclusions in nerve cells correlates with the expression of mutant 3R and 4R tau. However, the recently described mutations (R5H and R5L) in exon 1 of *tau* do not appear to fit into this scheme, since

they lead to a neuronal and glial tau pathology, despite all six tau isoforms being mutated.[7,18]

Here, we report a novel missense mutation (L315R) in exon 11 of *tau* in two families with frontotemporal dementia. At autopsy, clinically affected individuals showed a severe neuronal and glial tau pathology. However, the penetrance of this mutation was incomplete, since some individuals with the mutation failed to develop clinical symptoms. The L315R mutation reduced the ability of recombinant tau proteins to promote microtubule assembly, while it had no significant effect on heparin-induced assembly of tau into filaments.

Patient and methods

Case reports

The first five-generation family (Table 1 and Figure 1A) consisted of four affected members, and originated from a genetically isolated part of the Netherlands. Three patients (A V:1, A V:2, A V:3) presented with behavioural changes, memory problems or word-findings difficulties at ages 53, 55 and 56 years respectively. The mother of patient A V:1 (A IV:1) also suffered from a similar dementing illness and died at age 61. Perseverations, impaired executive function, and decreased attention and abstraction were found at neuropsychological testing in both of the tested patients (A V:1, A V:3). Neuroimaging showed asymmetric temporal atrophy in two patients (A V:2, A V:3), and frontotemporal atrophy in one patient (A V:1). Autopsy was performed in patient A V:1, who died after a disease duration of 8 years. The mother of two affected sibs (A IV:4) was cognitively normal at 82 years of age and CT-scanning did not reveal any abnormalities.

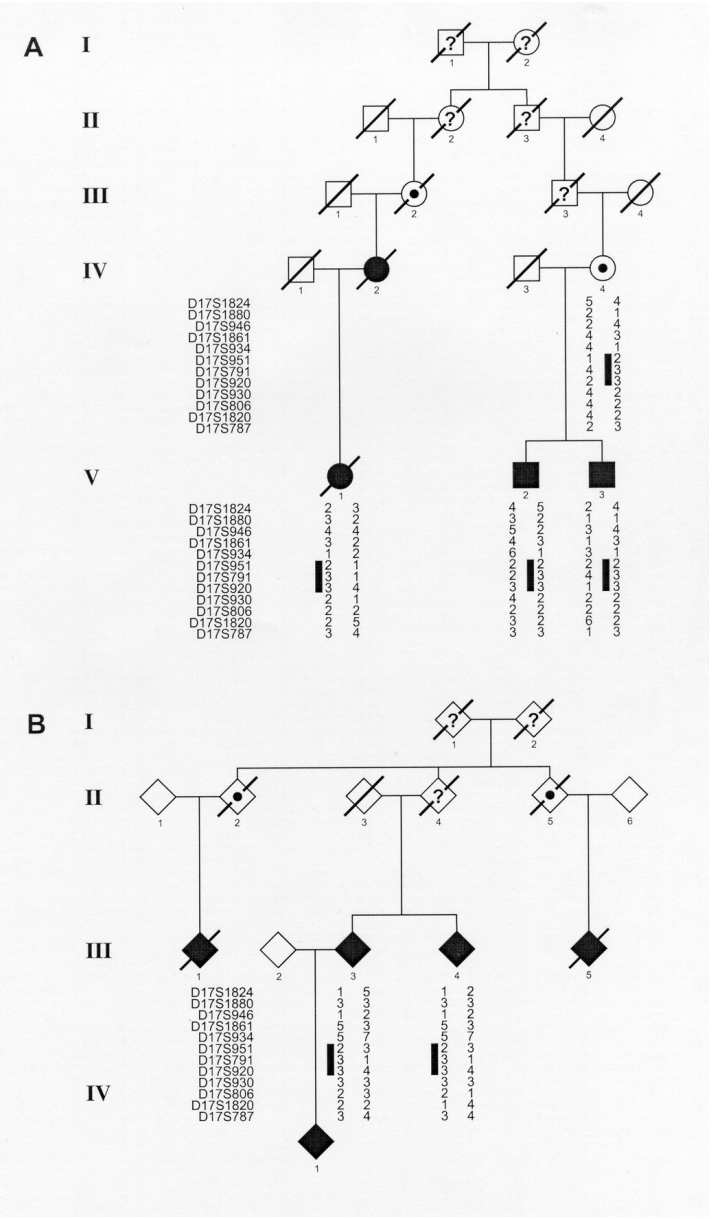
The second four-generation family (Table 1 and Figure 1B) came from the same area as the ancestors of the first family, and consisted of five affected family members. Two patients (B III:3, B III:4) had frontal lobe symptoms at similar age (59 and 64 years) as the affected members of the first family, whereas three other patients (B III:1, B III:5, and IV:1) had a much earlier onset (25, 29 and 39 years). Depression with suicidal tendencies and apathy were the prominent features in patient B III:3. Patient B III:5 was not able to complete primary school (mental retardation) and developed word finding problems, dysarthria and apathy from the age of 25 years and died at the age of 33 years. The parent of this patient (B II:5), according to the pedigree presumably the mutation carrier, died at age 70 without signs of dementia. The clinical presentation in patient B IV:1 consisted of impaired comprehension of language and depression, starting at the age of 39.

Table 1. Overview of clinical features and neuropsychological examination in families A and B

Patient	Age at onset	Age at death	Current age	Presenting symptoms	Neuropsychological findings	Atrophy on neuro-imaging
A IV:2	59	61*	-	NA	NA	NA
A V:1	55	63**	-	word finding problems, restless, interest ↓	executive functions, attention ↓	frontotemporal R > L
A V:2	53	-	57	memory ↓, hyperorality, disinhibition	NA	temporal R
A V:3	56	-	56	behavioural changes and memory problems	perseverations, loss of insight executive functions ↓	temporal R
B III:1	29	34	-	NA	NA	NA
B III:3	64	-	66	depression, apathy	perseverations	frontotemporal
B III:4	56	-	59	word finding problems, loss of insight , compulsive behaviour	memory, executive functions ↓ perseverations, loss of insight	temporal L
B III:5	25	33**	-	word finding problems	spontaneous speech ↓ motor stereotypes	generalised
B IV:1	39	-	41	depression, semantic difficulties	memory ↓ frontal signs	temporal

* Cause of death is not end stage dementia, ** Autopsy of the brain was performed and material was available for further experiments. NA = not available.

Figure 1. Pedigrees of the two families with haplotypes for the FTDP-17 region



Black bars indicate the shared alleles. Markers were obtained and ordered according to the Marshfield integrated linkage map and the April 2001 physical assembly of the Human Genome (http://genome.uc-sc.edu).

The pattern of atrophy on neuroimaging in all patients was temporal, frontotemporal or generalised. Little information is available on patient B III:1; at age 34 he died with a history of general brain atrophy. Individual B II:2, a possible carrier of the mutation according to the pedigree, died at age 85 without signs of dementia (see Table 1 for overview of patients and symptoms).

Tau sequencing

Genomic DNA of the patients was extracted and the *tau* exons were amplified and sequenced as described.[19] Restriction enzyme digestion was used to investigate the presence of the base change in exon 11 in 100 control chromosomes. *HpaII* digests specifically the mutant allele of the exon 11 PCR product, whereas *DdeI* digests specifically the normal allele. Enzymes were used according to the manufacturer's (InVitrogen) instructions.

Tau haplotype analysis

For the analysis we typed polymorphic short tandem repeat markers linked to the FTDP-17 locus: D17S1824, D17S1880, D17S946, D17S1861, D17S934, D17S951, D17S791, D17S920, D17S930, D17S806, D17S1820, D17S787. The markers were obtained and ordered according to the Marshfield integrated linkage map and the April 2001 physical assembly of the Human Genome (http://genome.uc-sc.edu). Fluorescently labelled markers were used as specified by the manufacturer and analysed using an ABI3100 automated sequencer with Genemapper 2.0 software (Applied Biosystems, Foster City, CA).

Histology and immunohistochemistry

Representative blocks through the brains of patients A V:1 and B III:5 were processed for histology and immunohistochemistry. Sections (4 μm) were cut and stained with hematoxylin and eosin (HE) and Bodian silver, or used for immunohistochemistry, as described.[20] Anti-tau antibodies consisted of the conformation-dependent, phosphorylation-independent antibody MC1 and the phosphorylation-dependent antibodies AT8, AT180, AT270, 12E8 and PHF1. MC1 (a kind gift from Dr Peter Davies, Albert Einstein College of Medicine, Bronx, USA) recognizes amino acids 7-9 and 312-322 of tau (in the numbering of the 441 amino acid isoform of human tau) and is sensitive to the conformation of tau. Antibodies AT8, AT180 and AT270 that recognize sites in tau phosphorylated, respectively, at S202/T205, T231 and T181 were obtained from Innogenetics (Ghent, Belgium). Antibody 12E8 (a kind gift from Dr P, Seubert, Elan Pharmaceuticals, San Francisco, USA) recognizes tau phosphorylated at

S262 and/or 356. Antibodies against α-synuclein, β-amyloid, ubiquitin and glial fibrillary acidic protein (GFAP) were also used as described.[20]

Tau protein analysis and immunoblotting

Soluble and sarkosyl-insoluble tau proteins were extracted from the cerebral cortex and cerebellum of patient A V:1, as described.[21] Tau proteins extracted from the frontal cortex of an Alzheimer's disease patient were used as a control. Dephosphorylation and immunoblotting using the phosphorylation-independent anti-tau antibody BR01 were done as described.[22]

Microtubule assembly

Site-directed mutagenesis was used to change L315 to arginine (in the numbering of the 441-amino acid isoform of human tau) in the 3R 381-amino acid isoform and in the 4R 412-amino acid isoform of human tau, expressed from cDNA clones htau37 and htau46, respectively. Wild-type and mutant tau proteins were expressed in *E. coli* BL21(DE3), as previously described.[10] 3R Tau with the K257T mutation and 4R tau with the ΔK280 mutation were expressed in parallel and used as controls. Tau proteins were purified and their concentration determined by densitometry, as described.[13] Purified recombinant and mutant 3R tau (0.3 mg/ml) and 4R tau (0.1 mg/ml) were incubated with bovine brain tubulin (1 mg/ml, 20 μM; Cytoskeleton, Denver, CO) in assembly buffer at 37°C, as described.[23] Assembly of tubulin into microtubules was monitored over time by a change in turbidity at 350 nm. In all experiments, wild-type and mutant tau proteins were expressed and purified in parallel. Numbers of separate experiments were as follows: htau37 and htau37L315R (n=5), htau46 and htau46L315R (n=5).

Tau filament assembly

Purified wild-type and L315R mutant forms of 3R tau (381 amino acid isoform) and 4R tau (412 amino acid isoform) (3 mg/ml) were incubated in the presence of 200 μg/ml heparin (British Drug House, Poole, U.K.) at 37° C for 48 h, as described (24). 3R Tau with the K257T mutation and 4R tau with the P301S mutation served as controls. Assembly was monitored semi-quantitatively by electron microscopy and quantitatively using thioflavin T fluorescence, as described.[23] Each filament assembly experiment made use of newly prepared batches of recombinant wild-type and mutant tau proteins that had been purified in parallel. Numbers of separate experiments were as follows: htau37 and htau37L315R (n=5), htau46 and htau46L315R (n=5).

Electron microscopy

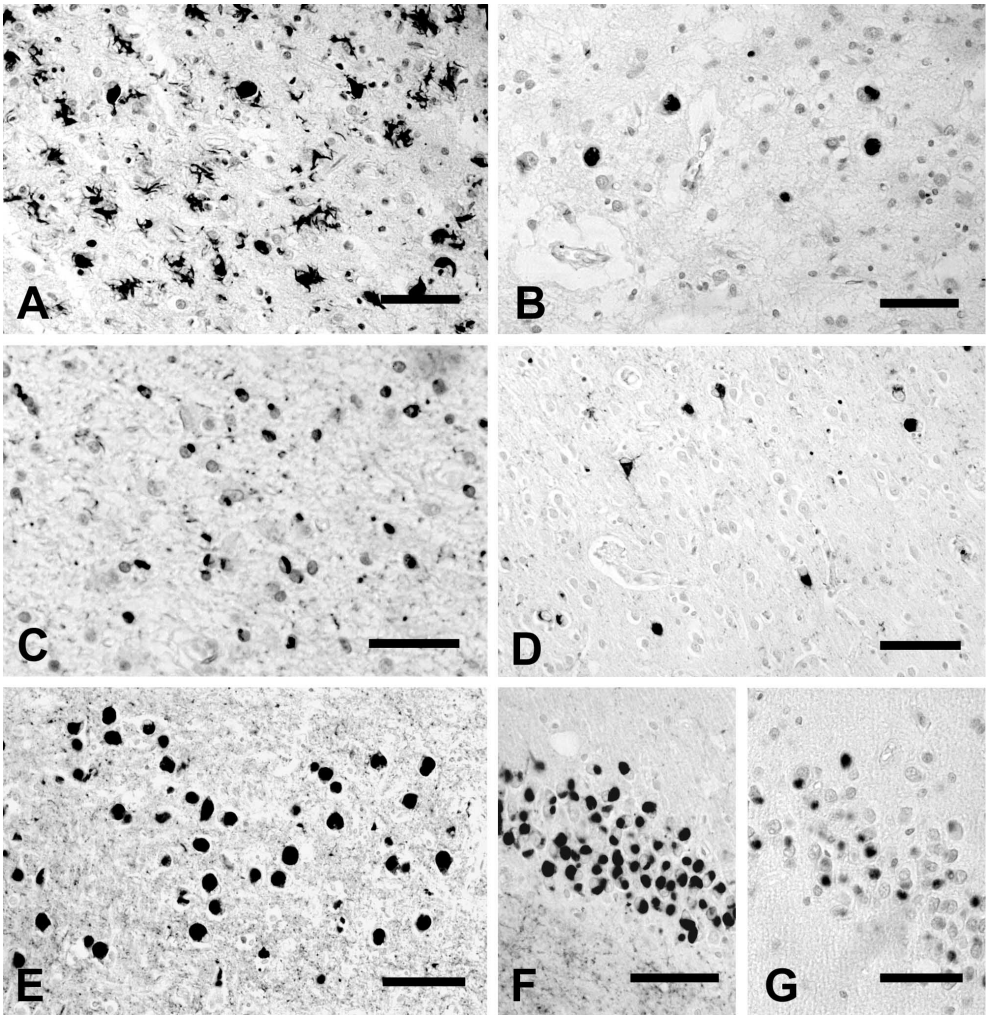
Aliquots of sarkosyl-insoluble, dispersed filament preparations and of synthetic tau filament assemblies were processed for electron microscopy, as described.[21] Anti-tau antibody AT8 was used at a dilution of 1:100. Procedures for immunoelectron microscopy were as described.[21]

Results

Neuropathological examination of both brains, A V:1 and B III:5 (brain weight 818 g and 863 g resp.) showed severe atrophy of the frontal and temporal lobes. HE staining in brain A V:1 showed mild neuronal loss and reactive astrocytosis in the frontal cortex, but severe in the temporal cortex, as well as in hippocampus, substantia nigra, red nucleus, caudate nucleus and amygdala. The dentate gyrus of the hippocampus was almost completely degenerated, with only a few Pick bodies recognised by Bodian silver staining. Brain B III:5 (Figure 2) showed most severe neuronal loss and reactive gliosis in the frontal cortex and underlying white matter, and less severe involvement of the temporal cortex, hippocampus, substantia nigra, caudate nucleus and striatum. Staining with phosphorylation-dependent antibodies of brain A V:1 showed numerous Pick bodies of variable intensity and size in the frontal cortex, with some tau deposits in the dendrites of astrocytes. In contrast, the severely affected temporal cortex contained extensive tau deposits in the glial cells, probably of astrocytic origin, with only a few Pick bodies. The subiculum of the parahippocampal gyrus also showed numerous Pick bodies, stained positive with all tau antibodies, except for 12E8. Tau staining of brain B III:5 revealed severe astrocytic tau deposits and some Pick bodies in the frontal cortex, whereas more
numerous Pick bodies were present in the deeper layers of the temporal cortex, with less severe tau pathology in the underlying white matter. AT8 antibodies stained Pick bodies in nearly all granule cells of the dentate gyrus, and in neurons of the caudate nucleus, putamen, locus coeruleus, and olivary nuclei, but much less in the thalamus. Glial tau staining was intense in the substantia nigra, and less in the other nuclei. Notably, the AT180 and AT270 antibodies gave a weaker signal, especially in staining the astrocytic tau pathology. The staining with ubiquitin, β-amyloid and α-synuclein antibodies was negative in both brains. A detailed overview of the pathology of both autopsied patients can be found in Table 2.

Sequence analysis of the genomic DNA of all affected family members revealed a T to G transition at the second base position of codon 315 (CTG to CGG), leading to a leucine to arginine amino acid substitution in exon 11 of the *tau* gene.

Figure 2 . Immunohistochemistry of brain of patient B III:5



A. Immunostaining of the frontal cortex with the phosphorylation-dependent antibody AT8 shows some Pick bodies and multiple tau-positive reactive astrocytes. B. Immunostaining of the same area with the phosphorylation-dependent antibody AT180 shows only the neuronal tau staining. C. Numerous AT8 positive glial cells are present in the white matter of the frontal cortex. D. Immunostaining of the temporal cortex with AT8 shows only neuronal pathology. E. Typical Pick bodies in the subiculum, immunoreactive with AT8. F, G. Immunostaining of the dentate gyrus, showing immunoreactivity with AT8 (F) and AT180 (G) antibodies.

Table 2. Overview of neuropathology of patients A V:1 and B III:5

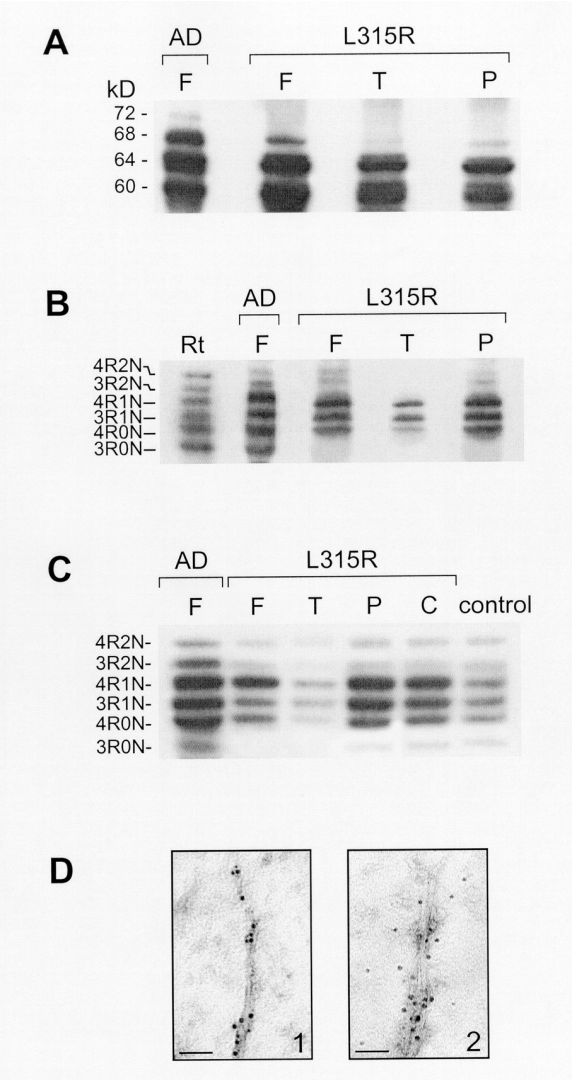
Brain regions	Neuronal loss		Gliosis		Pick bodies		Astrocytic tau deposits	
	A V:1	B III:5	A V:1	B III:5	A V:1	B III:5	A V:1	B III:5
Frontal cortex	++	+++	+++	+++	+++	+	+	+++
Temporal cortex	+++	+	+++	+++	+	+++	+++	+
Parietal cortex	++	-	++	-	++	++	+	-
Hippocampus	+++	-	-	-	+++	+++	+	+
Substantia nigra	+++	+	+	+	+	++	-	-
Caudate nucleus	-	+	-	-	+++	++	-	-
Putamen	-	-	-	-	-	++	-	-
Thalamus	-	-	+	-	-	+	-	-
Locus coeruleus	-	-	-	-	++	+++	-	-
Medulla	-	-	-	-	-	-	-	-

This base substitution was also observed in the unaffected 82-years old mother of two affected sibs. The base substitution was not observed in 200 control chromosomes. Haplotype analysis (Figure 1) showed a common allele in families A and B in the region of the *tau* gene, strongly suggesting a common ancestor for both families.
Immunoblotting of sarkosyl-insoluble tau extracted from the brain of the proband of family A (A V:1) showed two major bands of 60 and 64 kDa and a minor band of 68 kDa (Figure 3A). After dephosphorylation with alkaline phosphatase, these bands resolved into five bands that corresponded with human tau isoforms 4R2N, 3R2N, 4R1N, 3R1N and 4R0N (Figure 3B). Immunoblotting of dephosphorylated sarkosyl-soluble tau resolved into all six tau isoforms in a pattern identical to that found in AD and control brain, with a 4R to 3R ratio of 1.2 (Figure 3C).
Electron microscopy of sarkosyl-insoluble material extracted from the frontal cortex showed a small numbers of filaments. The majority of filaments (~ 80%) were irregularly twisted filaments with a long periodicity of more than 130 nm and a diameter of 13-15 nm, similar to the slender twisted filaments found in PiD. The minority (~ 20%) of the filaments were straight filaments. The phosphorylation dependent anti-tau antibody AT8 decorated both types of filaments (Figure 3D1 and 2).
Recombinant 3R and 4R tau proteins with the L315R mutation showed a reduced ability to promote microtubule assembly, when compared with the corresponding wild-type proteins (Figure 4 A,B). Thus, the L315R mutation led to a 20-30% reduction in the rates of microtubule assembly, when expressed as the optical density at 2 min (Figure 4C). The effects of the L315R mutation on filament assembly of recombinant 3R and 4R tau proteins were investigate by using heparin to induce assembly. Assembly was assessed quantitatively by thioflavin T fluorescence (Figure 4D) and semi-quantitatively by electron microscopy (not shown). Under the conditions of the experiments, no significant effect of the L315R mutation was detected (Figure 4D).

Discussion

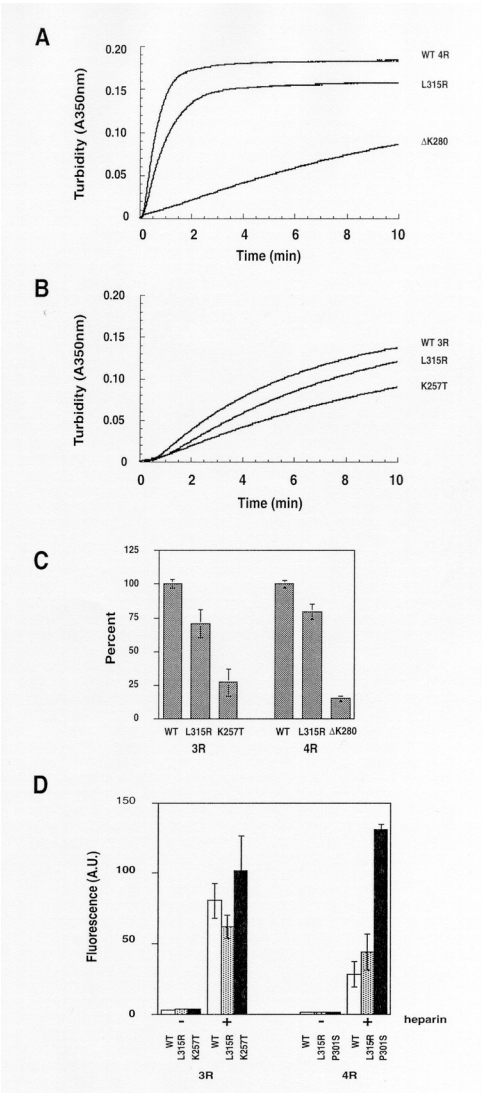
The novel *tau* mutation L315R was found to lead to a variable clinical expression, including three cases of non-penetrance, an extensive tau pathology in nerve cells and astrocytes in clinically affected individuals, and a relatively small reduction in the ability of tau to promote microtubule assembly. The two families with the L315R mutation could not be linked genealogically, because relevant archive material pre-dating the year 1800 was lost during the Second World War. This notwithstanding, it appears likely that the affected members from both families shared a common ancestor. Thus, they

Figure 3. Sarkosyl-insoluble, sarkosyl soluble and electron microscopic analysis of patient A V:1 brain



A, B. Analysis of sarkosyl-insoluble tau before (A) and after (B) alkaline phosphatase treatment. C. Analysis of sarkosyl-soluble tau after alkaline phosphatase treatment. Immunoblotting was performed using the phosphorylation independent anti-tau antibody BR01. AD = Alzheimer's disease; F = frontal cortex; T = temporal cortex; P = parietal cortex; C = cerebellum; Rt = recombinant tau. D,1-2. Electron microscopic images of tau filaments isolated from the frontal cortex of the patient's brain show twisted filaments decorated by the phosphorylation dependent anti-tau antibody AT8. (Scale bar: 50 nm).

Figure 4. Effects of the L315R mutation in the tau on microtubule assembly and heparin-induced filament formation



A. Polymerisation of tubulin induced by 3R wild-type and 3R L315R recombinant tau protein. B. Polymerisation of tubulin induced by 4R wild-type and 4R L315R recombinant tau protein. Microtubule assembly was monitored over time by turbidimetry. C. Optical densities for wild-type and mutant 3R and 4R tau protein at 2 min (expressed as percent of wild-type 3R and 4R tau protein taken as 100%). The results are expressed as means ± S.E.M. (n = 5). D. The effects of the L315R mutated 3R and 4R tau protein on filament assembly in the presence or absence of heparin.

originated from the same geographically and genetically isolated part of the Netherlands and haplotype analysis showed an allele shared by all the mutation carriers. The clinical phenotype of patients with the L315R mutation highlights the issue of variable penetrance. Thus, the presence of clinical symptoms in a 25 year-old mentally retarded patient with the mutation (B III:5) was in sharp contrast with the normal mental state of a 82 year-old mutation carrier (A IV:4). Previously, the P301S mutation was shown to lead to a similarly early age of onset of disease[25,26] and the issue of non-penetrance was raised in connection with several other mutations in *tau*.[27-29] However, compared with this earlier work, we were able to provide much stronger evidence in favour of non-penetrance. Thus, two affected individuals (A V:2 and A V:3) and their mother (A IV:4) were carrying the same mutation. Despite this, the mother was neurological normal and had a normal CT scan without lobar atrophy. Moreover, three additional cases of possible non-penetrance were present in both families, where subjects A III:2, B II:2 and B II:5 died aged 92, 70 and 85 years respectively, without clinical signs of a dementing illness.

Numerous Pick-like inclusions were present in cerebral cortex and hippocampus from both autopsied patients. They were stained by the Bodian silver stain and all phosphorylation-dependent anti-tau antibodies tested, with the exception of antibody 12E8. Similar findings have previously been reported for a number of other mutations in *tau*.[22,27,28,30-34] Unlike the latter, mutation L315R also resulted in a severe glial pathology, mainly astrocytic, in conjunction with the almost complete disappearance of nerve cells in some parts of the cerebral cortex. A similar neuronal and astrocytic pathology has recently been described for mutation L266V in exon 9 of *tau*[34] (and M. Hutton and D. Dickson, personal communication). Neuronal and glial, mostly oligodendrocytic, tau inclusions are characteristic of mutations R5H and R5L in exon 1 of *tau*.[7,18]

The presence of astrocytic inclusions in cases with mutation L315R is unexpected, since abundant tau inclusions in glial cells are normally associated with mutations that only affect 4R tau or that increase the relative amount of 4R tau, with the glial deposits being made of 4R tau.[4-6] Mutation L315R affects all six tau isoforms and does not change the ratio of 3R/4R tau. In the two brains studied here, there appeared to be an inverse relationship between the relative numbers of neuronal and glial tau inclusions. Thus, in a given brain region, abundant neuronal inclusions were accompanied by a much smaller number of glial deposits. Conversely, abundant astrocytic tau inclusions were found in conjunction with few neuronal inclusions and extensive nerve cell loss. This suggests that astrocytic inclusions may develop later than neuronal deposits, or that they may be longer-lived. Phagocytosis of neuronal tau deposits by astrocytes is also

compatible with the observed staining pattern, which would imply the presence of 3R tau inclusions in astrocytes. Clarification will require the use of antibodies specific for 3R and 4R tau.

Sarkosyl-insoluble tau extracted from a L315R brain resolved into two major bands of 60 and 64 kDa and a minor band of 68 kDa, similar to the pattern seen in sporadic Pick's disease[35] and in cases with the K257T, L266V, S320F and G389R mutations in *tau*. [22,27,28,32,34] However, following dephosphorylation, the band corresponding to tau isoform 3R0 was missing. Five bands aligning with isoforms 4R0N, 3R1N, 4R1N, 3R2N and 4R2N were observed instead. As the 60 kDa band from AD brain corresponds to isoform 3R0N,[21,36] the presence of a 60 kDa band in the absence of 3R0N tau in the L315R case implies that the isoform composition of the sarkosyl-insoluble tau bands differed from that of AD. Previously, a similar tau isoform pattern was described in the cerebral cortex from a patient with the S320F mutation in exon 11 of *tau*.[22] This raises the intriguing possibility that the absence of 3R0N tau from the sarkosyl-insoluble fraction may be a general characteristic of mutations in exon 11. The underlying mechanisms remain to be discovered. Soluble tau from L315R brain consisted of all six tau isoforms, similar to what is seen in AD and some cases of FTDP-17.[6] Tau filaments similar to those present in sarkosyl-insoluble tau from the L315R brain have been described in some cases of sporadic PiD and cases with *tau* mutations and a Pick-like phenotype.[22,27,35]

L315R is only the second mutation to be described in exon 11 of *tau,* the other being S320F.[22] It is located in the third microtubule-binding repeat, where a leucine residue is present at this position in all known vertebrate proteins with tau repeats, indicating its functional relevance.[37] Accordingly, recombinant tau with the L315R mutation showed a reduced ability to promote microtubule assembly, suggesting that this may be its primary effect. However, when compared with other mutations, the effect of the L315R mutation was relatively small, indicating a possible explanation for its reduced penetrance.

Mutation L315R is located close to the hexapeptide sequence VQIVYK (residues 306-311) that has been proposed to initiate tau filament assembly by forming local β-structure.[38] Despite this proximity, we failed to detect a significant effect of the L315R mutation on tau filament assembly. Of the known *Tau* mutations, L315R is only the third to increase the overall charge of the protein, the other two being N279K and G389R. It has been proposed that a reduction in charge is a positive determinant in the protein aggregation associated with human diseases.[39] This appears unlikely in the case of tau, since most mutations do not affect its net charge. Of the 30 known mutations, only five (K257T, ΔK280, E342V, K369I and R406W) reduce the charge of tau.

In conclusion, the present study describes a novel mutation in exon 11 of *tau*, widening the spectrum of hereditary diseases that resemble Pick's disease. The variable clinical expression, in conjunction with the small functional effect of the L315R mutation, emphasises the need to identify other factors (modifying genes or environmental precipitants) that influence the development of tauopathies.

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Chapter
4.3

Coexistent tau and amyloid pathology in hereditary frontotemporal dementia with *tau* mutations

Abstract

Hereditary frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) is associated with different mutations in the *tau* gene. Pathological changes consist of accumulation of hyperphosphorylated tau protein in frontal and temporal cortex, hippocampus, and some subcortical nuclei. We describe the neuropathological findings in five patients with P301L mutation, and in two affected sibs with R406W mutation. The P301L brains all showed a pretangle-type tauopathy of the frontal and temporal cortices. One of these patients, however, also showed an Alzheimer-type tauopathy with neurofibrillary tangles (NFT), neuritic plaques, and amyloid angiopathy of the temporo-parietal cortex. Three tau bands (64, 68, and 72 kDa) were seen in the frontal cortex, while the temporal cortex revealed four bands (60, 64, 68, and 72 kDa), containing all six tau isoforms. The first R406W brain showed many neurofibrillary tangles in affected regions with only a few diffuse amyloid plaques. The second R406W brain contained a much higher density of NFT in affected regions, and an extensive amyloid deposition consisting of both diffuse and neuritic plaques with dense cores. An intriguing question is whether the FTD and Alzheimer's disease changes are concomitant, or whether there is an interaction between tau and amyloid pathology. An acceleration of NFT formation due to amyloid deposition has been observed in non-demented aging and preclinical Alzheimer's disease. The question whether this mechanism occurs in FTD with *tau* mutations remains to be elucidated.

The term frontotemporal dementia (FTD) covers a group of presenile dementias with progressive behavioural changes and often frontal or temporal atrophy on neuroimaging.[1] Several mutations in the *tau* gene have been found in families with hereditary fronto-temporal dementia and parkinsonism linked to chromosome 17q21-22 (FTDP-17).[2-8] These mutations explain the accumulation of hyperphosphorylated tau protein in neurons and glial cells in the cortex, hippocampus, and subcortical nuclei. Tau pathology shows considerable variation in the type and distribution of tau deposits, the physical structure of filaments, and tau isoform composition between the different FTDP-17 families.[8-14]

Methods

We have investigated tau pathology in five P301L and two R406W patients (Table 1). All brains showed moderate to severe atrophy of frontal and anterior temporal lobes. Sections from different cortical regions, hippocampus, substantia nigra, and other subcortical nuclei were processed for routine staining and for immunohistochemistry using phosphorylation-dependent (AT8, AT180, AT100, PHF1, 12 E8 and E10), phosphorylation-independent anti-tau (BR133, BR134, BR304, BR189), anti-β-amyloid (βA4), and anti-ubiquitin antibodies. Tissue sections were pre-treated with 90% formic acid for 5 minutes before immunohistochemistry with antibody against βA4 was performed. Sarkosyl-insoluble tau was extracted from fresh-frozen cortices and hippocampus, dephosphorylated as previously described, and run on 10% SDS-PAGE and blotted onto immobilon P [Millipore].[15,16] Blots were incubated overnight at 4°C with antisera, BR134 and BR133, and stained using the biotin-avidin Vectastain system (Vector Laboratories). Sarkosyl soluble tau was extracted using 2.5% perchloric acid. Aliquots of sarkosyl-insoluble tau were processed for electron microscopy (EM).[17]

Results

P301L brains

Neuronal loss and gliosis were moderate to severe in the frontotemporal cortex. The substantia nigra showed severe loss of pigmented cells. Tau deposits of the pretangle type were found in the frontal and temporal cortex, and to a lesser degree in the parietal cortex, the granular cells of dentate gyrus, and substantia nigra. In the first three P301L brains, no amyloid staining was observed, while in P301L brain 4, some diffuse plaques were seen, mainly in the temporal and occipital cortex; no neuritic plaques or amyloid angiopathy were found. In P301L brain 5, many neurofibrillary tangles (NFT), some

extracellular, and many diffuse and neuritic plaques with dense amyloid cores were present in all cortices, subiculum, and hippocampus, whereas some tau-positive glial cells were additionally found in grey and white matter. Immunoblots of sarkosyl-insoluble tau from P301L brain 5 showed two major bands (64 and 68 kDa) and a minor band of 72 kDa in frontal cortex, and four bands of 60, 64, 68 and 72 kDa in temporal cortex and hippocampus. EM study of sarkosyl-insoluble tau preparations from P301L brain 5 showed tau-containing filaments in hippocampal formation, which were structurally similar to paired helical filaments (PHFs) in Alzheimer's disease (AD), and slender twisted filaments in frontal cortex, similar to the other P301L family that we have recently described.[11]

R406W brains

In the first R406W brain, neuronal loss and gliosis were of variable intensity in frontal and temporal cortex. NFT were abundant in the frontal and temporal cortex, pyramidal layer of the hippocampus, and gyrus parahippocampalis. Substantia nigra showed only a few NFT and neuropil threads. Occasional tau-positive glial cells were seen in the grey and white matter. A few diffuse plaques and occasional classic plaques (stained by βA4 antibody) were present. The antibody against ubiquitin showed staining of numerous neurons and dystrophic neurites in cortices and hippocampus. The second R406W brain showed severe neuronal loss and gliosis in the frontal, temporal, and parietal cortex. Many NFT and extracellular tangles were present in all cortices (except for occipital), hippocampus, gyrus parahippocampalis, amygdala, and hypothalamus. The NFT density in this brain was much higher than in the R406W brain 2. Many diffuse and neuritic plaques with dense cores (stained by anti-βA4 antibody) were present in all cortices with moderate deposition of β-amyloid in blood vessels. Immunoblots of sarkosyl-insoluble tau from the R406W brain 1 showed four bands of 60, 64, 68 and 72 kDa, and after alkaline phosphatase treatment six immunoreactive bands corresponding to the six tau isoforms in AD. The pattern of soluble tau was similar to that from control brains. EM of sarkosyl-insoluble tau preparations from the R406W brain 2 showed tau-containing filaments in the frontal and temporal cortices and hippocampal formation, which were structurally similar to PHFs in AD, with a diameter of 8-20 nm and a cross-over spacing of approximately 80 nm. A minority of filaments consisted of straight filaments (SFs) of about 12 nm.

Table 1. Cortical distribution of tau and amyloid depositions

		Tau Deposition			Amyloid Deposition			
Brain	Age	Type	Localisation	Density*	Type	Localisation	Density†	Angiopathy
P301L 1	65	pretangles	F, T, P	++	none	–	–	–
P301L 2	66	pretangles	F, T, P	++	none	–	–	–
P301L 3	53	pretangles	F, T, P	++	none	–	–	–
P301L 4	76	pretangles	F, T, P	+++	diffuse	T, O	++	–
P301L 5	64	pretangles	F, T	++	neuritic	T, P, O	+	+
		NFT	T, P	+				
R406W 1	69	NFT	F, T	+	diffuse	F, O	+	–
R406W 2	70	NFT	F, T, P	++	neuritic	F, T, P, O	++	+

Abbreviations: F = frontal cortex, T = temporal cortex, P = parietal cortex, O = occipital cortex, NFT = neurofibrillary tangles.
* Mean tangle density (both NFT and pretangles; number per mm^2): – = 0 /mm^2, + = 1 – 50 /mm^2, ++ = 50 – 100 /mm^2, +++ = 100 or more /mm^2. † Mean plaque density (both diffuse and neuritic; number per mm^2): – = 0 /mm^2, + = 1 – 5 /mm^2, ++ = 5 – 10

Discussion

All seven brains of patients with *tau* mutations (five P301L, two R406W mutations) showed NFT or tau deposits of pretangle type. However, large numbers of neuritic plaques and amyloid angiopathy were present in P301L brain 5 and in R406W brain 2. There are at least two other reports of diffuse or classic plaques in patients with presenile dementia and P301L or splice donor mutations.[8,18].

Tau-positive pretangles (mainly perinuclear deposits) were seen in the frontal and temporal cortex, and dentate gyrus of the P301L brains, and this is similar to the Dutch family (HFTD1) with this mutation.[11] This P301L pathology is also supported by the presence of slender twisted filaments, two major bands of 64 and 68 kDa, and a minor band of 72kDa of extracted sarkosyl-insoluble tau from the frontal cortex, which is consistent with the pattern in other P301L families.[8,12,13] However, NFT, diffuse and senile plaques, PHFs, and a pattern of four bands (60, 64, 68 and 72 kDa) of sarkosyl-insoluble tau from the temporal cortex and hippocampus of the P301L brain 5 is consistent with the pathological diagnosis AD. This is further supported by the presence of all six tau isoforms following treatment with alkaline phosphatase. This implies that two different types of tau pathology coexist in the same brain, but were differently distributed in some brain regions.

The presence of NFT in nerve cells with occasional tau deposits in glial cells in both R406W brains is in agreement with studies carried out in another R406W family.[19] NFT, extracellular NFT, PHFs and SFs are found when all six tau isoforms are mutated, as in cases with coding mutations outside exon 10. Interestingly, the R406W brain 2 showed both a higher density of NFT and a higher number of diffuse and neuritic plaques than the R406W brain 1.

An intriguing question is whether the FTD and AD changes are concomitant, or whether there is an interaction between tau and amyloid pathology. An acceleration of NFT formation due to amyloid deposition has recently been observed in nondemented aging and preclinical AD.[20] The question, therefore, whether this mechanism occurs in FTD with *tau* mutations remains to be elucidated in future studies.

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Chapter

4.4

Total tau and phospho-tau 181 in CSF of patients with frontotemporal dementia due to P301L and G272V *tau* mutations

Abstract

In this study, we analysed total tau, phosphotau 181, and amyloid-β_{1-42} in CSF of 26 FTD patients, including nine with *tau* mutations (seven P301L, two G272V). Although CSF total tau was mildly increased in FTD ($p=0.05$), this increase was not seen in the subgroup with *tau* mutations. Furthermore, CSF phospho-tau 181 and $A\beta_{1-42}$ levels were not different compared with non-demented controls. Therefore, we conclude that the tau pathology present in P301L and G272V brain does not appear to be associated with an increase in either CSF total tau or phospho-tau 181.

Frontotemporal dementia (FTD) is a neurodegenerative disorder of the frontotemporal cortex, presenting with presenile onset of behavioural changes and cognitive decline.[1] Mutations in the *tau* gene have been identified in some, but not all patients with familial FTD, and are associated with the accumulation of hyperphosphorylated tau protein in the brain at neuropathological examination.[2,3] In contrast, only about 20% of cases with sporadic FTD show accumulation of tau protein, usually in the shape of classical Pick bodies. The remaining cases are characterised by neuronal loss, spongiosis and gliosis with or without ubiquitin-positive, but tau-negative inclusions in the frontotemporal cortex, and may occur in sporadic as well as in familial form.[4]

Because cerebrospinal fluid (CSF) is in direct contact with the extracellular space of the central nervous system, many studies have focussed on CSF in search of biomarkers with diagnostic significance in dementia. Most studies with FTD patients show a modest increase of total tau protein concentration in CSF.[5,6] However, the level of tau protein in CSF has not yet been investigated in FTDP-17 patients with *tau* mutations. Therefore, we measured levels of CSF total tau (t-tau), CSF tau phosphorylated at Thr181 (Ptau-181), and CSF-amyloid-β_{1-42} ($A\beta_{1-42}$) in 26 FTD patients, nine of whom showed missense mutations in the *tau* gene. We compared our findings with a group of patients with tauopathy due to Alzheimer's disease (AD) as well as non-demented controls. Our hypothesis was that CSF Ptau-181 levels would be elevated in FTDP-17 patients with *tau* mutations, as all known *tau* mutations lead to the deposition of hyperphosphorylated tau protein in neurons and/or glial cells, and these deposits are known to be phosphorylated at Thr181 in P301L and G272V brain.[7]

Methods

Subjects

Twenty-six patients with FTD (12 women, 14 men, mean age 54.7 ± 7.0 years [± standard deviation]) were recruited for CSF analysis from the outpatients clinic of the departments of Neurology of the Erasmus Medical Centre of Rotterdam and the VU University Medical Centre of Amsterdam, the Netherlands between January 1997 and December 2001 (Table 1). *Tau* mutations were identified in nine FTD patients with a positive family history: seven with the P301L mutation and two with the G272V mutation.[8] Family history of dementia was negative in the remaining 17 FTD patients. In addition to these patients, we included 18 patients with AD (seven women, 11 men, mean age 66.0 ± 7.5 years), and 13 non-demented controls (eight women, five men, mean age 57.3 ± 12.6). Non-demented controls were subjects who visited the

outpatients clinic for thunderclap headache without subarachnoidal haemorrhage, neuritis vestibularis, or non-progressive subjective memory complaints without cognitive impairment on extensive psychometric evaluation or imaging abnormalities.
The diagnosis probable FTD was made according to the Lund-Manchester criteria[1] and the diagnosis probable AD was made in accordance with the NINCDS-ADRDA criteria.[9] All patients underwent thorough clinical investigation, including detailed medical and family history, neurological examination, psychometric evaluations and neuroimaging, consisting of CT, MRI, and/or single-photon emission computed tomography (SPECT) scanning with 99mTc-hexamethyl propyleneamine oxime (HMPAO). In case of a diagnostic lumbar puncture and CSF examination, patients were asked to consent to the collection of additional 4 ml for research purposes. The study protocol was approved by the Medical Ethics Committee of Erasmus Medical Centre, Rotterdam, and the VU University Medical Centre, Amsterdam, the Netherlands.

CSF analysis

CSF samples were obtained by lumbar puncture and stored in polypropylene tubes at -80°C until biochemical analysis. CSF t-tau was determined by sandwich ELISA using the monoclonal antibody (Mab) AT120 as capturing antibody, and two Mabs (HT7 and BT2) as detection antibodies, recognising different epitopes (INNOTEST™ hTAU-Ag, Innogenetics, Gent, Belgium).[10] CSF Ptau-181 was determined by sandwich ELISA (INNOTEST™ PHOSPHO-TAU $_{(181P),}$ Innogenetics), using Mab HT7 as capturing antibody and biotinylated Mab AT270 as detection antibody, which is specific for a phosphotau-Thr181 epitope.[11] CSF $A\beta_{1-42}$ was determined by sandwich ELISA, using Mab 21F12 specific for the C-terminus of $A\beta_{1-42}$ as capturing agent, and a biotinylated Mab anti- $A\beta_{1-42}$ N-terminal antibody (3D6) for detection (INNOTEST™ β-amyloid $_{(1-42)}$, Innogenetics).[12]

Statistics

Statistical procedures were performed using Statistical Package of Social Sciences (SPSS) software. Data are presented as medians (25 and 75 percentiles) since CSF t-tau, CSF Ptau-181, and CSF-$A\beta_{1-42}$ were not distributed normally. For group comparisons, the Mann-Whitney *U*-test and the Kruskal-Wallis tests were used depending on the number of groups. Correlations were calculated using Spearman's rank correlation coefficient test with respective two-sided correlation.

Table 1. Clinical data and CSF analyses per diagnostic category

Diagnostic Category	n	Sex F/M	Age Mean ± SD	Duration Mean ± SD	T-tau, pg/ml Median (25-75 percentile)	Ptau181, pg/ml Median (25-75 percentile)	$A\beta_{1-42}$, pg/ml Median (25-75 percentile)
FTD	26	12/14	54.7±7.0	3.0±1.8	299 (179-499)	33 (25-43)	683 (458-771)
- *Tau* mutation	9	4/5	52.3±7.0	2.1±1.4	330 (184-338)	31 (28-42)	528 (409-708)
- Sporadic	17	8/9	56.0±6.9	3.5±1.8	298 (178-706)	34 (22-71)	756 (441-831)
AD	18	7/11	66.0±7.5	3.0±2.1	479 (360-698)	80 (54-101)	280 (222-312)
Non-demented	13	8/5	57.3±12.6	—	171 (117-310)	31 (21-42)	547 (421-625)

T-tau = total tau; Ptau181 = tau phosphorylated at Thr. 181, $A\beta_{1-42}$ = β-amyloid$_{1-42}$ protein, FTD = frontotemporal dementia, AD = Alzheimer's disease, SD = standard deviation, F = female, M = male.

Results

The age at examination in patients with FTD was similar to that of non-demented controls ($p=0.4$), while the AD patients were significantly older ($p<0.001$). The mean age of the nine FTD patients with *tau* mutations (52.3±7.0 years) did not differ from that of the remaining FTD patients (56.0±6.9 years, $p=0.2$). The mean duration of disease at lumbar puncture in both FTD and AD patients was 3.0 years. CSF t-tau, CSF Ptau-181, and CSF $A\beta_{1-42}$ levels were similar in men and women. There was no significant correlation between CSF t-tau, CSF Ptau-181 or CSF $A\beta_{1-42}$, and age at lumbar puncture or duration of disease in any of the patient groups. The correlation between CSF t-tau and CSF Ptau-181 was high in all groups.

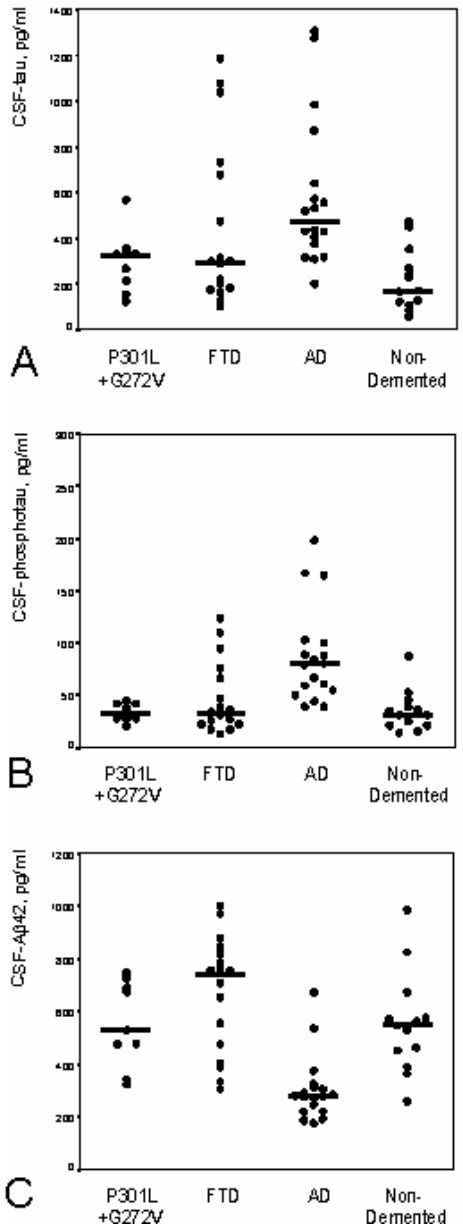
CSF t-tau in FTD patients was slightly higher than in non-demented controls ($p=0.05$), whereas CSF t-tau in the subgroup of FTD patients with *tau* mutations did not differ significantly from that in controls (Figure 1). CSF t-tau levels were increased in patients with AD ($p<0.001$) compared with non-demented controls . CSF Ptau-181 in the total FTD group, as well as in the two subgroups separately, did not differ from that in controls, while CSF Ptau-181 levels in AD patients were significantly higher than in non-demented controls ($p<0.001$). The ratio of CSF Ptau-181 over CSF t-tau was not different in any of the groups. There was no correlation between the duration of the symptoms in patients with *tau* mutations and CSF t-tau or CSF Ptau-181 (Table 2). FTD patients, including patients with *tau* mutations, had similar levels of CSF $A\beta_{1-42}$ compared with non-demented controls, in contrast to AD patients, who had lower levels of CSF $A\beta_{1-42}$ compared with non-demented controls ($p< 0.001$).

Discussion

To our knowledge, this study is the first to address CSF tau levels in FTD patients with *tau* mutations. Although CSF t-tau was slightly higher in FTD patients than in non-demented controls, this elevation was not found in the subgroup of patients with P301L or G272V *tau* mutations. CSF Ptau-181 was not increased in FTD patients, neither in FTD with *tau* mutations or in sporadic FTD. At the same time, we confirmed previous findings of increased CSF t-tau and CSF Ptau-181 levels, and lowered CSF $A\beta_{1-42}$ in AD patients.

The observation of mildly increased levels of CSF t-tau in the total FTD group is similar to findings in previous studies regarding FTD.[5,6] Increased CSF t-tau has also been found in a variety of other neurological disorders, including AD, corticobasal degeneration, Creutzfeldt-Jakob disease and acute stroke.[13,14] Therefore, these elevated levels probably

Figure 1. Scatterplots of CSF analyses



A. Total-tau, B. CSF Ptau-181, C. CSF $A\beta_{1-42}$, per diagnostic category (bar represents median).

Table 2. Characteristics of patients with *tau* mutations

	Sex	Age*	Duration*	t-tau (pg/ml)	Ptau-181 (pg/ml)	$A\beta_{1-42}$ (pg/ml)
P301 L *tau* mutation						
1.	F	49	0.5	266	27	528
2.	M	58	2.0	345	44	674
3.	M	52	0.8	569	42	725
4.	F	62	5.0	214	34	475
5.	F	53	1.1	332	31	692
6.	M	63	2.0	155	28	324
7.	F	45	2.3	331	28	478
G272V *tau* mutation						
8.	M	45	3.2	330	42	748
9.	M	45	2.2	123	20	343

* years. T-tau = total tau; Ptau-181= tau phosphorylated at Thr. 181; $A\beta_{1-42}$ = β-amyloid$_{1-42}$ protein, F = female, M = male.

reflect aspecific neuronal and axonal degeneration, and are not merely a consequence of neurofibrillary tau pathology. It has been proposed that hyperphosphorylated tau protein, which is the neuropathological substrate that all tauopathies have in common, may be more specific in differentiating tauopathies such as AD and corticobasal degeneration from other neurodegenerative disorders. Indeed, studies regarding CSF phospho-tau using antibodies directed against different phospho-epitopes (Threonine-181, Serine-199, and Threonine-231) have all shown an increase in CSF phospho-tau, specifically in AD.[15-17]

Surprisingly, CSF Ptau-181 levels in the present study were not increased in FTD patients with *tau* mutations. These findings indicate that accumulation of phosphorylated tau in brains of patients with tauopathy does not necessarily lead to an increase in CSF phospho-tau. Additional factors are probably involved in determining why certain other

tauopathies, such as AD, are indeed associated with an increase in CSF phospho-tau. A possible explanation for this observation may be that the tau deposits in P301L and G272V brain are located intracellulary (in the shape of pretangles or Pick-like inclusions respectively) and might not reach the CSF, while the extracellular ghost tangles found in AD are not seen.[18] It will be interesting to examine CSF of patients with R406W *tau* mutations, as the neurofibrillary pathology found in this mutation closely resembles AD.[19]

In the current study, CSF was only available from patients with P301L and G272V missense mutations, which both reduce the ability of mutant tau protein to interact with microtubules and other molecules.[20] Intronic and some coding region mutations in exon 10 have a primary effect at the mRNA level, resulting in a change in ratio of 3- to 4-repeat tau isoforms, without affecting the binding-properties of the protein. Because of the different mechanisms by which *tau* mutations lead to neurodegeneration, it is unpredictable whether CSF t-tau or CSF Ptau-181 levels may be altered in patients with *tau* mutations that affect the alternative splicing of exon 10. Analysis of CSF of patients with different types of *tau* mutations, as well as various other types of tauopathy, may contribute to our understanding of which factors determine the selective increase of different CSF proteins in neurodegenerative disorders in general.

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5

FTD families without tau mutations

Chapter

5.1

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

Abstract

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 initiative loss 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 localisation 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 characterisation of the ubiquitin-positive inclusions may clarify the neurodegenerative pathways involved in this subtype of FTD.

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),[1-4] and subsequently in patients presenting with other clinical phenotypes, including progressive supranuclear palsy and corticobasal degeneration.[5-7] All families with *tau* mutations have in common the accumulation of hyperphosphorylated tau protein in affected neurones or glial cells.[8,9] However, at least three FTDP-17 families have not shown *tau* mutations, despite significant linkage to the *tau*-containing region on chromosome 17q21-22.[10-12] Linkage to another locus on the centromeric region of chromosome 3 has been reported previously in a single Danish FTD kindred.[13] Recently, locus heterogeneity in FTD has been emphasised by the identification of a new locus on chromosome 9q21-22 in families with amyotrophic lateral sclerosis and FTD-type dementia.[14]

Two FTD families have shown ubiquitin-positive, tau-negative inclusions, which suggests an alternative pathophysiological mechanism.[15,16] 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,[17] and in semantic dementia.[18] 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.[19,20]

Here we describe the clinical and neuropathological features of a large Dutch FTD family (family HFTD3) reported earlier,[10] which is characterised 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.

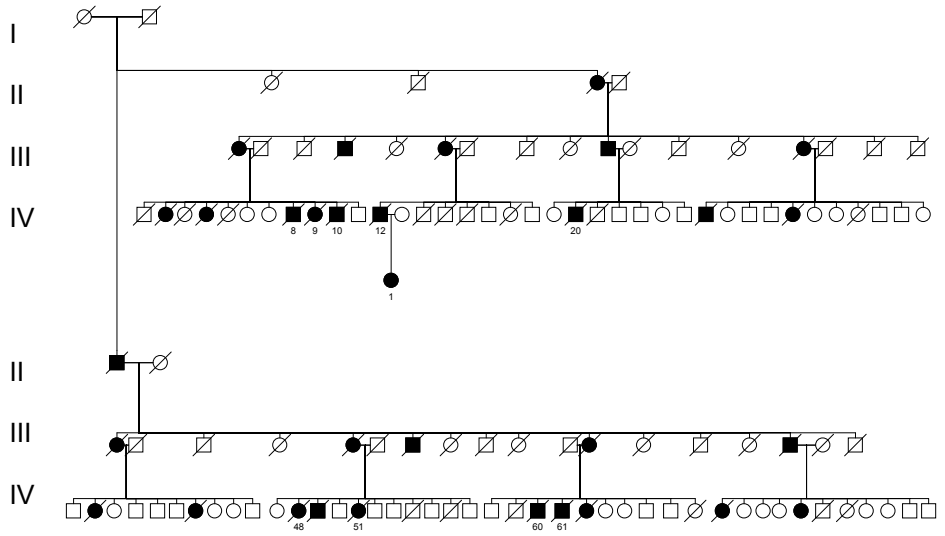
Methods

Clinical data

This four-generation FTD family with 32 affected members (19 women, 13 men) shows an autosomal dominant pattern of inheritance (Figure 1) and has been reported briefly.[10]

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[21,22] was established in 10 patients, and unspecified dementia was diagnosed in the remaining 22 patients because of limited clinical information.

Figure 1. Pedigree of family HFTD3



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.[10] 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 *et al.*[23] The short tandem repeat polymorphisms D17S945, D17S953, D17S946, D17S934, D17S951, D17S950, and D17S791 for chromosome 17q21-22 were selected based

on previous linkage results,[10] 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.[13] 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 0.4U of AmpliTaq Gold DNA polymerase. Initial denaturation was 15 min at 95°C followed by 32 cycles of 30s denaturation at 95°C, 30s annealing at 55°C and 90s 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 (filterset 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.[24] 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.[25] 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 10mM Tris-HCl, pH 8.3, 50mM KCl, 1.5mM $MgCl_2$, 200μM 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 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. 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 silver, 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[26]), 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), parkin (1:500, Chemicon). Heat-induced antigen retrieval was performed by heating slides at 80°C for 30 minutes in 0.1M 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 using 2.5% perchloric acid as described previously[27] 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[8]) 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 microscopy was used to evaluate the presence of sarkosyl-insoluble tau filaments as described previously.[28]

Total RNA was isolated from frontal cortex tissue of Patient IV:60 and two healthy control brains using RNAzolB kit (Campro Scientific, Berlin, Germany) according to manufacturer's specifications. Reverse transcription (RT)-PCR was performed using the Superscript Preamplification 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 visualised 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 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 summarised in Table 1.

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

Patient	Sex	Age at onset	Dur-ation	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	Disinterest, 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

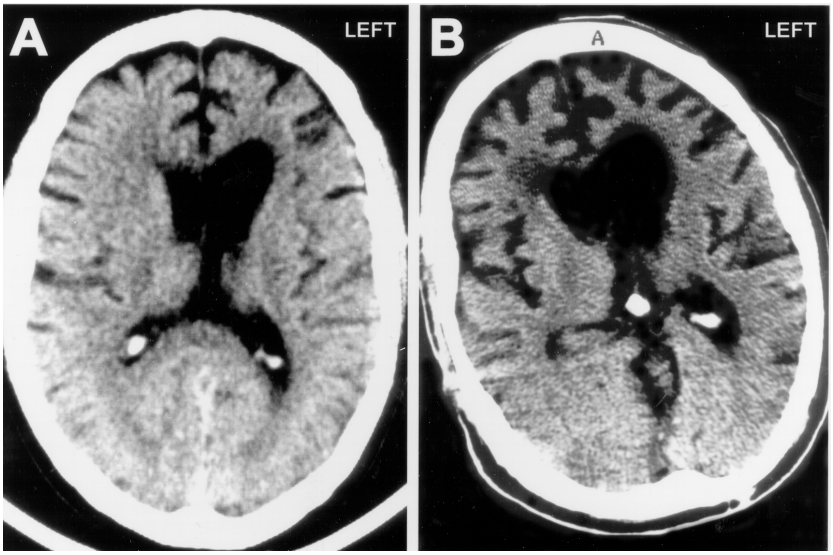
* Ongoing; ** SPECT scan performed and showing hypoperfusion of anterior parts of the brain. M = male, F = female.
FT = frontotemporal, T = temporal, F>T = frontal atrophy more severe than temporal atrophy.

Extensive neuropsychological evaluation in two patients (1 and 2 years after onset, respectively) showed evidence for cognitive impairment compatible with the diagnosis FTD. Both patients were co-operative, 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 of 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 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 asymmetric reduction in dopamine D_2 receptors in the striatum.

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 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). HMPAO-SPECT showed evident hypoperfusion of 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.

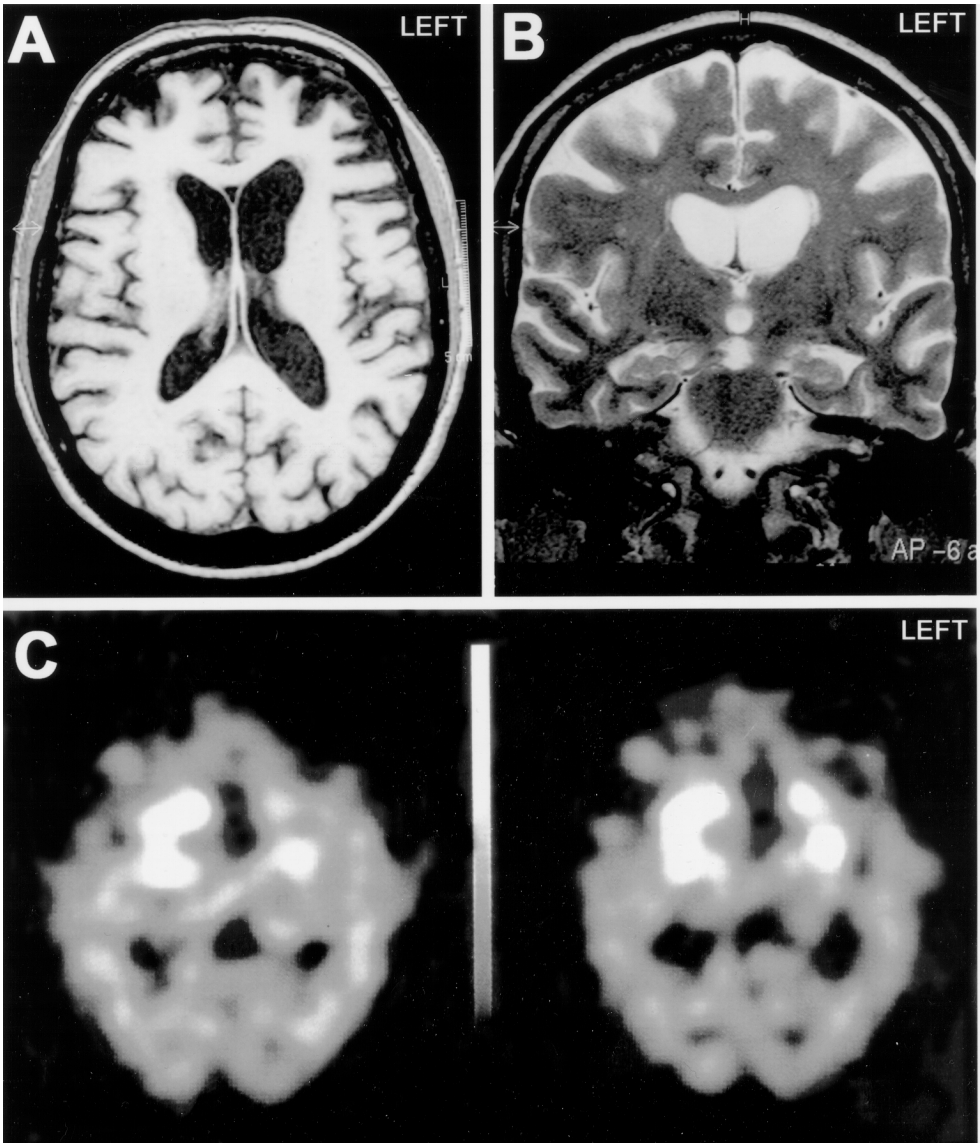
Figure 2. CT scans of patient IV:60



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.

Patient V:1 This 53-year-old woman complained of memory problems and difficulty in scheduling her work as a specialised 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 symmetric 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 of striatal dopamine D_2 receptors, greater on the left than on the right (Fig. 3C). The symptoms showed a subjective partial and transient response to levodopa treatment.

Figure 3. Neuroimaging 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 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.

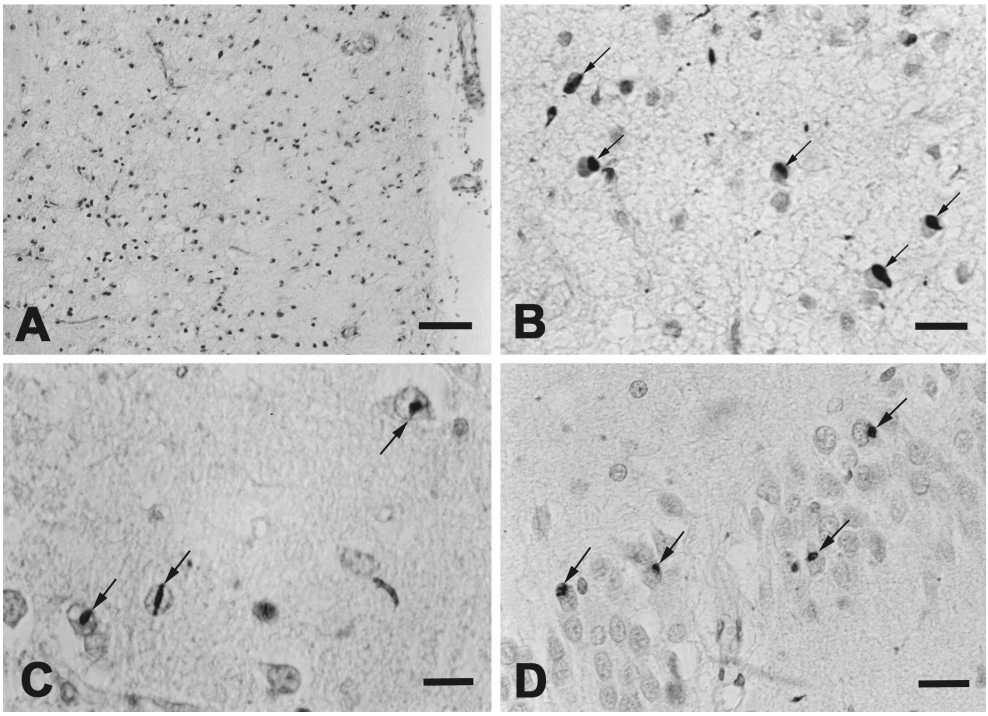
Genetic studies

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 to 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 brain of Patient IV:60, as can be expected at the age of 76.
Both brains showed 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 hematoxylin-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 at thorough examination. These inclusions had a cat's eye or target shape and were occasionally grouped together in small fields (Fig. 4C).

Figure 4. Microscopic findings in patient IV:60



A. Hematoxylin and eosin staining of frontal cortex, showing severe neuronal loss and microvacuolation. B. Ubiquitin immunostaining of 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 (A) and 100μm (B-D).

Furthermore, some granular cells of the dentate gyrus contained cytoplasmic ubiquitin-positive inclusions, which were round and less dense than the inclusions found in 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 chord (only available for Patient IV:60) did not show any ubiquitin inclusions.

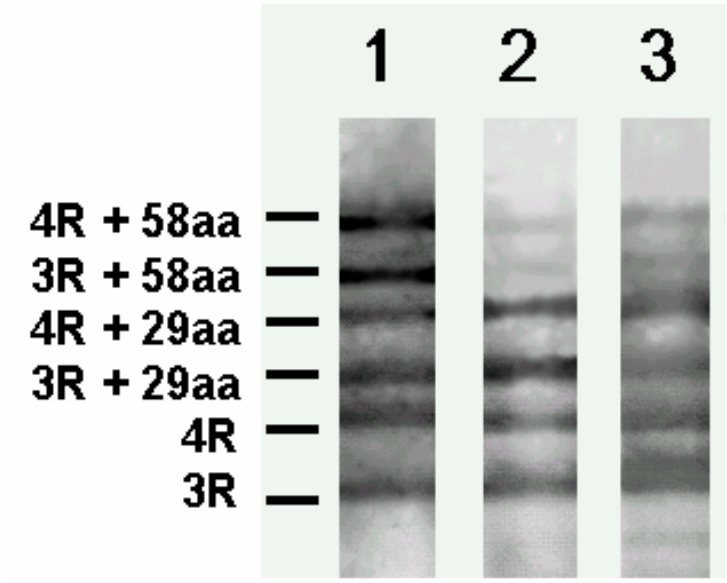
Phosphorylation-dependent and independent antibodies against tau protein and antibodies against α-synuclein, neurofilament (SMI-32), β-tubulin , MAP2, and polyglutamine (1C2) did not stain the inclusions. No amyloid plaques or ballooned cells were detected with βA4 antibody or αB-crystallin, respectively.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

with GFAP antibody showed very severe reactive astrocytosis in all 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 frontal cortex of Patient IV:60 (data not shown).

Figure 5. Biochemical studies of patient IV:60



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 recombinant human brain tau isoforms (lane 1).

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.[9,29-31] 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 the age at onset – from 53 to 71 years. This differs from that seen in most families with tau-related FTD.[30] 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.[15,32] 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 P301S mutation in the *tau* gene.[33]

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.[15-18] 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 localisation. 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 localisation can only be determined by electron microscopic studies, which were hampered by the scarcity of the lesions and the altered morphology of post-mortem tissues. It would be 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 localisation of inclusions found in Huntington's disease and other triplet repeat diseases,[34,35] 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 may 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 patient IV:60 was insufficient for the detection of sarkosyl insoluble tau. Interestingly, Zhukareva *et al.* have found reduced levels of sarkosyl-soluble tau protein in sporadic FTD cases and in a family with hereditary dysphasic disinhibition dementia (HDDD2), which has shown linkage to the same chromosomal region and absence of *tau* mutations.[36] However, we did not find a striking qualitative difference in the amount and ratio of three- versus four-repeat isoforms as compared with control brain. However, more patients are needed to determine the exact ratio's.

Kertesz *et al.* 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.[15] 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 is located within intron 9. 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.[16,32] 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, characterised 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.[11] *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 P301L, G272V and R406W mutations.[9] However, astrocytosis is an non-specific phenomenon and only reflects severe neuronal loss. Another candidate gene might be the nerve growth factor receptor gene,[11] 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.[37,38] 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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6

General Discussion

Although frontotemporal dementia (FTD) is the second most common cause of presenile dementia after Alzheimer's disease (AD), it is still a relatively rare disorder. Taking into account recent epidemiological studies (chapter 2.1),[1] we expect there to be less than a thousand patients in a population like the Netherlands of about 15.5 million inhabitants. Nevertheless, research in this field has evolved enormously over the past decade, especially after the delineation of the first clinical and neuropathological criteria for FTD by the Lund and Manchester groups in 1994.[2] However, it was not until the identification of the first mutations in the *tau* gene in 1998,[3-5] that the major importance of this disorder was recognised by specialists in other neurodegenerative fields. Tauopathy is the hallmark of a number of related disorders, including progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD), and elucidation of the pathophysiological pathways leading to neurodegeneration in these disorders may ultimately lead to therapeutic and preventive action.

Further research regarding FTD is of importance for a number of reasons. First of all, accurate diagnosis of FTD at an early stage of the disease is necessary to improve the value of clinical studies. Knowledge regarding both epidemiology and the spectrum of clinical manifestations resulting from this disease is indispensable in this respect, as well as the development of novel diagnostic tools, such as biomarkers. This presenile disorder invariably has great impact on close family members, as the severe behavioural and personality changes often lead to distressing situations at home. Early diagnosis is therefore also of great importance for the family to understand and accept the changes in behaviour in the patient. Secondly, although much has been learnt over the past few years regarding the *tau* gene, identification of new *tau* mutations may give more insight into the different mechanisms through which tau dysfunction can lead to neurodegeneration. Subsequent development of functional studies and animal models yields invaluable information on the pathophysiological mechanisms involved in each type of mutation. Thirdly, little is known regarding the aetiology of FTD in patients without *tau* mutations, over 80% of the FTD population. Other genetic and possibly environmental causes still have to be identified.

In this chapter the studies described in this thesis are discussed in the context of our current knowledge regarding neurodegeneration in general and FTD in particular. After some methodological considerations, the implications of the main findings are discussed, and suggestions for future research are made.

Methodological considerations

Epidemiological studies

Because FTD is a rare disorder, the patients in this study were collected from throughout the Netherlands, which has a population of more than 15 million inhabitants. We regularly requested all hospital-based neurologists and psychiatrists, as well as nursing home physicians, to refer patients with suspected FTD to us. In this way we hoped to ascertain all patients in the Netherlands with FTD. Unfortunately, as shown in chapter 2.1, this approach was not entirely successful, with estimated prevalences in Zuid-Holland being twice as high as in other provinces. As there is no evidence for familial clustering in Zuid-Holland, this difference is probably due to underascertainment in other provinces. As is discussed in chapter 2.1, explanations for underascertainment may be found in either misdiagnosis or non-referral, both of which occurred in our study.

The only other study regarding the prevalence of FTD used a different approach and investigated all patients with presenile dementia in a small, but well-defined population in Cambridge, United Kingdom (UK).[1] Underascertainment is less frequent in such a study, with misdiagnosis only occurring if a dementia syndrome is not recognised by the primary physician. However, there are also drawbacks to this study. First of all, neuropathological verification was not available, making it impossible to determine the accuracy of clinical diagnosis. A second drawback is the fact that this population was small, and may not be representative for the general UK population. For instance, the prevalence of Huntington's disease was extremely high in the Cambridge area, because of the presence of a specialised nursing home. Environmental factors may also affect prevalence estimates in a small population. The extreme overrepresentation of men in the Cambridge study (14 men, three women) may point in the direction of occupational risk factors, e.g. mining or exposure to chemicals in factories, which may be more common within specific populations.

The province of Zuid-Holland is suitable for epidemiological studies, because of the population size (over 2 million inhabitants), density (relatively small area) and diversity (occupations, ethnic backgrounds). To improve the degree of ascertainment in our study, multiple sources of case ascertainment should be employed to minimise non-referral. On top of the approaches already taken, general practitioners should be regularly requested to participate, so that patients not referred by their primary specialist or patients referred to hospitals outside the province would still be identified. Psychological practices and psychiatric hospitals should also be involved, as many patients are mistakenly diagnosed with psychiatric disorders such as depression. Finally, in order to reach patients and family directly, information regarding the study should be

available on the internet. By comparing the degree of ascertainment for each method and application of the capture-recapture technique propagated by Laporte,[6] an estimate of the degree of under-ascertainment and subsequent correction may be attempted.
Ideally, to completely resolve the issue of underascertainment in our study, a more rigorous approach to patient ascertainment would have to be pursued. Indeed, an epidemiological study regarding the prevalence of PSP in the U.K. investigated the effect of different methods of case ascertainment, a so-called 'Russian doll' design.[7] Best results were achieved with an active method of case ascertainment, in which all computerised records of general practitioners were screened for PSP in a community based setting. However, this approach would be extremely time-consuming if applied to a large population such as Zuid-Holland, and may not be as efficient as in PSP, as relatively objective physical findings or therapeutic options are absent in FTD. In my opinion the amount of time and labour involved in obtaining an accurate estimate of the prevalence should be carefully weighted against the relevance of the information gained.

Temporal variant of FTD

Ascertainment of patients with the temporal variant of FTD in the current study has been difficult due to the fact that the clinical criteria were not established until 1998.[8] The temporal variant of FTD may present as primary progressive aphasia or semantic dementia, depending upon the distribution of temporal atrophy on neuroimaging,[9,10] and patients with either of these syndromes were not included into our study until 1999. Patients who had been seen before that time were re-evaluated, and a number were included retrospectively. However, the percentage of patients not referred to us prior to 1999 due to the atypical presentation of FTD, is unknown.
Alternatively, recently an increasing number of patients with language difficulties were referred because of suspected FTD. However, language difficulties may also be prominent in other neurodegenerative diseases, such as Alzheimer's disease (AD), making the clinical distinction quite difficult.[11,12] Typical in FTD patients, including those with the temporal variant, is the focal and often asymmetric aspect of atrophy on neuroimaging.[13] If the atrophy tends to generalise, other disorders as AD are more likely. The chance of misdiagnosis is higher in the temporal variant of FTD, as AD also may present with atrophy of the medial temporal lobe, possibly resulting in an overestimation of the prevalence of FTD. Our approach, using strict criteria for focal and asymmetric atrophy, was found to be successful, as shown by the fact that the diagnosis FTD was confirmed in all 13 patients with the temporal variant of FTD who came to autopsy during the course of the study.

Genetic studies

During the nineties, linkage to chromosome 17q21-q22 was found in a number of large pedigrees with an autosomal dominant pattern of inheritance of variable dementia syndromes. Remarkable names were used for these syndromes, such as disinhibition-dementia-parkinsonism-amyotrophic complex (DDPAC),[14] pallido-ponto-nigral degeneration (PPND),[15] familial progressive subcortical gliosis,[16] familial multiple system tauopathy,[17] rapidly progressive familial frontotemporal dementia,[18] and others. In 1997, at a consensus meeting in Ann-Arbor, the similarities between these syndromes induced the collective term frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17).[19] In 1998 mutations in the *tau* gene were identified in a large number of these families, confirming the common origin of these syndromes.[3-5] However, the great clinical diversity, both within families and between families, has complicated ascertainment of suitable patients for genetic studies.

During this study, we regularly requested participating physicians to refer patients with suspected FTD. However, as can be derived from the names of above mentioned syndromes, familiar FTD may present with a variety of clinical features not typical of FTD. Indeed, the novel S320F mutation described in chapter 4.1 was found in a patient initially diagnosed with AD. Only after neuropathological examination showing tauopathy without any amyloid deposition, mutation analysis of the *tau* gene was performed. This clearly shows how other familial FTD cases with atypical presentation may have been missed. Indeed, the virtual absence of intronic *tau* mutations in the Dutch population may be explained non-referral or exclusion of patients with prominent parkinsonian features. However, intronic mutations were also absent in the French population.[20,21] Furthermore, sequencing of the *tau* gene in a series of patients with AD did not reveal any mutations, making the S320F patient probably an exception.[22]

In establishing the frequency of *tau* mutations in the Dutch FTD population, we were faced with the problem of referral bias. FTD patients with a positive family history are more likely to be referred, as family members are more keen to gain information and to contribute to research for future generations. This bias leads to an overestimation of familiar forms of FTD, and is even present in pathology series, as next-of-kin are often more willing to consent to autopsy in familial disorders. Most studies report similar findings of positive family history in about 40% of FTD patients, but the percentage of *tau* mutations ranges considerably.[23-25] Both the Dutch and the Swedish FTD population have a positive family history of dementia in about 35 to 40% of patients, but mutations were found in 14% in the Dutch (chapter 2.1) and in none of the Swedish patients.[25] Although population-dependent factors cannot be excluded (founder effect of common mutations), this difference may also be due to application of stringent diagnostic criteria

in our study, as well as the evaluation of family history. We considered the family history only to be positive if a first degree relative developed signs of dementia before the age of 80, in order to exclude FTD patients with a family member with AD at old age. The criteria used in the Swedish population are unclear and may be less strict.[25]
A final methodological consideration concerns the evaluation of the pattern of inheritance in familiar cases. Up to date, nearly all mutations in the *tau* gene have an autosomal dominant effect with a high degree of penetrance. Only one pedigree has been reported with PSP patients homozygote for a *tau* mutation (ΔN296), with heterozygotes being less severely affected (late onset parkinsonism).[26] Recessive forms of FTD may exist and consanguinity of parents may point in this direction. However, the finding of non-penetrance in the family with the L315R mutation (chapter 4.2) emphasises that family history should be extensively evaluated. If multiple siblings are affected and both parents reached old age without dementia, this may be due to non-penetrance in the parents due to a dominant mutation. Further evaluation of second degree relatives may reveal which type of inheritance it concerns if DNA is not available.

Main findings and their implications

The *tau* gene in hereditary frontotemporal dementia

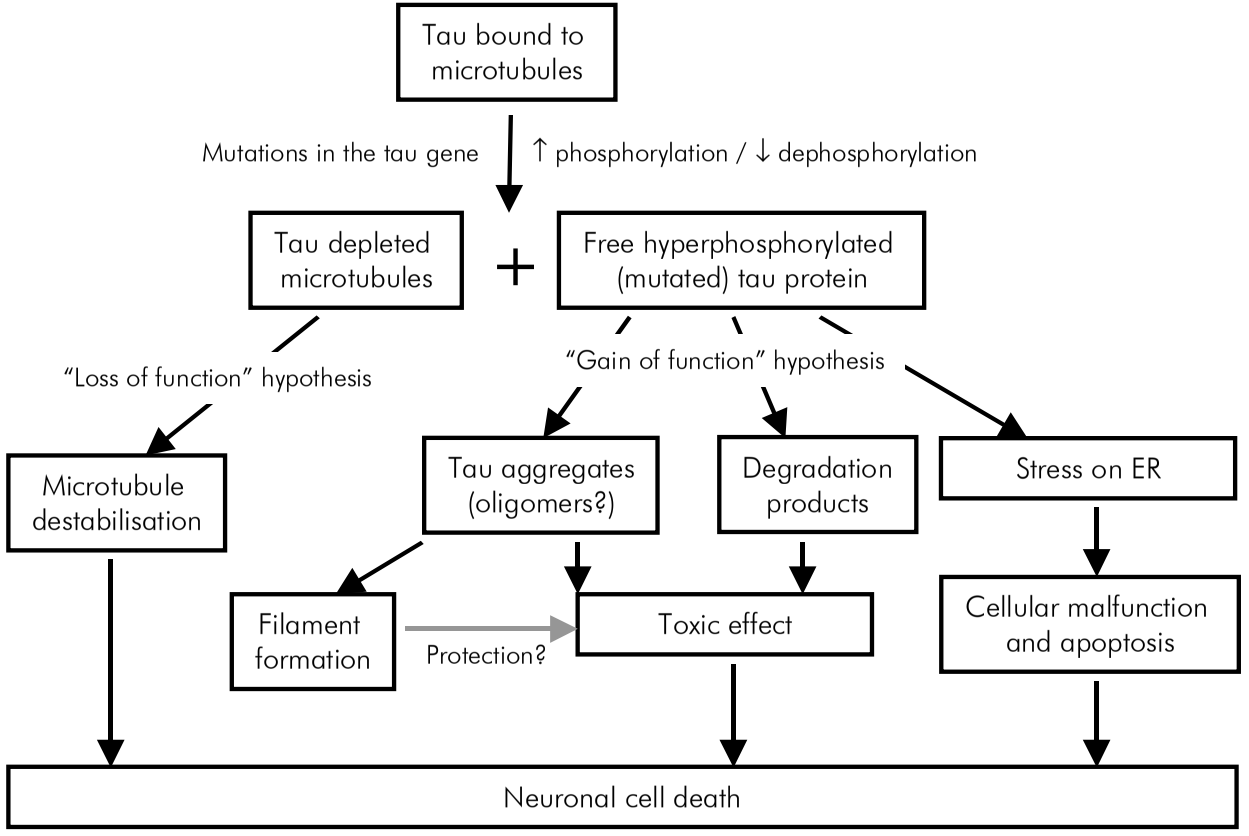
At the present, over 20 different *tau* mutations have been identified in patients with familiar FTD (chapter 1.3).[27] Although much has been learnt since 1998 regarding the effect of *tau* mutations, it is currently still unknown how they lead to neuronal cell death. The complexity of tau function, effects of mutations and involvement in neurodegeneration is reviewed amongst others by Shahani *et al*.[28]
Most mutations cluster around the 5'-end of the *tau* gene in exons 9 to 13, although mutations in a single codon in exon 1 have been reported.[29,30] Mutations may affect the alternative splicing of exon 10 (measured by exon-trapping and resulting in an altered ratio of 3 to 4 repeat tau), alter the binding properties of tau with respect to tubulin (resulting in a decreased microtubule assembly rate), or enhance the self-aggregation of tau into filaments. Functional studies are available to measure all three effects *in vitro*, and are frequently used to evaluate whether an observed base-change may be pathogenic. Unfortunately, the severity of the effect of the mutation does not correlate well with the observed phenotype in patients. For instance, the R406W mutation causes a severe reduction of microtubule assembly rate,[31] while patients with this mutation have a relatively late age at onset and longer duration of disease.[32]
The two novel *tau* mutations reported in chapter 4.1 (S320F) and 4.2 (L315R) are the only two mutations found to date in exon 11 of the *tau* gene. Both mutations were

associated with extensive tau pathology with Pick-like inclusions, although the astrocytic tau pathology observed in L315R was not present in S320F. These mutations are only 5 amino acids separated from each other, but have a very different effect on the binding properties of the tau protein. The amino acid change in S320F is located within the microtubule binding domain of the protein and subsequently results in a large (95%) reduction of the microtubule assembly rate. The L315R mutation, however, is located outside the microtubule binding domain and the microtubule assembly rate was reduced by only 20 to 25%. The filament assembly rate was also mildly affected in L315R, with just a 15% increase in only the 4 repeat isoforms. Together with the extreme variance in age at onset in patients with the L315R mutation, this suggests that other mechanisms probably play a role in the pathogenic effect of *tau* mutations in general.

Mutations in most dominant disorders usually result in a gain of function of the mutated protein. Loss of function of the tau protein due to a dominant *tau* mutation is less likely, as experimental studies with *tau* knock-out mice do not reveal a severe phenotype.[33] The fact that tau mutations lead to a neurodegenerative disorder with onset at adult age is also more consistent with a slow accumulation of a toxic process over time. A gain of function of mutated tau protein may be found in a toxic effect of either the unbound tau protein itself (for instance in hyperphosphorylated state), tau aggregates (dimers, oligomers or filaments), or degradation products of the mutated tau protein. The different mechanisms by which tau mutations may lead to neurodegeneration are schematically represented in Figure 1. Identification of the correct pathway to neurodegeneration may lead to the development of functional studies that measure an effect of *tau* mutations which shows more correlation to the clinical phenotype.

Neuropathologically, all *tau* mutations are characterised by the accumulation of filamentous tau in the cytoplasma of affected neurons and glial cells. Aggregation of tau into insoluble filaments may however be a mechanism by which neurons neutralise mutant tau protein. Similar observations have been made in experimental models of polyglutamine disease, where interruption of inclusion formation by mutant polyglutamine results in enhanced toxicity.[34] Recently, excessive accumulation of proteins (such as synucleins, serpins, amyloid and tau) became recognised as a common cause for stress of the endoplasmic reticulum (ER), and subsequent cellular malfunction and apoptosis. This interesting observation might explain part of a common pathogenic pathway of accumulation of apparently heterogeneous proteins in different neurodenerative disorders.[35-37]

Figure 1. Possible pathways leading to neurodegeneration in FTD due to *tau* mutations*



*Adapted from Shahani and Brandt, 2002.[28]

Interaction between tau and other proteins, such as beta-amyloid ($A\beta_{1-40}$ and $A\beta_{1-42}$), is also subject of much research at this moment. AD is neuropathologically characterised by both deposition of Aβ and tau in affected brain regions. Mutations found in hereditary AD (amyloid precursor protein gene (APP), presenilin 1 and 2 genes) all lead directly to increased production of Aβ, making this probably one of the first steps in the cascade to neurodegeneration in AD, with tau deposition playing a secondary role. FTD patients with *tau* mutations rarely develop Aβ deposition (amyloid plaques), suggesting that these mutations intervene at a later stage and that the cascade to neurodegeneration is a one-way street.[38] Induction of tau pathology by Aβ has been observed in experimental studies. Lewis *et al.* showed that double mutant tau/APP transgenic mice have a much more severe phenotype and degree of tau pathology than transgenic mice with only *tau* mutations.[39] This observation is especially interesting when one takes into account that APP transgenic mice show only Aβ deposition and never tau pathology, unlike AD patients. Secondly, Aβ injected into the cortex of mice transgenic for the P301L *tau* mutation showed a severe increase in tau deposition.[40] This increase was not found at the cortical injection site, but in the amygdala from where the axons project. In light of this knowledge, the Aβ deposition in FTD patients with *tau* mutations (chapter 4.3), probably due to an unrelated cause such as old age or co-occurrence of AD, may well have accelerated tau deposition in these patients, resulting in more severe pathology than in other patients with the same mutation and comparable disease duration.

FTDP-17 without *tau* mutations

Tau mutations account for only 10 to 50% of all cases of familiar FTD[23,24,41] with the genetic cause for the remaining patients still unknown at this point in time. A number of families with linkage to the tau-containing region of chromosome 17q21-22 show neither *tau* mutations, nor tau pathology at neuropathological examination.[42-44] Although this is in sharp contrast to all of the patients with known *tau* mutations, it cannot be excluded that these patients have a novel type of *tau* mutation (for instance in the regulatory region of the gene) which may lead to neurodegeneration through a different mechanism. Zhukareva *et al.* reported that patients from one of these families (HDDD2), as well as a number of sporadic FTD patients with Dementia Lacking Distinctive Histology (DLDH), were characterised by loss of normal brain tau protein.[45] Although this observation might explain the lack of tau deposition in these patients, it is hard to imagine that a dominant mutation (present in only one of the two *tau* gene copies) may lead to less than 50% production of tau protein. Furthermore, transcription of the *tau* gene in these patients was normal, making mutations in the regulatory regions

unlikely. The authors suggest that the problem would therefore be post-transcriptional, at the level of translation or messenger RNA stability.[45] Remarkably, the loss of brain tau was found both in brain regions with and without neurodegeneration. This is in contrast to FTD patients with *tau* mutations, in whom tau deposition takes place only in the regions with neurodegeneration, mainly the frontotemporal cortex. So far, the observation of reduced levels of tau protein in subgroups of FTD patients has not been reproduced by other groups.[46] In my opinion, it is more likely that a second gene, located close to the tau gene, is involved in this type of FTD.

The Dutch HFTD3 family (chapter 5.1) shows ubiquitin-positive inclusions, which are not found in DLDH, and normal levels of normal brain tau, suggesting a different aetiology. A family with similar pathological features, also from the Netherlands, has been reported by Rademakers *et al.*[47] Linkage to chromosome 17q21-q22 was also found in this family, with a maximal lod-score of 5.5. When we combine genetic information of both of these families, assuming a common genetic defect, linkage is restricted to a 4 Mbp region containing about 130 genes, including the *tau* gene. Before mutations in the *tau* gene were identified, a number of candidate genes were sequenced in the search for pathogenic mutations in FTDP-17. The gene for Glial fibrillary acidic protein (GFAP), is still located within the critical region of linkage, but was sequenced by Isaacs *et al.* without mutations being found in a number of FTD families, including HFTD3 and HDDD2.[48] Two other genes which were sequenced at this time, NIK protein kinase and C17orf1, now lie outside the critical region of linkage.[49] However, a number of interesting candidate genes remain.

First of all, a recently discovered gene named Saitohin is located within intron 9 of the *tau* gene.[50] A single nucleotide polymorphism that results in an amino acid change (Q7R), in complete linkage disequilibrium with the well-defined extended *tau* haplotype, was associated with late-onset AD (RR genotype).[50] Although this could not be replicated in two subsequent studies with larger AD populations,[51,52] a trend towards an association between the QQ genotype and FTD was found.[51] The saitohin Q allele can be considered as a novel determinant of the *tau* H1 haplotype, which has previously also been implicated in FTD,[53] as well as a number of tauopathies such as PSP and CBD.[54,55] The function of the saitohin gene is largely unknown, although it's expression profile is similar to that of tau. The reported association might be due to the influence of the *tau* gene, and more work is clearly needed to clarify the association.

One of the most promising candidate genes within the region of linkage on 17q21-q22 is the Gamma-tubulin gene. The protein is probably a universal component of the microtubule organising centers and although gamma-tubulin is present at less that 1% of the level of alpha- and beta-tubulin, it is limited to the centrosome.[56] In particular, it is

associated with the pericentriolar material, the microtubule-nucleating material of the centrosome. This is of particular interest with respect to the localisation of the ubiquitin-positive inclusions found in the HFTD3 family, which are preferentially located directly adjacent to the nucleus. Another interesting candidate gene is the Proteasome activator subunit 3 (PSME3) gene.[57,58] The corresponding protein is part of the proteasome activator 28 (PA28) complex, which is an alternative proteasome activator that does not employ the use of ubiquitin in protein degradation. Hypothetically, mutations in this gene may lead to disruption of the PA28 complex, resulting in excessive use of the ubiquitin-dependent pathway of protein degradation, which may ultimately lead to accumulation of ubiquitinated proteins in inclusions or stress on the ER.

Other forms of hereditary frontotemporal dementia

Genetic heterogeneity is further emphasised by the identification of three additional loci in pedigrees of patients with FTD and related disorders. First of all, linkage to the pericentromeric region of chromosome 3 was found in a large Danish pedigree described by Brown *et al.*[59] The clinical features in this family fulfil the Lund-Manchester criteria for probable FTD, although involvement of the parietal lobes was quite prominent on neuroimaging.[60] In contrast to our HFTD3 family (chapter 5.1), the neuropathological changes in the Danish family are compatible with the diagnosis DLDH, without tau- or ubiquitin-positive deposits. The region of linkage is about 23.6 Mbp, but contains only about 125 genes, mostly of unknown function making it difficult to identify a good candidate gene. However, some of the genes with known function show some promise. For instance, the gene for the serotonin receptor, as reduced levels of post-synaptic serotonin have been found in post-mortem studies of FTD patients.[61-64] Furthermore, a partial treatment response to SSRIs was found in a small sample of FTD with behavioural problems.[65]

Hosler *et al.* found linkage to a 12 Mbp locus on chromosome 9q21-q22 in a group of 22 families with both FTD and amyotrophic lateral sclerosis (ALS).[66] Linkage to this locus was not found in a subset of families with only ALS, suggesting a specific phenotype for this locus. Features of ALS or motor neurone disease (MND) occurred in only about 4% of our total population, although six of these patients came from families with autosomal inheritance of both dementia and ALS. Unfortunately, it is difficult to obtain DNA for linkage analysis in these families, as patients with ALS or MND have a very rapid disease progression. Neuropathologically, FTD with MND is characterised by ubiquitin-positive inclusions, similar to those found in family HFTD3 (chapter 5.1), although none of the family members had any signs of MND. The region of linkage on chromosome 9q21-q22 contains 94 genes, none of which show any functional similarity to the candidate

genes discussed for chromosome 17q21-q22. It may be interesting to compare genes with unknown function in both regions of linkage for homology.
Finally, linkage to another region on chromosome 9 (9p13.3-p12) was found by Kovach *et al.* in four families with autosomal inheritance of a unique syndrome: premature FTD, inclusion body myopathy (IBM), and Paget's disease of bone.[67] Neuropathological features from these families are incompletely established, making it difficult to classify the dementia into one of the subgroups. The association with IBM is however very interesting, as deposition of both tau and amyloid has been described in muscle biopsies of patients with sporadic IBM, accentuating the similarity to neurodegenerative disorders such as AD.[68,69] Mutations in the UDP-N-acetylglucosamine-2-epimerase/N-acetyl-mannosamine kinase (GNE) gene, located within the critical region of linkage, have recently been found in recessive forms of IBM without FTD.[70] It is possible that different types of mutations within the same gene may lead to different phenotypes (allelic variants), making the GNE gene also a good candidate for this autosomal dominant disorder.

Suggestions for future research

The Lund-Manchester criteria (in combination with neuroimaging and psychometric evaluation) are very accurate in distinguishing FTD from other neurodegenerative disorders, reaching a sensitivity and specificity of about 97%.[71] However, it is difficult to accurately diagnose FTD at early stages of the disease, even with the aid of new functional imaging techniques. In light of the progression made regarding new therapeutic interventions, it is of the utmost importance to identify biological markers that can differentiate early signs of FTD from both psychiatric and other neurodegenerative disorders. Much attention is currently being paid to the analysis of CSF, with promising results for AD with respect to the application of total tau, phospho-tau and amyloid measurement in a diagnostic setting.[72,73] CSF analysis in patients with FTD has lead to some contrasting results. For instance, increased CSF total-tau has been found by some groups, but not by others.[25,74,75] This is not very surprising, given the fact that FTD is a clinical syndrome that may be caused by a number of different neuropathological disorders. Unfortunately, there are currently no accurate ways to predict the subtype of pathology based on clinical or radiological features. As shown in chapter 4.4, patients with *tau* mutations (known to be associated with deposition of tau protein in affected brain regions), did not have an increase in either total tau or phospho-tau181 in CSF. Therefore, it is of importance that findings from CSF analyses

are related to the post-mortem diagnosis, as the relationship between neuropathology and subsequent protein changes in CSF is more complex than previously expected.
The novel *tau* mutation L315R (chapter 4.2) is associated with a very variable phenotypic expression, with one carrier dying at the age of 33 of Pick's disease and another carrier being cognitively intact at age 82. This is in contrast to most other *tau* mutations, which show a high degree of penetrance and less variation in the age at onset of FTD.[31] Therefore, this family is extremely interesting with respect to identification of possible modifying factors, either of genetic or environmental origin. Identification of protective or detrimental modifying factors will lead to more understanding regarding the effect of *tau* mutations on neuronal cell loss, and may eventually lead to development of new therapeutic interventions.
Regarding family HFTD3 (chapter 5.1), if sequencing of candidate genes does not yield any new information, it will be important to identify which proteins accumulate within the ubiquitinated inclusions. New techniques for analysing protein composition of tissue, for instance mass spectrometry, may be of value in the identification these proteins. Because fresh-frozen brain tissue from this family is very scarce, it may be prudent to analyse brain tissue from unrelated patients with FTD with ubiquitin inclusions, as the pathology of both subtypes is remarkably similar.
Finally, the main question which arises when studying neurodegenerative disease is: why are specific cell populations more vulnerable in specific disorders? The fact that different diseases present with typical clinical symptoms is largely related to the subgroup of neurons which are affected by the disease. The neurons of the frontal and anterior temporal lobes progressively degenerate in FTD, with relative sparing of the parietal and occipital lobe at even late stages of the disease. Although we have learnt much regarding tau function and dysfunction, it is currently still unclear why a germ-line mutation in a gene coding for a protein expressed in neurons throughout the brain, should lead to problems is such specific groups of neurons. Although this will be a difficult question to answer, with the development of microchip arrays we have a powerful tool to screen for a large number of transcription factors and proteins in different brain regions, giving more insight in the differences between specific cell populations, and possibly resolving this enigma.

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7

Summary & Samenvatting

Summary

Frontotemporal dementia (FTD) is a neurodegenerative disorder of mainly presenile onset, characterised by progressive behavioural disturbance, aphasia, and a decline in frontal cognitive functions. Although FTD is a relatively rare disorder compared to the more common Alzheimer's disease, research regarding FTD is of importance for a number of reasons. First of all, accurate diagnosis of FTD at an early stage of the disease is necessary to improve the value of clinical studies, especially in light of future therapeutic interventions. Secondly, elucidation of the pathophysiological pathways leading to neurodegeneration in FTD may yield knowledge regarding a number of related neurodegenerative disorders. The identification of mutations in the *tau* gene in hereditary FTD is an important example in this respect.

This aim of this thesis was to describe epidemiological and clinical characteristics of the FTD patient population in the Netherlands, with emphasis on hereditary forms of FTD. This study was part of an ongoing population-based genetic-epidemiological study on FTD in the Netherlands, which was started in 1994 at the Erasmus University Rotterdam.

Chapter 1.1 gives an general introduction to the thesis. This is followed by an introduction to FTD in general in **chapter 1.2**, and an introduction to hereditary forms of FTD in **chapter 1.3**.

Chapter 2 describes two epidemiological studies on FTD. **Chapter 2.1** presents prevalence estimates on FTD in the Netherlands, with emphasis on the prevalence in the province Zuid-Holland where the study centre is located. FTD was diagnosed in 245 patients (51% female), the largest FTD patient population described to date. The prevalence of FTD in the province Zuid-Holland was higher than reported in previous studies, with a maximum of 9.4 per 100.000 inhabitants at age 60 to 69 years. Tau mutations were found in 34 patients, all with a positive family history for dementia (14% of the total population; 32% of patients with a positive family history). **Chapter 2.2** concerns a retrospective case-control study with 80 patients with the sporadic form of FTD and 124 age, sex, and surrogate informant matched control subjects, regarding various medical and environmental risk factors. Head trauma was associated with a 3.3 times increased risk of FTD (95% confidence interval: 1.3 to 8.1) and thyroid disease with a 2.5 times increased risk (95% CI: 0.9 to 7.9). This study is the first to address non-genetic risk factors for sporadic FTD, and further studies will be important to confirm the observed associations.

Chapter 3 focuses on FTD patients with mainly temporal atrophy, a clinical subgroup of FTD not formally recognised until the new criteria for FTD in 1998. **Chapter 3.1**

investigates the correlation between complex compulsive behaviour (CCB) and the distribution of atrophy in a group of 90 FTD patients. Logistic regression analysis showed that temporal lobe atrophy ($p<0.005$), as well as asymmetry of atrophy ($p<0.05$) were independently associated with CCB, after adjusting for age at onset, gender, duration of symptoms at the time of imaging, severity of atrophy, and bicaudate and bifrontal ratio. This suggests that temporal lobe atrophy may mediate CCB in patients with FTD, especially if asymmetry of atrophy is present. **Chapter 3.2** is a letter on the association between the apolipoprotein E4 allele and distribution of atrophy in FTD patients. Although the frequency of the ApoE4 allele was not increased in FTD patients compared with non-demented controls, in the subgroup of FTD patients with temporal atrophy the ApoE4 allele was significantly more common, suggesting that apoE genotype may be involved in determining the distribution of atrophy in FTD.

The role of the tau gene in hereditary FTD is explored in **Chapter 4**. In **chapter 4.1 and 4.2** we describe families with presenile dementia due to novel missense mutations in exon 11 of the *tau* gene, namely S320F and L315R. To our knowledge, they are the first mutations to be described in exon 11 of *tau*. Clinical, pathological, biochemical data are presented, as are functional studies regarding the effect of the mutations on tau function *in vitro*. These mutations highlight the importance of variable clinical penetrance and the need to develop functional studies which measure effects of *tau* mutations which correlate with the clinical phenotype. **Chapter 4.3** describes the neuropathological findings in five patients with P301L and in two patients R406W *tau* mutations. Amyloid pathology is observed in two patients (one P301L and one R406W), both with deposition sarkosyl-insoluble tau protein, consisting of four bands (60, 64, 68, and 72 kDa) and containing all six tau isoforms, in the temporal cortex. Although the FTD and Alzheimer's disease-type changes are probably concomitant, there may be an interaction between tau and amyloid pathology, in the form of acceleration of tau pathology due to amyloid deposition. In **chapter 4.4** we analysed total tau, phosphotau 181, and amyloid-β_{1-42} in CSF of 26 FTD patients, including nine with *tau* mutations (seven P301L, two G272V). Although CSF total tau was mildly increased in FTD, this increase was not seen in the subgroup with *tau* mutations. Furthermore, CSF phospho-tau 181 and $A\beta_{1-42}$ levels were not different compared with non-demented controls. Thus, we conclude that the tau pathology present in P301L and G272V brain does not appear to be associated with an increase in either CSF total tau or phospho-tau 181, and that the relationship between neuropathological changes in the brain and subsequent protein alternations in CSF is more complex than expected.

Chapter 5 deals with hereditary forms of FTD that do not show any *tau* mutations. In **chapter 5.1** 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. Pathological examination of the brains of two affected family members showed non-specific neuronal degeneration with ubiquitin-positive inclusions in neurones. Biochemical analysis of soluble tau did not reveal abnormalities in tau isoform distribution. Therefore, we suggest that the FTD in this family may be caused by a gene other than the tau gene itself, located within the same region on chromosome 17q21-q22.

In **chapter 6** methodological considerations of these studies are presented, focussing mainly on different aspects of study design in epidemiological studies. The most important findings of the study are also reviewed and finally suggestions for future research are made.

Samenvatting

Frontotemporale dementie (FTD) is een neurodegeneratieve aandoening met een voornamelijk preseniele presentatie, gekarakteriseerd door progressieve gedragsstoornissen, afasie, en een teloorgang van de frontale cognitieve functies. Hoewel FTD een relatief zeldzame aandoening is vergeleken met de veel vaker voorkomende ziekte van Alzheimer, is onderzoek naar FTD van belang voor een aantal redenen. Ten eerste, een betrouwbare diagnose van FTD in een vroeg stadium van de ziekte is belangrijk voor de verbetering van de betrouwbaarheid van klinische onderzoeken, vooral met het oog op toekomstige therapeutische opties. Ten tweede, verheldering van de pathofysiologische mechanismen van neurodegeneratie in FTD kan leiden to inzicht in een groot aantal andere verwante aandoeningen. De identificatie van mutaties in het *tau* gen in erfelijke FTD is hier een belangrijk voorbeeld van.

Het doel van dit proefschrift was het beschrijven van epidemiologische en klinische eigenschappen van de Nederlandse FTD populatie, met nadruk op de erfelijke vormen van FTD. Deze studie is onderdeel van een lopende genetisch-epidemiologische studie naar FTD in Nederland, welke in 1994 is opgezet aan de Erasmus Universiteit Rotterdam.

Hoofdstuk 1.1 is een algemene inleiding van het proefschrift. In **hoofdstuk 1.2** wordt een introductie over FTD in het algemeen gegeven, terwijl **hoofdstuk 1.3** zich richt op met name de erfelijke vorm van FTD

Hoofdstuk 2 beschrijft een tweetal epidemiologische studies naar FTD. **Hoofstuk 2.1** presenteert prevalentie schattingen van FTD in Nederland, met nadruk op de prevalentie van FTD in Zuid-Holland, de provincie waar de Erasmus Universiteit Rotterdam zich bevindt. De diagnose FTD werd gesteld in 245 patiënten (51% vrouwen), de grootste FTD populatie tot op heden beschreven. De prevalentie van FTD in Zuid-Holland was hoger dan eerder werd gerapporteerd, met een maximum van 9.4 per 100.000 inwoners in de leeftijd van 60 tot 69 jaar. *Tau* mutaties werden gevonden in 34 patiënten, allen met een positieve familie anamnese voor dementie (14% van de totale populatie, 32% van de patiënten met een positieve familie anamnese). **Hoofdstuk 2.2** betreft een retrospectieve case-control studie met 80 patiënten met de sporadische form van FTD en 124 leeftijd, geslacht en heteroanamnese gematchde controle personen, aangaande diverse medische en omgevingsfactoren. Hoofdtrauma was geassocieerd met een 3.3 maal verhoogde kans op FTD (95% betrouwbaarheids interval: 1.3 to 8.1) en schildklier aandoeningen met een 2.5 maal verhoogde kans (95% BI: 0.9 to 7.9). Deze studie is tot op heden de eerste die zich richt op non-

genetische risico factoren voor FTD, en verder onderzoek zal van belang zijn om de gevonden associaties te bevestigen.

Hoofstuk 3 betreft FTD patiënten met vooral temporale atrofie, een klinische subgroep die pas sinds de nieuwe criteria van 1998 officieel wordt herkend. **Hoofdstuk 3.1** onderzoekt de correlatie tussen complex dwangmatig gedrag en de verdeling van atrofie op structurele beeldvorming in een groep van 90 FTD patiënten. Met logistische regressie analyse werd aangetoond dat met name temporale atrofie ($p<0.005$), maar ook asymmetrische atrofie ($p<0.05$) onafhankelijk van elkaar zijn geassocieerd met dwangmatigheid, na aanpassing voor beginleeftijd, geslacht, duur van de symptomen tijdens beeldvorming, ernst van de atrofie, bicaudatus ratio en bifrontale ratio. Dit suggereert dat de temporaal kwab een rol speelt bij het ontstaan van dwangmatigheid in FTD, vooral als er sprake is van asymmetrische atrofie. **Hoofdstuk 3.2** is een brief over de associatie tussen het apolipoproteïne E4 allel en de verdeling van atrofie in FTD patiënten. Hoewel de frequentie van het ApoE4 allel niet verhoogd was bij patiënten met FTD vergeleken met niet-dementerende controle personen, was in de subgroep van FTD patiënten met temporale atrofie het ApoE4 allel significant vaker aanwezig. Dit suggereert dat het ApoE genotype een rol speelt in het bepalen van de distributie van atrofie in FTD.

De rol van het *tau* gen in de erfelijke vorm van FTD wordt bestudeerd in **Hoofdstuk 4**. In **hoofdstuk 4.1 en 4.2** beschrijven we een aantal families met preseniele dementie ten gevolge van nieuwe mutaties in exon 11 van het *tau* gen, namelijk S320F en L315R. Tot op heden zijn dit de enige mutaties gevonden in exon 11 van *tau*. Klinische, pathologische en biochemische bevindingen worden beschreven, als ook functionele studies die het effect van de mutaties *in vitro* meten. Deze mutaties benadrukken het belang van variabiliteit in klinische penetrantie en de noodzaak om functionele testmethoden te ontwikkelen die effecten van mutaties meten die correleren met het klinisch fenotype. **Hoofdstuk 4.3** beschrijft neuropathologische bevindingen in vijf patiënten met P301L en twee patiënten R406W *tau* mutaties. Amyloid pathologie werd gevonden in twee patiënten (een P301L en een R406W), beiden met depositie van sarkosyl-onoplosbaar tau eiwit, bestaande uit vier banden (60, 64, 68, en 72 kDa) die alle zes de tau isoformen bevatten, in de temporale cortex. Hoewel deze FTD en AD-achtige veranderingen waarschijnlijk concomitant zijn, is het mogelijk dat er een interactie tussen tau en amyloid pathologie optreedt in de vorm van een acceleratie van de tau pathologie door amyloid depositie. In **hoofdstuk 4.4** analyseerde we totale tau, phosphotau 181, and amyloid-β_{1-42} concentraties in liquor cerebrospinalis van 26 FTD patiënten, waarvan negen mutaties in het *tau* gen hadden (zeven P301L, twee G272V). Hoewel de totale tau concentratie licht verhoogd was in de FTD groep, bleek

deze toename niet aanwezig te zijn in de subgroep van patiënten met *tau* mutaties. Bovendien, phospho-tau 181 en $A\beta_{1-42}$ concentraties in de liquor cerebrospinalis waren niet verschillend vergeleken met niet-dementerende controle personen. Hierdoor concluderen wij dat de tau pathologie die gevonden wordt in hersenen van patiënten met P301L en G272V mutaties niet gepaard gaat met een toename van totale tau of phospho-tau 181 in de liquor, en dat de relatie tussen neurodegeneratie en de daaropvolgende eiwit veranderingen in de liquor meer complex is dan verwacht.

Hoofdstuk 5 richt zich op de erfelijke vormen van FTD waarbij geen mutaties in het *tau* gen worden gevonden. In **hoofdstuk 5.1** beschrijven we de klinische en neuropathologische kenmerken van een grote familie met FTD. Genetisch onderzoek leverde bewijs voor koppeling aan het *tau*-bevattende deel van chromosoom 17q21-q22 (maximale lod score 3.46, $\theta=0$ voor marker D17S950), maar mutaties in het *tau* gen werden niet gevonden. Pathologisch onderzoek van de hersenen van twee aangedane familieleden toonde aspecifieke neuronale degeneratie met ubiquitine-positieve inclusies in neuronen. Biochemisch onderzoek van oplosbaar tau eiwit liet geen afwijkingen zien in de distributie van de tau isoformen. Het is daarom goed mogelijk dat de FTD in deze familie wordt veroorzaakt door een ander gen, dat gelegen is in de nabijheid van het *tau* gen zelf.

In **hoofdstuk 6** worden methodologische aspecten van deze studie besproken, met nadruk op de verschillende technieken in epidemiological studies. De belangrijkste bevindingen van de studie worden doorgenomen en als laatste worden suggesties voor toekomstig onderzoek gemaakt.

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