Frontotemporal Dementia

clinical, genetic, and pathological heterogeneity

Harro Seelaar

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Frontotemporal Dementia

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Frontotemporale Dementie

klinische, genetische en pathologische heterogeniteit

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Introduction

Chapter 1.1

Introduction to the Thesis

The current clinical syndrome frontotemporal dementia (FTD) was first described in 1892 by the Czech psychiatrist Arnold Pick.(1) He described a patient with aphasia and behavioural changes with on macroscopic examination marked left frontotemporal atrophy. In 1911, Alois Alzheimer described the detailed microscopic changes, including argyrophilic neuronal inclusions, which are still known as Pick bodies.(2) The term Pick's disease was introduced in 1926 and was used till the early 90's to describe the clinical and pathological entity.(3) To date, Pick's disease is used for a neuropathological subgroup of FTD patients.

FTD encompasses distinct canonical syndromes: the behavioural variant of FTD (bvFTD), and two language variants, semantic dementia (SD), and progressive non-fluent aphasia (PNFA).(4-6) FTD is accompanied by motor neuron disease (MND) in 5 – 15 % of the cases. FTD patients characteristically present at presenile age with variable behavioural changes and language disturbances.

The clinical syndrome FTD is part of a wide clinicopathological spectrum designated by the term frontotemporal lobar degeneration (FTLD). The last few years have seen major advances in our understanding of FTD, its genetic causes and pathological substrates.

In 1994, a genetic-epidemiological study on FTD was started at the Erasmus University Medical Center of Rotterdam. Since then, over 400 patients have been included in our FTD cohort.

The aim of this thesis was to describe and determine the relationship between the clinical presentation, genetics and pathology of FTD, with emphasis on the hereditary form of FTD.

After a general introduction to FTD (**chapter 1.2**) the clinical presentation in familial FTLD and their brain perfusion patterns on single nucleotide computed tomography (SPECT) scans will be described in **chapter 2.1**. Survival profiles of patients with FTD and motor neuron disease were assessed in **chapter 2.2**. Hereditary FTD is described in **chapter 3**. **Chapter 3.1** reports the *Progranulin* mutations in our cohort, whereas in **chapter 3.2** we describe the clinical and pathology of patients with unknown genetic defect. In **chapter 4.1**, we assessed the clinicopathological findings of five patients from families with FTLD and motor neuron disease. In **chapter 4.2** the frequency of a novel pathological FTLD subtype with ubiquitin-, and FUS-positive, TDP-43 negative inclusions (FTLD-FUS) is described, including a detailed description of the clinical, neuroimaging and pathological features of this pathological subtype. The findings, limitations and possible implications of this thesis and suggestions for further research are presented in **chapter 5**.

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Chapter 1.2

Clinical, Genetic and Pathological Heterogeneity of Frontotemporal Dementia

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Abstract

Frontotemporal dementia (FTD) is the second most common young-onset dementia, and is clinically characterized by progressive behavioural change, executive dysfunction and language difficulties. Three clinical syndromes, behavioural variant FTD, semantic dementia and progressive non-fluent aphasia, form part of a clinicopathological spectrum named frontotemporal lobar degeneration (FTLD).

The classical neuropsychological phenotype of FTD has been enriched by tests exploring Theory of Mind, social cognition and emotional processing. Imaging studies have detailed the patterns of atrophy associated with different clinical and pathological subtypes. These patterns offer some diagnostic utility while measures of progression of atrophy may be of use in future trials.

Between 30–50 percent of FTD is familial and mutations in two genes, *MAPT* and *Progranulin* (*GRN*), account for about half of these cases. Rare defects in *VCP*, *CHMP2B, TARDP* and *FUS* genes have been found in a small number of families. Linkage to chromosome 9p13.2-21.3 has been established in familial FTD with motor neuron disease, although the causative gene is yet to be identified.

Recent developments in the immunohistochemistry of FTLD, and also in amyotrophic lateral sclerosis (ALS), have led to a new pathological nomenclature. The two major groups are those with tau-positive inclusions (FTLD-tau) and those with ubiquitin-positive and TDP-43 positive inclusions (FTLD-TDP). Recently a new protein involved in familial ALS, fused in sarcoma (FUS), has been found in FTLD patients with ubiquitin-positive and TDP-43-negative inclusions.

In this review we discuss recent clinical, neuropsychological, imaging, genetic and pathological developments that have changed our understanding of FTD, its classification and criteria. The potential to establish an early diagnosis, predict underlying pathology during life and quantify disease progression will all be required for disease-specific therapeutic trials in the future.

Introduction

Frontotemporal dementia (FTD) is the second most common early-onset dementia and is characterized clinically by progressive behavioural changes and frontal executive deficits and/or selective language difficulties. Although recognised over a century ago the last few years have seen rapid advances in our understanding of FTD, its genetic causes and pathological substrates.(1-5) In 1892 Arnold Pick described a patient with progressive aphasia and lobar atrophy,(6) and in 1911 the presence of argyrophilic neuronal inclusions at neuropathological examination, later known as 'Pick bodies', was reported by Alois Alzheimer.(7)

The selective involvement of the frontal and/or temporal cortices with relative preservation of more posterior cerebral regions determines the presentation, and gives rise to the terms FTD as a clinical syndrome with distinct subtypes, and the term frontotemporal lobar degeneration (FTLD) to describe the pathological syndrome. The disease progresses from an insidious onset of behavioural change or language impairment and cognitive decline to a severe and more generalised dementia, accompanied by progressive cerebral hypometabolism and atrophy of frontal and temporal lobes preferentially.

The clinical spectrum of FTD encompasses distinct canonical syndromes: the behavioural variant of FTD (bvFTD) and the language variants, semantic dementia (SD) and progressive non-fluent aphasia (PNFA). There is also overlap of FTD with motor neuron disease (FTD-MND or FTD-ALS), as well as the parkinsonian syndromes, progressive supranuclear palsy (PSP) and corticobasal syndrome (CBS).(8) Recent advances in FTD have identified novel genetic defects and a chromosomal locus in hereditary forms of FTLD,(9-14) as well as novel neuropathological associations, e.g. the proteins TAR DNA binding protein of 43 kDa (TDP-43) and fused in sarcoma (FUS) are now recognized in the pathological classification of FTLD.(15-19)
In this review we will discuss the different clinical variants, neuropsychological aspects, neuroimaging, hereditary forms, pathological subtypes and the clinicopathological associations of FTD with the focus on recent developments.

Epidemiology

The exact prevalence of FTD remains uncertain, as there have been only a few studies and these have produced a wide range of estimates. The highest prevalences have been reported from two independent studies in the UK and one Italian study with an estimated prevalence of FTD of 15 – 22 per 100,000 inhabitants aged 45 – 64 years,(4-5, 20) which was almost half the prevalence of AD in this age group.(5) However, a study from the Netherlands estimated the prevalence of FTD to be significantly lower (9.4 per 100,000 in the age group of 60 – 69 years).(21) The lower prevalence relative to AD in that series is consistent with some pathological series.(22-23) The estimated prevalence in a Swedish population-based sample of 85-year-olds was 3.1 per 100 inhabitants.(24)

Two reported incidence studies of FTD were remarkably consistent; 3.5 and 4.1 cases per 100,000 person-years in the age-group of 45 – 64 years.(25-26) There do not appear to be clear gender differences in susceptibility.(21, 23, 26-27)

The average age at onset is around 50 – 60 years, although approximately 10 percent have an age at onset over 70 years (up to 89 years).(9) There is a wide range in durations of illness (2 – 20 years) partly reflecting different underlying pathologies. Patients with FTD-MND have the shortest survival with a mean of three years.(9, 28)

Clinical presentation

BvFTD, SD and PNFA all share an insidious onset and inexorably progressive but variable decline. Each clinical syndrome is associated with topographically distinct cerebral involvement: with bvFTD associated with symmetrical (or right-sided) frontal and anterior temporal dysfunction, PNFA left frontotemporal dysfunction and SD anterior temporal (typically left more than right) deficits. BvFTD is the most common of these subtypes and accounts for about half of all cases (9, 29) without any clear differences in presentation between sporadic and familial bvFTD, or between late and early onset bvFTD. (30) While all of the subtypes can occur in conjunction with motor neuron disease (FTD-MND), it is most commonly seen with bvFTD, occasionally with PNFA and only very rarely with SD.

Emotional blunting, loss of empathy, apathy, selfishness and neglect of personal hygiene are typical of bvFTD, but may be seen in all subtypes.(31) Other frequently reported symptoms are disinhibition, irritability, gluttony, altered preference for foods (particularly for sweets), wandering, pacing, motor and verbal stereotypies, and hoarding.(31) It has been suggested that bvFTD can be subdivided into apathetic and disinhibited variants depending on initial presentation,(32) however these symptoms frequently co-occur and the usefulness of this distinction is questionable.(30) A stereotyped-compulsive syndrome has been recognized as a third variant.(33)

A significant correlation with specific topographic patterns of atrophy, hypoperfusion or hypometabolism has been found for several of these symptoms. Apathy is associated with atrophy and dysfunction of the right anterior cingulate cortex and superior frontal gyrus,(34) disinhibition with the right subgenual cingulate cortex and orbitofrontal cortex,(34-36) overeating with an orbitofrontal-striatal circuit,(37) and executive dysfunction with the dorsolateral and prefrontal cortex.(38)

The core features of current clinical criteria for bvFTD encompass an insidious onset and gradually progressive course, early disruption of social and personal conduct, early emotional blunting and lack of insight.(2-3) Stereotypic behaviour, alterations in eating behaviour, and loss of social awareness particularly support a diagnosis of FTD, while more posterior symptoms such as difficulty with spatial orientation and locating objects suggest Alzheimer's disease (AD).(31, 39-40) Apathy, mood changes, and dysexecutive symptoms occur in both and have not been found to be effective discriminators of FTD from AD.(39, 41-42)

Table 1: Language characteristics in PPA variants (52, 55, 120, 209-211)

	Spontaneous Speech	Motor speech	Single word comprehension	Grammar/Sentence comprehension	Sentence Repetition	Naming/ word retrieval	Fluency	Reading
SD	Fluent Grammatically correct Empty and circumlocutory Semantic errors	Spared	Impaired	Initially spared, becomes impaired as single word comprehension deteriorates	Spared	Anomia (nouns > verbs)	Impaired (categorical > letter)	Surface dyslexia
PNFA	Decreased fluency Articulatory errors Apraxia of speech and/or Agrammatism	Impaired	Initially spared, becomes affected in late disease	Impaired for complex sentences	Can be impaired	Spared initially but anomic as disease progresses (verbs > nouns)	Impaired (letter > categorical)	Phonological dyslexia
LPA	Slow output with word-finding pauses Phonemic paraphasias	Spared	Relatively spared	Impaired for simple and complex sentences	Impaired	Impaired	Impaired (letter ≈ categorical)	Phonological dyslexia

The clinical criteria focus on behavioural changes rather than cognitive disturbances, and therefore might equally apply to a number of psychiatric syndromes, including (late-onset) depression and schizophrenia. However, virtually no studies have focussed on the differentiation of bvFTD from psychiatric disorders. With this in mind, an interesting group of patients is the "non-progressive", "benign" or "slow" bvFTD, who do not (or only slowly) progress over time, and do not show definite structural atrophy or hypometabolism many years from symptom onset.(43-45) Behavioural symptoms may appear to progress according to carer description but without measurable cognitive change.(45) As these patients with a non-progressive bvFTD appear to have a normal life expectancy and seldom come to autopsy, the underlying pathology is still unknown.(44-45) Possible diagnoses that have been suggested include decompensated Asperger syndrome or personality disorder, mild bipolar syndrome, or an otherwise previously undescribed neuropsychiatric syndrome with functional disruption of the same orbitofrontal-amygdala-polar network.(44)

Autopsy-proven studies have shown that the current clinical criteria correctly classify approximately 80-90 percent of bvFTD cases, whereas 3 to 17 percent are pathologically-proven AD.(46-49) However, the criteria lack sensitivity (37 percent) in the early phase of bvFTD.(50) Therefore, revised criteria for bvFTD have been proposed in light of recent advances.(51) The most important revisions are the incorporation of neuroimaging and genetic findings within the criteria and expansion of the role of supportive behavioural features for the diagnosis of bvFTD.(51)

The nosology of the language variants of FTD remains controversial. PNFA and SD are the canonical subtypes of what is collectively often termed primary progressive aphasia (PPA). Fluent speech, progressive impairment of single-word comprehension, preserved articulatory abilities, and a multimodal breakdown of semantic memory are the characteristic features of SD.(52) Patients with SD may show behavioural changes in the course of the disease similar to bvFTD.(53) In particular they may become egocentric and develop fixed daily routines.(53) PNFA patients present with apraxia of speech and/or expressive agrammatism: single-word comprehension and object knowledge are relatively preserved and behavioural symptoms are less common. However, there are patients with progressive language impairment who do not fit into the SD and PNFA: a third, more recently defined, subtype of PPA is the logopenic or phonological variant (LPA) which is characterized by a slow rate of speech output, word-finding difficulties, deficits in sentence repetition, and occasional phonemic errors in spontaneous speech and naming, whereas motor speech, expressive grammar and single-word comprehension are relatively spared.(52, 54) It remains unclear whether there are further subtypes of PPA although there is some evidence that patients with *GRN* mutations have a non-fluent PPA syndrome distinct to either PNFA or LPA.(55)

PPA subtypes have an association with different types of underlying pathology. SD is associated most commonly with FTLD-TDP type 1 (17) pathology and only rarely with FTLD-tau or AD pathology.(49, 56-57) PNFA is commonly associated with FTLD-tau,(56, 58-59), although AD and to a lesser extent FTLD-TDP pathology have also been described.(49, 57-58) Finally, LPA is predominantly associated with AD pathology.(58-59) Although these are relatively strong associations, they are not absolute, and it is currently not possible to predict with certainty the underlying pathology of specific PPA syndromes.(57) However, using multimodal predictors including qualitative clinical features, neuropsychological test scores, and atrophy on MRI improve the noninvasive prediction of the underlying pathology in non-fluent PPA.(60)

Motor neuron disease (MND) may occur early or late in the disease course in a subset of FTD patients. (61-62) Muscle atrophy, weakness, and fasciculations are often most prominent in the upper extremities and the tongue. The disease has a rapidly progressive course with a mean survival of three years. It is now accepted that FTD and MND are part of the same clinicopathological spectrum. A third of all FTD-MND cases have a positive family history for dementia, FTD, MND, or FTD-MND. The causative gene defect in FTD-MND has yet to be discovered.

Some patients with predominantly right temporal lobe atrophy (RTLA) present with prominent behavioural changes, episodic memory disturbances, topographical disorientation and prosopagnosia. (63-64) Patients with RTLA are usually diagnosed clinically with either bvFTD or SD.(64) It has been suggested that patients with bvFTD and RTLA have FTLD-tau pathology, whereas patients with SD and RTLA have FTLD-TDP pathology.(64)

Neuropsychology and social cognition

Impairment of executive function including planning, organisation, judgement, problem solving and mental flexibility, is characteristic of FTD,(65) whereas memory, visual perception, and spatial skills are usually relatively well-preserved.(50, 66-70). However, executive dysfunction may be absent or overshadowed by pronounced behavioural changes in early disease, and may also be seen in AD.(71) Verbal fluency (letter and categorical) is usually impaired in bvFTD and PNFA, but to a lesser degree also in AD.(72-74) In SD patients, semantic fluency is more impaired than letter fluency.(72, 74)

Early episodic memory impairment, a characteristic feature of AD, has also been reported in pathologically-proven bvFTD cases, and in patients with *GRN* gene mutations.(75-76) Though orientation in time and place, delayed free recall and delayed recognition are more often impaired in AD than in FTD at initial assessment, it still remains difficult to differentiate FTD from AD in the early phase using standard neuropsychological tests.(77)

As standard neuropsychological tests cannot reliably differentiate bvFTD from AD,(78-80) several investigators have explored the utility of emotional processing and social recognition tasks in the clinical diagnosis of FTD over recent years. Social dysfunction and emotional blunting commonly

occur in FTD.(44, 71, 81-87) Theory of Mind (ToM) tests require the interpretation of social situations and ascribing mental state to oneself and others, and may reveal subtle deficits not detected with standard neuropsychological testing.(71) Recent reports suggest that the neural basis for ToM tasks, social cognition and empathy lies within the medio- and/or orbitofrontal cortex, which is affected early in bvFTD.(88) Patients with bvFTD have impaired scores on these tests of social judgments and cognitive flexibility, and express concrete, literal interpretations.(71, 89) Performances in ToM tests do not correlate with executive functioning on standard neuropsychological testing in the early phase.(81, 90-91) However, in a more advanced stage, when atrophy spreads to dorsolateral frontal structures, the ToM ability and executive functioning become strongly related.(71, 89-90) Empathy as rated by caregivers is clearly impaired in bvFTD and SD patients and correlates with ToM tasks.(81, 92)

In line with these observations, recognition of facial emotions is impaired in patients with bvFTD, in particular for negative emotions (anger, fear, sadness and disgust).(81, 83-87, 93) The same applies for the recognition of vocal emotions, in particular for anger and sad voices.(85) Interpretation of sarcastic statements is impaired in FTD, and is correlated with the ability to recognize negative emotions.(44) Self-conscious emotions, such as embarrassment and amusement, are another important aspect of emotion functioning which may be disrupted in FTD.(94-95) Social cognition tests also seem to help to differentiate bvFTD patients with imaging abnormalities from the non-progressive bvFTD with normal neuroimaging.(81, 92)

It will be interesting to investigate further whether impaired social cognition is a very early feature of familial FTD as has already been described in a single case study of a presymptomatic mutation carrier; studying presymptomatic mutation carriers may allow identification of sensitive (even preclinical) cognitive predictors of decline and its neural substrate.(96)

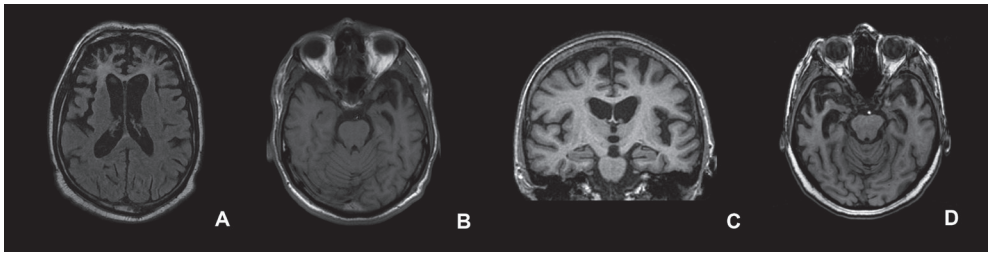
Neuroimaging

Patients with FTD classically have frontal and temporal atrophy and hypometabolism which is often asymmetric. In the clinical setting this is often best seen using volumetric structural MRI scans (with coronal T1 images being particularly useful for assessing asymmetry) or with functional imaging using either FDG-PET or, less commonly, HMPAO-SPECT. More recently, however, neuroimaging studies have aimed to refine these initial findings, mostly in clinical cohorts, but also in pathologically and genetically-confirmed FTD.

Studies of mild bvFTD in clinically-defined cohorts show involvement particularly of frontal and paralimbic areas,(97) namely the anterior cingulate cortex and frontal insula as well as medial frontal and orbitofrontal cortices, hippocampus, striatum and thalamus, more in the right than left hemisphere. With increasing disease severity, more diffuse atrophy in similar areas is seen with involvement of more lateral frontal areas and subsequently more posterior temporal and anterior parietal atrophy.(98) It remains unclear whether this pattern of atrophy is a feature of bvFTD independent of the underlying pathology (which is heterogeneous) or whether different pathologies have distinct patterns of atrophy.

Unfortunately, there are currently no studies which directly compare all of the pathological subtypes. Patients with FUS pathology seem to have a similar pattern of frontal paralimbic atrophy to that described above but in addition have severe caudate involvement compared to FTLD-tau or FTLD-TDP.(99-101) Studies comparing genetically-defined FTD patients with either *GRN* or *MAPT* mutations (102-103) have described different patterns between the two groups: *GRN* mutations are associated with asymmetrical frontal, temporal and inferior parietal lobe atrophy whereas *MAPT* mutations are associated with relatively symmetrical anteromedial temporal lobe and orbitofrontal grey matter atrophy.(102-103)

Figure 1 Imaging of FTD subtypes



(A) Frontal atrophy on axial fluid attenuated inversion recovery MRI of a patient with behavioural variant of FTD (bvFTD). (B) Axial T1-weighted image with left temporal lobe atrophy in a patient with semantic dementia (SD). (C) Coronal T1-weighted MR image of a patient with progressive non-fluent aphasia (PNFA) and left inferior frontal and superior temporal atrophy. (D) Axial T1-weighted MR image in a patient with predominant right temporal lobe atrophy.

The presence of early parietal lobe atrophy in *GRN* mutations, a feature which may distinguish such cases from other FTD patients, has been shown in studies of presymptomatic mutation carriers.(96, 104) Whether patients with different mutations in the same gene have distinct patterns of atrophy is unclear although one small study suggests there may be a difference between patients with *MAPT* mutations that affect splicing of exon 10 (more medial temporal lobe atrophy) compared with mutations that do not affect splicing of exon 10 (more lateral temporal lobe atrophy).(105) Bringing these findings together, one study that used a cluster analysis to investigate bvFTD suggested there may be four types of bvFTD anatomically: a temporal-dominant subtype associated with *MAPT* mutations; a temporofrontoparietal subtype that can be associated with *GRN* mutations but also with other pathologies such as corticobasal degeneration (CBD); as well as frontal-dominant and frontotemporal subtypes.(106) Larger studies of pathologically-proven patients are needed to confirm these findings.

Early voxel-based morphometry (VBM) studies of SD showed asymmetrical atrophy of the anterior and inferior temporal lobes,(107-108) usually affecting the left more than the right hemisphere. These findings were supported by subsequent region of interest (ROI) studies of the temporal lobe, which identified involvement particularly of the temporal pole and anterior parts of the entorhinal cortex, fusiform gyrus, inferior temporal gyrus, amygdala and hippocampus with relative sparing of the superior temporal gyrus.(109-110) Most studies have used clinically-defined cohorts but one study looking at

measurement of cortical thickness in patients with left greater than right temporal lobe atrophy showed a similar pattern of involvement in a pathologically-confirmed cohort of patients, all with FTLD-TDP. (111). This study also showed that areas within the left hemisphere outside the temporal lobe are involved with increasing disease severity, namely orbitofrontal, inferior frontal, insular, and anterior cingulate cortices.(111) Increasing involvement of the temporal lobe in the right hemisphere is seen with disease progression.(111-112) A mirror-image pattern of initial atrophy and disease progression seems to occur in those with right greater than left temporal lobe involvement (RTLA).(113) Although SD is characteristically FTLD-TDP pathologically, in a small number of cases, Pick's disease (FTLD-tau) and occasionally AD pathology can be seen. One small study showed similar patterns of atrophy in the FTLD-TDP and FTLD-tau but with a qualitatively different pattern in those with AD who had mostly hippocampal involvement, lack of the knife-edge anterior temporal atrophy seen in the other groups and without the sparing of the superior temporal gyrus.(114)

Studies of PNFA are fewer and more heterogeneous, which reflects the clinical heterogeneity of this group. As with SD, there is asymmetrical involvement with more atrophy in the left hemisphere and most significant involvement of the inferior frontal lobe and anterior insula.(52, 112, 115-116) With increasing severity there is involvement of left superior temporal, middle and superior frontal and anterior parietal lobes.(112) ROI studies have also shown involvement of the caudate in PNFA.(117) There are few pathologically-confirmed studies of PNFA and these are usually in mixed pathological groups but they show similar findings to the clinical cohort studies.(112, 118) Some small studies suggest there may be different patterns of atrophy in PNFA patients with PSP clinically (and therefore likely pathologically) compared to those without PSP,(119) and also in those with *GRN* mutations (FTLD-TDP pathologically) compared to those without.(120) More detailed studies of PNFA subgroups will be needed to confirm these findings.

Being able to distinguish FTD from AD is important clinically and recent studies have suggested that atrophy or cortical thinning of precuneus, posterior cingulate, posterior temporal and parietal areas is characteristic of AD pathology independent of clinical diagnosis and is therefore helpful in distinguishing those with atypical AD presentations (which may include bvFTD or a progressive aphasia) from those with FTLD pathology.(121-123) Clinically, however, VBM or cortical thickness studies are unlikely to be available and simpler techniques such as visual rating scales have been developed which can help to differentiate FTD from AD.(124) More sophisticated methods using techniques such as support vector machines are being developed which allow automatic classification of patients into FTD or AD groups with little user input necessary, although currently these are computationally demanding.(125) Another possibility is to use support vector machine-based MRI analyses that integrate gray matter and diffuse tensor imaging (DTI), which has shown accurate pathological or CSF-defined categorization of FTLD and AD.(126)

A different neuroimaging tool that accurately differentiates FTLD from AD is arterial spin labeling (ASL) perfusion MRI, which reveals noninvasive quantification of cerebral blood flow, without the use of ionizing radiation as in SPECT or PET.(127) Patients with AD pathologically can also be defined using amyloid molecular imaging (e.g. PIB-PET) (128) but the availability of such scans is currently limited to a few large research centres.

One of the more novel concepts to emerge from recent neuroimaging studies of FTD using the technique of resting-state fMRI is the idea that FTD is caused by degeneration within specific intrinsic functional connectivity networks that are selectively vulnerable to FTLD pathologies.(129) Consistent with earlier VBM findings in structural MRI studies of bvFTD, resting-state fMRI studies show attenuated connectivity within an anterior 'salience' network of dorsal anterior cingulate and frontoinsular cortices which has connectivity to subcortical and limbic structures.(130) In contrast there appears to be enhanced connectivity in the more posterior 'default' network which has been shown to be affected in AD.(130) These findings have been linked to specific neuropathological findings (early involvement of von Economo neurons in FTD),(131) and further work will be needed to look at whether specific pathological subtypes can be linked to specific and distinct neural network degeneration.

Cerebrospinal fluid and plasma biomarkers

Currently cerebrospinal fluid (CSF) biomarkers have limited ability to identify FTD reliably. This might be explained by both the pathological heterogeneity and the large variation in neurodegenerative severity. Levels of CSF tau in FTD are normal, increased or even decreased.(132) Levels of CSF phosphorylated tau are essentially normal in FTD, in contrast with AD. Levels of CSF amyloid beta(1-42) have been found to be either decreased or in the normal range. An indication of lower amyloid beta(1-40) levels in FTD compared with AD and control subjects, might particularly be useful to distinguish FTD patients from subjects without a neurodegenerative disorder.(132)

Decreased levels of progranulin protein are found in plasma, serum and cerebrospinal fluid (CSF) by enzyme-linked immunosorbent assay (ELISA), and may reliably differentiate *GRN* mutations carriers from non-carriers.(133-137)

It remains to be investigated if measurement of plasma or CSF levels of TDP-43 is useful diagnostically, as plasma phosphorylated TDP-43 levels have been found to be correlated with the extent of TDP-43 pathology in FTLD.(138-139)

Recent biomarker studies on CSF are using multi-analyte profiling to derive novel biomarkers for neurodegenerative disorders and have delivered some promising neuropeptides (agouti-related peptide (AgRP), adrenocortotrophic hormone (ACTH), IL-17 and IL-23 and Fas) which are useful in distinguishing FTLD-TDP from FTLD-tau patients.(140-141)

Genetics

A positive family history has been found in 30 – 50 percent of patients with bvFTD, whereas patients with SD or PNFA have a much lower frequency.(9-11, 21, 142-143) The heritability in FTD-MND differs between studies from 10 – 60 percent.(9, 11, 143) An autosomal dominant mode of inheritance is found in 10 – 27 percent of all FTD patients.(9-11, 21, 142-143).

Genetic heterogeneity of FTLD is reflected by the identification of mutations in the *microtubule associated protein tau* (*MAPT*) and *progranulin* (*GRN*) genes in approximately 50 percent of the familial cases, whereas mutations in the *valosin containing protein* (*VCP*), *charged multivesicular body protein 2B* (*CHMP2B*), *TAR-DNA binding protein* (*TARDP*), and *fused in sarcoma* (*FUS*) genes are found in less than five percent. Familial FTD-MND has been linked to chromosome 9, but the causative gene defect has yet to be discovered.

MAPT

More than 40 mutations in the *MAPT* gene have been identified in families with FTD and parkinsonism linked to chromosome 17q (FTDP-17) with accumulation of hyperphosphorylated tau protein in neurons and/or glial cells (http://www.molgen.ua.ac.be/FTDmutations).(12) Alternative splicing of exons 2, 3 and 10 of the *MAPT* gene gives rise to six isoforms: three isoforms containing three amino-acid repeats (3R), and three isoforms with four repeats (4R).(144) Mutations can be distinguished into missense mutations in exon 9 -13 affecting the normal function of the tau protein to stabilize microtubules, and intronic and some coding mutations affecting the splicing of exon 10 at the mRNA level, resulting in a change of the ratio of 3R to 4R tau isoforms.(145)

The mean age at onset is 55 years and usually shows a small intrafamilial variation between 45 and 65 years, although the disease may present before the age of 40 years or after 70 years in a few mutations. (146) The mean duration of illness is approximately nine years, but varies between five and twenty years. There exists a dementia-dominant phenotype with prominent behavioural changes including disinhibition and obsessive-compulsive behaviour,(146) and a parkinsonism-dominant phenotype with CBS or PSP-like syndromes.(76) Patients may develop language problems e.g. mild semantic impairment during the illness.(147)

Progranulin

More than 60 mutations in the *GRN* gene on chromosome 17 (1.7 Mb centromeric to the *MAPT* gene) have been identified to date, and account for approximately 5 – 10 percent of all FTD patients, and up to 22 percent in familial FTD.(9, 13-14, 148-150) Its frequency is similar to that of *MAPT* gene mutations in hereditary FTD.(9, 143) *GRN* gene mutations are occasionally reported in sporadic cases.(9, 148-150) Whether this is due to a low penetrance of the *GRN* mutation in one of the parents or to a spontaneous mutation in the patient is unknown.

GRN encodes the progranulin protein, which is a growth factor implicated in wound healing and tumour growth inflammation, and is abundantly expressed in specific neuronal subsets.(151) The neuropathology of patients with *GRN* mutations is characterized by tau-negative, and ubiquitin- and TDP-43-positive inclusions.(152)

The mean age at onset is around 60 years ranging from 35 to 89 years, with a penetrance of 90 percent by the age of 70 years.(76, 148) Within families, the onset age shows considerable variation with a difference of up to 20 years between consecutive generations.(9, 76) The mean duration is eight years (range 3 – 22 years).
Apathy and social withdrawal are the most common behavioural changes. Twenty-five percent of patients presents with early isolated language dysfunction, suggestive of an anomic non-fluent type (without motor speech impairment)and with relatively early single word comprehension impairment. (153) Hallucinations and delusions are frequently reported.(154-156) Episodic memory deficits occur in 10 – 30 percent, and may lead to the clinical diagnosis of amnestic variant of MCI or together with parietal deficits, like dyscalculia, visuospatial dysfunction, and limb apraxia to AD.(154-155, 157-159)
Extrapyramidal features are frequently seen, and include CBS with limb apraxia, asymmetric parkinsonism, and dystonia.(154-155, 160-162) ALS is only a very rare part of the clinical spectrum within *GRN* families,(9, 148, 158, 160, 163) e.g. it has been reported in a single patient of a large Canadian family.(13, 164)

Other hereditary forms

The genetic heterogeneity of FTD is further emphasized by the rare occurrence of mutations in the *VCP*, *CHMP2B*, *TARDP* and *FUS* genes.(165-168) *VCP* gene mutations are associated with inclusion body myopathy (90%), Paget's disease of the bone (45%) and FTD (38%) (IBMPFD), presenting between the age of 40 and 60 years.(165, 169)
The clinical presentation of *CHMP2B* gene mutations consists of a frontal lobe syndrome and a more global cognitive impairment, with parkinsonism, dystonia, pyramidal signs, and myoclonus later in the course of the disease.(170) ALS has been reported in only two patients.(171)
TARDBP gene mutations on chromosome 1 are found in five percent of familial ALS,(167, 172-179) and occasionally in FTD or FTD-MND cases.(167-168) Also *FUS* gene mutations are found in five percent of the familial ALS cases,(180-183) and in one clinical bvFTD patient, but not in FTD-MND.(184) However, for the majority of the FTD-MND families, the genetic defect has yet to be identified. A number of these families have been linked to a locus on chromosome 9p13.2-21.3, but at time of writing an exhaustive sequencing of all genes in this region has not revealed any coding or splice-donor site mutations.(185-190)
There still remains a subgroup of FTD patients with a positive family history without known gene mutations. These patients usually have bvFTD and memory problems with or without MND, TDP-43 pathology and neuronal loss and gliosis of the cornu ammonis 1 and subiculum of the hippocampus (hippocampal sclerosis) at neuropathological examination.(9)

Genetic risk factors

Several investigators have tried to identify genetic risk factors for FTD. Homozygosity for the T allele of the SNP rs5848 was found to have a 3.2 fold increased risk for developing FTLD-TDP,(191) but this observation could not be replicated in other studies.(192-193) The same is true for three other SNPs of the *GRN* gene, which were initially found to be associated with younger onset age or shorter survival in FTLD or ALS.(9, 191) Also, an association of the *Cystatin C* gene (*CST3*) haplotype B, the ε4 allele of the apolipoprotein E gene (*APOE*), and heterozygosity of the codon 129 polymorphism of the *prion protein* gene (*PNRP*) could not be confirmed in further studies.(194-198) Finally, SNPs in the *Ubiquitin associated protein 1* (*UBAP1*) gene have been associated with FTD,(199) which was supported by a reduced mRNA expression from the disease-associated haplotype in a quantitative analysis.(199)

Recently, an international genome wide association study (GWAS) with pathologically proven FTLD-TDP patients has demonstrated a significant association with three SNPs within the *TMEM106B* gene on chromosome 7p21.(200) *TMEM106B* variants also contribute to genetic risk for FTLD-TDP in individuals with *GRN* gene mutations.(200) *TMEM106B* encodes an uncharacterized transmembrane protein of 274 amino acids.(200) Expression data showed increased *TMEM106B* expression in the frontal cortex of FTLD-TDP than in controls, suggesting that increased *TMEM106B* expression in the brain might be linked to mechanisms of disease in FTLD-TDP and that risk alleles at *TMEM106B* confer genetic susceptibility by increasing gene expression.(200)

Genetic screening in clinical practice

The benefit of genetic screening in FTD depends on the strength of the family history and the clinical subtype. Genetic defects, either *MAPT* or *GRN*, are most often found in patients with an autosomal dominant form of bvFTD.(9, 143) Genetic screening in SD is unlikely to be useful, although patients who develop semantic impairment later in the illness may have a *MAPT* gene mutation,(9, 143) whereas a *GRN* mutation can be found in a familial form of PNFA.(9, 143, 153) Current gene defects are very rare in FTD-MND, and genetic screening is therefore likely not to be useful at present.(9, 143) Screening in sporadic patients will be of little value as a very few mutations have been found in sporadic patients, except for those with a concealed or incomplete family history. In this latter group, careful consideration is necessary before embarking on and genetic screening.(143)

Pathology

FTLD is the common underlying pathology of clinical FTD subtypes, and also includes ALS, PSP, and CBD. The major pathological hallmark of FTLD is selective atrophy of the frontal and temporal cortex, with neuronal loss, gliosis and spongiosis of the superficial layers, especially of layer II. The nomenclature has been changed several times since it was first described by Arnold Pick over a hundred years ago.(6) The term Pick's disease is now reserved for cases of FTLD with intraneuronal argyrophilic inclusions, the so-called Pick bodies, which consist of abnormal three-repeat tau protein (FTLD-tau).

FTLD is a neuropathologically heterogeneous disorder, which can be divided into two major subtypes; FTLD with tau-positive inclusions (FTLD-tau), and FTLD with ubiquitin-positive and TDP-43-positive, but tau-negative inclusions (FTLD-TDP).(18) FTLD-tau includes patients with *MAPT* mutations, Pick's disease, PSP, CBD, argyrophilic grain disease (AGD), and multiple system tauopathy with dementia (MSTD).(18) *MAPT* mutations are associated with different types of tau inclusions (Pick bodies, neurofibrillary tangles and pretangles) in the frontal and temporal cortex, hippocampus and subcortical nuclei, and sometimes in midbrain, brainstem, cerebellum and spinal cord.(146) Glial tangles and coiled bodies in white matter are found in a few *MAPT* mutations and consist predominantly of four-repeat tau isoforms.(146)
FTLD-TDP is the second major subtype of FTLD, with ubiquitin-positive inclusions, which have the TDP-43 protein as major constituent.(201) The further classification into four different FTLD-TDP subtypes according to the morphology and distribution of the inclusions (15-16) can be predicted to some extent by the clinical picture: SD is strongly associated with abundant dystrophic neurites (type 1), FTD-MND with numerous neuronal cytoplasmatic inclusions in both superficial and deep cortical laminae (type 2), *GRN* mutations are characterized by numerous cytoplasmatic inclusions, dystrophic neurites and neuronal intranuclear inclusions (type 3), and *VCP* mutations are characterized by numerous intranuclear and infrequent number of neuronal cytoplasmatic inclusions and dystrophic neurites (type 4).(15, 17) It remains unclear what differences in underlying pathophysiology determine the distinction between these TDP-43 subtypes.

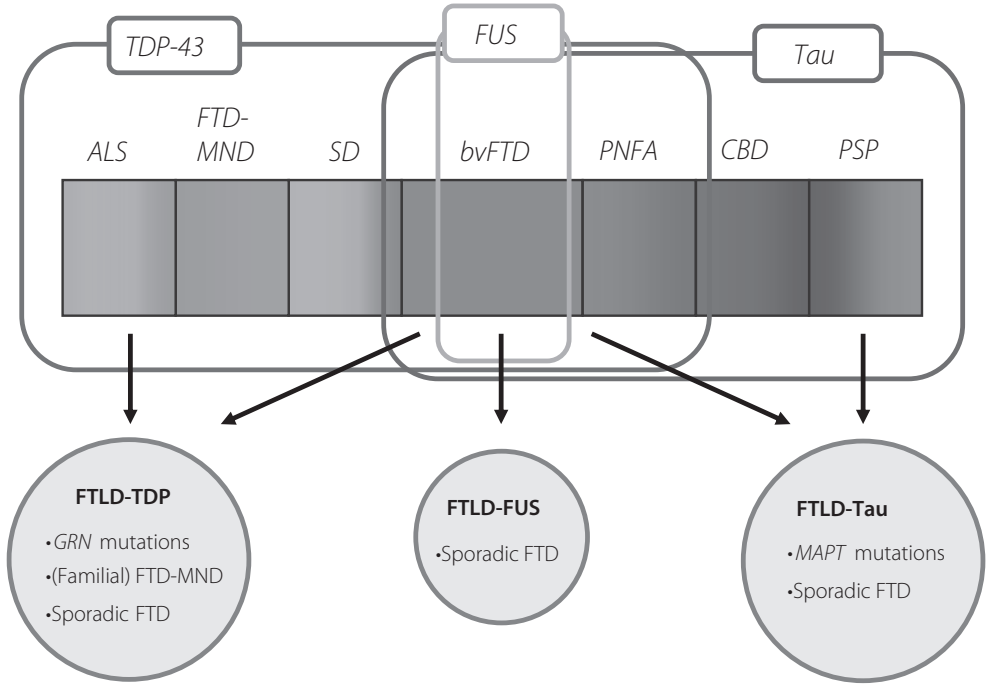
A small number of FTLD cases with ubiquitin-positive, TDP-43 negative pathology,(202-205) have recently shown immunoreactivity with the FUS antibody.(19, 99) None of these FTLD-FUS cases had *FUS* gene mutations.(205) FTLD-FUS cases are characterized by a young age at onset, bvFTD, a negative family history, and caudate atrophy on MRI.(99-100) FUS-positive inclusions are also found in patients with neuronal intermediate filament inclusion disease (NIFID).(206) NIFID patients mostly present with bvFTD symptoms, a negative family history, and pyramidal and/or extrapyramidal movement disorder.(206)
The FUS protein contains 526 amino-acids and is as nuclear protein involved in DNA repair and the regulation of RNA splicing.(180-181) Mutations in the *FUS* gene on chromosome 16 have emphasized its pathogenetic role in the clinicopathological spectrum of FTD and ALS.(184)
Finally, the pathological heterogeneity of FTLD has been further emphasized by cases with ubiquitin-positive, TDP-43 and FUS-negative inclusions, termed FTLD-UPS. Most of the FTLD-UPS cases carry a *CHMP2B* mutation,(207) but there remains a few without *CHMP2B* mutations.(205). Further research on FTLD-UPS is necessary to elucidate the full complement of FTLD pathologies.(205)

Future clinicopathological studies, including neuroimaging and genetics are necessary to improve the prediction of the underlying pathology. Especially the prediction of the underlying pathology in (sporadic) bvFTD will be important, as tau-, TDP-43-, or FUS-pathology could be the disease-modifying protein in these patients.

Future directions

Important advances in the field research on FTD over the last decade have led to an impressive change in the clinical recognition of this disease. Future scientific efforts should focus on three major lines of research: 1. to improve the early detection of the disease 2. to develop reliable markers in predicting the underlying pathology, and 3. to unravel its pathophysiology in order to develop therapeutic strategies preventing or delaying the disease process.

Figure 2 Clinical, genetic and pathological spectrum of FTLD



Concerning the clinical diagnosis, an international study has been initiated to revise the clinical criteria based on a large sample of pathologically-proven cases. The aim is that neuroimaging and genetic data, and the most salient clinical features should be incorporated in a revised set of simplified criteria of bvFTD. A second clinical issue will be to monitor the progression of the disease in individual patients, which has now become available by the recent introduction of FTD rating scale (FRS)(208) characterizing the features of different severity stages. Finally, the use of social cognition tasks will help in the early detection of bvFTD and discrimination from non-progressive bvFTD. Their use offers us the opportunity to investigate the relative contributions of individual brain regions to social cognition in FTD.

Although relatively specific atrophy patterns have been found in clinical FTD subtypes, neuroimaging features as biomarkers for underlying pathology in bvFTD have yet to be determined. Support vector machines and arterial spin labeling are new neuroimaging tools to accurately differentiate FTD from AD. Another novel and promising neuroimaging technique is resting-state fMRI, which has shown changes in the salience network in FTD. An interesting question will be whether the early (or even presymptomatic) *MAPT* or *GRN* mutation carriers can be detected using this technique.

The differentiation of PPA into SD, PNFA, and LPA has proven to be an important step in predicting underlying pathology in these groups: TDP-43 pathology is most commonly found in SD, tau-pathology in PNFA, and AD-pathology in LPA. Multimodal predictors, including clinical parameters, neuropsychological test scores, and atrophy patterns, will improve the prediction of the underlying pathology in clinical PPA syndromes. However, radioactive compounds to detect tau or TDP-43 pathology in the brain with PET scanning would be of great help to differentiate bvFTD into its two major pathological subtypes during life. The recent recognition of the FUS protein as a pathological component of neuronal inclusions in a specific subtype of FTLD emphasizes the existence of different pathways, and will also contribute to further understanding of the underlying pathophysiology. Another strategy would be the development of new CSF biomarkers, which could be derived by large-scale proteomics analysis.

Several common (*MAPT*, *GRN*) and rare (*VCP*, *CHMP2B*, *TARDBP*, *FUS*), genetic factors have been found in hereditary FTD over recent years. However, we still have to identify one or more gene defects in familial FTD with and without MND. Whole exome sequencing as innovative genetic technique might reveal new genetic defects in small families with FTD and for pathologically well-characterized FTLD subtypes (like FTLD-FUS). Identification of novel genetic defect(s) will help to understand the pathophysiology of TDP-43 in hereditary and probably also of the sporadic FTLD-TDP. A large genome-wide association study of more than 3000 DNA samples is currently underway and may hopefully reveal additional genetic factors with a small effect on the disease process.
All these small steps in unravelling the pathophysiology should finally lead to the development of therapeutic interventions for FTD.

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Chapter 2

Clinical Presentation in Frontotemporal Dementia

Chapter 2.1

Brain Perfusion Patterns in Familial Frontotemporal Lobar Degeneration

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Abstract

Frontotemporal lobar degeneration (FTLD) is a clinically, genetically and pathologically heterogeneous disorder. The aim of this study was to compare clinical features and perfusion patterns on SPECT of patients with familial FTLD-TDP and *MAPT* mutations. In this study, patients were included if they had *MAPT* or *GRN* mutations, positive family history with pathologically-proven FTLD in the patient or first-degree relative, or were part of FTD-MND families. All patients and ten age- and gender-matched controls underwent measurement of brain perfusion using ^{99m}Tc-HMPAO SPECT. We used SPM8 to perform image preprocessing and voxel-based group analyses ($p<.001$). Gender and age were included as nuisance variables in the design matrices. Of the 29 patients with familial FTLD, 19 had familial FTLD-TDP (*GRN* mutations in six), and 10 had *MAPT* mutations. At clinical presentation, familial FTLD-TDP patients were older at onset ($p=.030$) and had more memory deficits ($p=.011$), whereas *MAPT* had more naming deficits ($p<.001$) and obsessive-compulsive behaviour ($p=.001$). The between groups SPECT analyses revealed significantly less perfusion in the right frontal lobe, precuneus, cuneus and inferior parietal lobule in familial FTLD-TDP, whereas significantly less perfusion was found in the left temporal and inferior frontal gyri in *MAPT*. Post-hoc analysis with familial FTLD-TDP with unknown genetic defect (UGD) versus *MAPT* patients revealed less perfusion in the right frontal and parietal lobe. Familial FTLD-TDP shows relatively more posterior hypoperfusion, including the precuneus and inferior parietal lobule, possibly related to significant memory impairment. *MAPT* patients were characterised by impaired perfusion of the temporal regions and naming deficits.

Introduction

Frontotemporal dementia (FTD) is the second most common form of presenile dementia, and is characterized by a variable clinical presentation of progressive behavioural, language and executive dysfunction. Motor neuron disease (MND) accompanies FTD in 5–15 percent of the cases.(1-2)

A positive family history is found in approximately 40 percent of all patients with FTD.(3-4) Two major gene defects have been found in familial FTD; mutations in the *microtubule associated protein tau* (*MAPT*) and *progranulin* (*GRN*) genes.(5-7) However, there still remain one or more familial forms of FTD with unknown gene defect (UGD), in particular familial FTD-MND.(3-4)

FTD is part of a broad clinicopathological spectrum named frontotemporal lobar degeneration (FTLD). *MAPT* is associated with FTLD with tau-positive inclusions (FTLD-tau), whereas *GRN* and familial forms with unknown genetic defect are associated with ubiquitin and TDP-43 positive inclusions (FTLD-TDP).(3-4)

MAPT and *GRN* patients differ in several aspects from each other; disinhibition and obsessive-compulsive behaviour, together with semantic deficits in *MAPT* (8) versus apathy, social withdrawal, and less spontaneous, non-fluent speech, with wordfinding difficulties in *GRN* mutations.(9) Episodic memory deficits in the initial stage have been reported in patients with familial FTLD-TDP with and without *GRN* mutations.(3, 9)

Structural imaging using voxel-based morphometry (VBM) on MRI has shown symmetrical and predominantly temporal and frontal atrophy in *MAPT* mutations versus asymmetrical frontotemporoparietal atrophy in *GRN* mutations.(10-11) Functional imaging using SPECT has also revealed asymmetrical frontotemporoparietal hypoperfusion in patients with *GRN* mutations, whereas perfusion patterns of *MAPT* patients have not been investigated yet.(12-15)

The aim of this study was to use statistical parametrical mapping (SPM) to determine differences in brain perfusion on ^{99m}Tc-HMPAO SPECT scans of patients with familial FTLD-TDP and *MAPT* mutations. Furthermore we studied the brain perfusion pattern of patients with familial FTLD-TDP UGD.

Methods

Participants

Patients with FTD were ascertained in an ongoing genetic-epidemiologic study conducted in the Netherlands since 1994,(1, 3) after referral to the out-clinic department of the Erasmus MC- University Medical Center, or after a visit to nursing homes or psychogeriatric hospitals by a research physician. Detailed clinical history was obtained from the spouses and first-degree relatives using a checklist of behavioural and cognitive changes, and motor symptoms.

The age at onset was defined as the age at which the first symptom, compatible with FTD diagnosis, was observed by a close relative or caregiver. During the neurological examination carried out in all patients, special attention was paid to the presence of extrapyramidal and motor neuron disease signs.

Data on family history were obtained using a structured questionnaire fulfilled by a spouse or first-degree relative. Family history was defined positive if patients had at least one first-degree relative with dementia, parkinsonism, or MND, irrespective of their age at onset.
Ten age and gender matched controls were selected. Control patients had no neurological disorders or abnormalities at neurological examination and no abnormalities on ^{99m}Tc-HMPAO SPECT scan. This study was approved by the Medical Ethical Committee at the Erasmus MC – University Medical Centre Rotterdam. For each patient, a spouse or first-degree relative of the patient gave written informed consent.

Inclusion criteria

For this study we included patients with a positive family history, an available ^{99m}Tc-HMPAO SPECT scan, and at least one of the following characteristics: 1) confirmed neuropathological diagnosis of FTLD in the patient or a first-degree relative, 2) positive DNA-screening for *MAPT* or *GRN* mutations, 3) being part of a family with FTD-MND and therefore suggestive for FTLD-TDP pathology.

Clinical features

The presence of the following clinical features was scored as absent or present: apathy, disinhibition, obsessive-compulsive behaviour, memory deficits and motor neuron disease (MND). For a correlation analysis between clinical features and relative cerebral hypoperfusion, we semiquantitatively scored every clinical parameter, except for MND, as follows: 0 (absent), 1 (clinical feature present, although not initially or predominantly present) or 2 (clinical feature was initially or predominantly present).

Neuropsychological data

Neuropsychological evaluation was performed in 21 patients and ten controls. The test battery included the Mini-Mental State Examination, Trailmaking A and B, Stroop I, II and III, the Dutch 15-Word Verbal Learning Test (15-WVLT)(16), Boston Naming Test, Phonological or letter (DAT) fluency, semantic fluency (naming animals and occupations), Clock Drawing long or short, and Orientation for time and place. T-scores were used to correct for age, education and gender, based on Dutch norms.

Structural imaging data

The presence and severity of frontal, temporal and parietal atrophy on structural imaging data (CT or MRI) were reviewed and scored by H.S. and J.C.v.S. as: 0) no atrophy, 1) mild atrophy, 2) moderate or severe atrophy.

Genetic screening

Mutation screening of all exons and exon-intron regions of *MAPT*, *GRN* and *CHMP2B* genes were performed in all patients as previously described.(3)

Pathology

Brain autopsy was carried out in 12 patients and of two first degree relatives included in this study according to the Legal and Ethical Code of Conduct of the Netherlands Brain Bank. The pathology protocols have been described before (3, 17-18). A panel of antibodies including AT-8, ubiquitin, TDP-43 and FUS were used for the diagnoses made by the neuropathologist (A.J.M.R.). FTLD-TDP cases were classified as proposed by Sampathu et al.(19)

^{99m}Tc-HMPAO SPECT scanning and image processing

Brain perfusion ^{99m}Tc-HMPAO SPECT scans were exclusively performed at the department of nuclear medicine of the Erasmus MC – University Medical Centre. Technetium-99m - hexamethyl-propylenamine-oxime (^{99m}Tc HMPAO) SPECT scans were acquired on a Prism 3000XP Philips (Picker) three-headed system, with a fan-beam collimator. Acquisition was performed 5 or 30 minutes after injection of ^{99m}Tc HMPAO (750 MBq, 20 mCi) in a quiet room with eyes open. The difference in time is due to the clinical setting in which the study has been performed, where two patients were injected at the same time. Duration of scanning was 30 minutes. Hundred-twenty projections (40 steps of 3º, and 20 seconds per step) were acquired. Image reconstruction was performed by a ramp-filtered back projection and three-dimensionally smoothed with a Metz Filter.

After gross manual image reorientation and approximate definition of the image centre point, the SPECT scans were spatially processed using the Statistical Parametric Mapping SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/, Wellcome Trust Centre for Neuroimaging, London, UK) implemented in Matlab 7.4.0 (MathWorks, Natick, MA, USA). Estimation of individual SPECT normalisation parameters were constraints by a source weighting image defined by the SPM(default brain mask. Then all spatially normalised images were visually checked to make sure that SPECT were not misregistrated with the SPM8 SPECT template and smoothed using a 16 mm FWHM kernel.
All images were checked visually after normalisation to ensure that no cerebral region was incorrectly normalised in MNI space. Voxel-based image analyses were conducted using SPM8 in the framework of the General Linear Model.(20) The effect of variations in global image intensity was removed by using proportional scaling to the mean global image intensity. The method of using a regional reference (21) was not suitable for our data since we were unable to obtain a reference area where perfusion was relatively preserved in all patients groups. Group comparisons of spatially normalised and smoothed images were performed on a voxel-by-voxel basis using the "full factorial design" in SPM8. Age and gender were entered as nuisance variables in the design matrix.

We performed the following contrasts: Familial FTLD-TDP minus controls; *MAPT* minus controls, familial FTLD-TDP minus *MAPT* and inclusively masked by familial FTLD-TDP minus controls; *MAPT* minus familial FTLD-TDP and inclusively masked for *MAPT* minus controls. We performed these analyses with an inclusive mask ($p<.05$) to show that our findings were not related to a relative increase of brain perfusion

when one patients' group is compared to the other.

Results were all characterized in terms of the probability that the difference in magnitude value in a given voxel could occur by chance under the null hypothesis. Significance level was set at p<.001, uncorrected for multiple comparisons in all analyses. Only clusters containing at least 20 contiguous voxels either with or without masking were considered as significant.

Table 1 Demographic, clinical and structural imaging data of familial FTLD-TDP, *MAPT* and controls

	Familial FTLD-TDP (n=19)	*MAPT* (n=10)	Controls (n=10)	P-value*
Male : Female	11 : 8	5 : 5	6 : 4	.569
Age at onset, yrs (sd)	56.9 (8.9)	49.8 (5.9)	-	**.030**
Age at SPECT, yrs (sd)	59.4 (9.4)	52.1 (6.3)	56.7 (7.8)	**.039**
Time onset till SPECT ,yrs (sd)	2.4 (1.6)	2.3 (1.3)	-	.869
Age at death, yrs (sd)	64.3 (10.6) (n=14)	58.6 (7.5) (n=10)	-	.154
FTD subtype				
bvFTD (%)	15 (79)	10 (100)	-	1.000
FTD-MND (%)	4 (21)	0 (0)	-	.268
Clinical presentation				
Apathy (%)	15 (79)	8 (80)	-	1.000
Disinhibition (%)	17 (84)	8 (80)	-	.592
Obsessive-Compulsive (%)	3 (16)	8 (80)	-	**.001**
Memory impairment (%)	16 (84)	3 (30)	-	**.011**
Language				
Wordfinding difficulties (%)	10 (53)	3 (30)	-	.433
Economy of speech (%)	10 (53)	2 (20)	-	.126
Comprehension deficits (%)	0 (0)	2 (20)	-	.111
Naming deficits (%)	0 (0)	6 (60)	-	**<.001**
Apraxia (%)	4 (21)	0 (0)	-	.268
Parkinsonism (%)	4 (21)	1 (10)	-	.633
Motor neuron disease (%)	4 (21)	0 (0)	-	.268
Atrophy on structural imaging				
Frontal atrophy				
0 (%)	0 (0)	1 (10)	-	.161
+ (%)	10 (47)	1 (10)	-	**.025**
++ (%)	9 (53)	8 (80)	-	.090
Temporal atrophy				
0 (%)	1 (6)	1 (10)	-	.632
+ (%)	10 (47)	2 (20)	-	.215
++ (%)	8 (47)	7 (70)	-	.153
Parietal atrophy				
0 (%)	2 (12)	5 (50)	-	**.018**
+ (%)	12 (59)	4 (40)	-	.233
++ (%)	5 (29)	1 (10)	-	.303

*Familial FTLD-TDP compared to *MAPT*; Atrophy is rated as: 0, no atrophy; +, mild atrophy; ++, moderate-to-severe atrophy.

Statistical analysis

SPSS 15.0 for Windows (SPSS, Chicago, Ill. U.S.A.) was used for statistical analysis of clinical and demographic data. Data of age at onset, age at brain SPECT, time between onset, time of SPECT were analysed using independent sample t-test. The Chi-square test was used to analyse differences between gender, clinical and structural neuroimaging parameters. The non-parametric Mann-Whitney U test was used to analyse neuropsychological data.

Results

Clinical data

A group of 29 patients was included in this study and consisted of 19 patients with familial FTLD-TDP and 10 patients with *MAPT* mutations. Of the 19 familial FTLD-TDP patients, six had a *GRN* gene mutation (Ser82ValfsX174 in five, Gln125X in one) with pathological confirmation in one (FTLD-TDP type 3)(19). Of the other 13 patients from 10 families, seven were pathologically diagnosed as FTLD-TDP type ,(19) four had a first-degree relative with pathologically confirmed FTLD-TDP, and the remaining two patients were part of a large FTD-MND family without pathological confirmation.

The *MAPT* mutation group consisted of ten patients (P301L in eight, G272V in two) and brain specimens were available in four cases. There were no patients with FTLD-tau pathology and a positive family history without a *MAPT* mutation.

Demographic, clinical and structural imaging data of familial FTLD-TDP, *MAPT* and controls are summarized in Table 1. Patients with *MAPT* mutations were younger at time of SPECT-scan (p=.039). Four patients of the familial FTLD-TDP group had a clinical presentation of MND (bulbar in three). Memory impairment was more often present in familial FTLD-TDP (p=.011), whereas obsessive-compulsive behaviour (p=.001) and naming deficits (p<.001) were more often present in *MAPT* (Table 1).

Neuropsychological data revealed significant differences on the Boston Naming Test when *MAPT* was compared to familial FTLD-TDP (p=.019). There were no other significant differences between familial FTLD-TDP and *MAPT*. Using T-cores did not reveal significant differences between *MAPT* and familial FTLD-TDP. All neuropsychological data and analyses between the two distinct patient groups compared to controls are presented in Table 2.

Perfusion comparisons

Familial FTLD-TDP, MAPT and controls

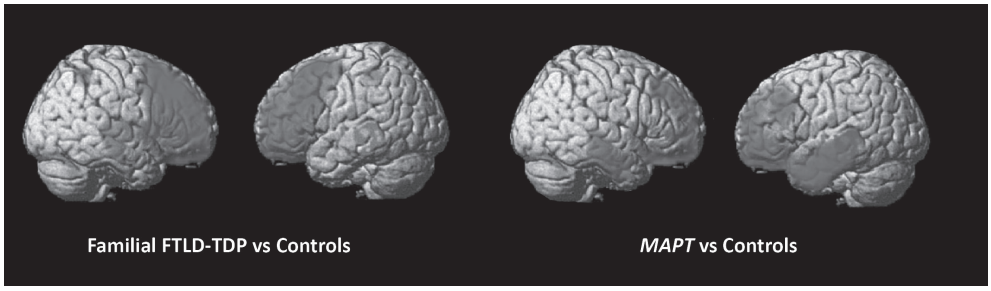
On SPECT, familial FTLD-TDP patients directly compared to controls (p<.001) showed relative hypoperfusion of both frontal lobes, including the precentral gyrus, the right superior temporal gyrus, left and right inferior parietal lobule, left postcentral gyrus, caudate and thalamus (Figure 1). *MAPT* patients compared to controls showed left bifrontal and bitemporal (predominantly left), caudate and thalamus hypoperfusion compared (Figure 1). A direct comparison of brain perfusion was performed between familial FTLD-TDP and *MAPT*.

Table 2 Neuropsychological data

	n	FTLD-TDP	n	*MAPT*	n	Controls	P-value*
MMSE	14	21.9 (12-30)[b]	5	23.4 (14-28)[b]	10	28.6 (27-30)	.559
Processing Speed							
Trail Making A, sec	14	88.8 (30-179)[b]	7	82.6 (38-179)	9	42.0 (22-60)	.585
Stroop I, sec	12	74.0 (50-109)	6	73.8 (34-109)	8	58.5 (41-91)	.820
Executive Function							
Trail Making B, sec	14	218.9 (127-258)[b]	7	200.3 (57-258)	9	126.2 (48-258)	.856
Stroop III , sec	12	215.6 (94-281)[a]	6	191.7 (90-281)	8	130.9 (79-208)	.358
Trailmaking ratio; TMT B/ TMTA	14	3.1 (1.4-5.5)	7	3.3 (1.4-5.7)	9	3.0 (1.7-5.9)	.971
Stroop ratio; Stroop III/Stroop II	12	2.2 (1.3-3.6)	6	1.8 (1.3-2.1)	8	1.9 (1.1-3.2)	.335
WCST, concepts	13	1.8 (0-6)[b]	6	1.7 (0-6)[a]	10	4.5 (1-6)	.918
Memory							
15-WVLT immediate recall, max 75	10	23.8 (9-38)[b]	4	33.3 (9-43)	9	41.9 (25-62)	.142
15-WVLT delayed recall, max 15	10	4.8 (0-10)	4	6.5 (0-11)	8	7.6 (3-13)	.454
Language							
Boston naming test, max 60	8	48.9 (36-56)[a]	5	32.2 (24-45)[b]	9	54.6 (39-59)	**.019**
Phonological Fluency DAT	11	10.2 (0-27)[c]	5	12.4 (0-51)	9	29.7 (22-40)	.320
Semantic Fluency Animal	15	10.7 (1-25)[b]	7	7.4 (0-22)[b]	7	22.3 (15-37)	.210
Semantic Fluency Occupations	15	7.3 (1-17)[c]	7	5.7 (0-17)[b]	7	15.1 (8-22)	.407
Visuo-constructive function							
Clock drawing long, max. 14	6	12.0 (8-14)	0	-	7	12.7 (12-14)	-
Clock drawing short , max. 3	9	2.0 (0-3)	6	2.2 (0-3)	0	-	.607
Orientation							
Orientation time, max 5	15	3.3 (0-5)[a]	6	3.7 (0-5)	10	4.7 (3-5)	.677
Orientation place, max 5	15	4.2 (1-5)	6	4.2 (0-5)	10	4.9 (4-5)	.569

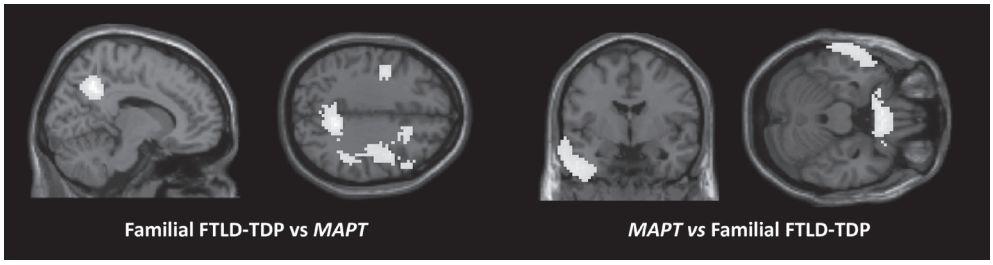
Range of each test is given between brackets. *Familial FTLD-TDP versus *MAPT*; a, versus controls p≤0.05; b, versus controls p≤.01; c, versus controls p≤.001; Non-parametric Mann-Whitney U test was used.

Figure 1 Perfusion pattern of familial FTLD-TDP and *MAPT* compared to controls



Significant relative hypoperfusion superimposed on a SPM8 canonical single subject template, with a threshold p<.001.

Figure 2 Perfusion pattern of familial FTLD-TDP compared to *MAPT*



Significant relative hypoperfusion superimposed on a SPM8 canonical single subject template, with a threshold p<.001.

Familial FTLD-TDP showed relative hypoperfusion of the right and left precuneus, right cuneus, precentral gyrus, inferior parietal lobule, inferior frontal and middle frontal gyri, cingulate gyrus, lingual gyrus and cerebellum. Relative hypoperfusion of the left hemisphere was found in the cingulate gyrus and middle frontal gyrus (Figure 2, Table 3). To make sure that these differences occurred in brain areas where perfusion was indeed lower than in controls, we performed the same analysis while inclusively masking by the contrast familial FTLD-TDP compared to controls (at p<.05). When masked at this relatively liberal threshold, the right precuneus, cuneus, inferior parietal lobule, precentral gyrus, inferior and middle frontal gyri and cingulate gyrus remained significant (Table 3).

When *MAPT* was directly compared to familial FTLD-TDP, significant relative hypoperfusion was found of the left inferior, middle and superior temporal gyri, right inferior and medial gyri, right rectal gyrus and left inferior occipital gyrus (Figure 2). All temporal and frontal regions remained significant after masking by the contrast *MAPT* compared to controls (at p<.05)(Table 3).

Table 3 Brain regions with relative hypoperfusion in familial FTLD-TDP compared to *MAPT*

	Coordinates (mm)			
	x	y	z	Z-score
Familial FTLD-TDP (n=19) compared to *MAPT* (n=10)				
R Precentral Gyrus BA 6*	42	-4	37	3.74
L Cingulate Gyrus BA 24	-12	5	40	3.69
R Inferior Frontal Gyrus BA 9*	54	20	28	3.65
R Middle Frontal gyrus BA 46*	48	17	25	3.60
R Inferior Parietal Lobule BA 40*	45	-52	58	3.50
R Precuneus BA 7*	9	-64	40	3.45
L Precuneus BA 31	-6	-52	37	3.44
L Middle Frontal BA 6	-24	-10	49	3.43
R Cingulate Gyrus BA 32*	18	23	37	3.41
R Lingual Gyrus BA 19	18	-64	-2	3.41
R Cuneus BA 7*	6	-70	31	3.21
***MAPT* (n=10) compared to Familial FTLD-TDP (n=19)**				
R Medial Frontal Gyrus BA 25†	3	20	-20	4.10
R Rectal gyrus BA 11†	3	23	-26	3.96
L Inferior Temporal Gyrus BA 20†	-51	-4	-38	3.97
L Inferior Occipital Gyrus BA 18	-33	-97	-14	3.89
L Middle Temporal Gyrus BA 21†	-63	2	-20	3.64
L Superior Temporal Gyrus BA 38†	-30	11	-29	3.38
L Inferior Frontal Gyrus BA 47†	27	23	-23	3.20

*, Areas that remained significant after masking by Familial FTLD-TDP compared to Controls; †, Areas that remained significant after masking by *MAPT* compared to Controls

Post-hoc analyses

Familial FTLD-TDP with unknown genetic defect (UGD), GRN, MAPT and controls

To determine whether posterior cortical hypoperfusion in familial FTLD-TDP was not driven by *GRN* gene mutations, we divided the familial FTLD-TDP group into patients with *GRN* gene mutations (n=6) or with unknown genetic defect (UGD) (n=13), and also included *MAPT* (n=10) and controls (n=10) in the design matrix. FTLD-TDP UGD directly compared to controls showed relative hypoperfusion bifrontally, including precentral gyrus, in right middle temporal lobe, right precuneus, superior parietal lobule, inferior parietal lobule, thalamus and caudate nucleus (Figure 3).

FTLD-TDP UGD was then directly compared to *MAPT*, and masked by the analysis FTLD-TDP UGD compared to controls. The right precentral gyrus, middle frontal gyrus, right and left inferior frontal gyrus, right precuneus, cuneus, and superior parietal lobule showed significant relative hypoperfusion (Figure 3, Table 4).

When familial FTLD-TDP UGD was directly compared to *GRN* and vice versa, no significant differences of relative hypoperfusion were found.

Table 4 Brain regions with relative hypoperfusion in familial FTLD-TDP UGD compared to *MAPT*

	Coordinates (mm)			
	x	y	z	Z-score
R Precentral Gyrus BA 6*	57	-7	43	3.79
R Middle Frontal Gyrus BA 6*	48	2	40	3.62
L Cerebellum	-12	-58	-8	3.53
R Postcentral Gyrus BA 2	48	-28	31	3.51
R Lingual Gyrus BA 19	18	-64	-2	3.48
R Inferior Frontal Gyrus BA 9*	54	20	28	3.46
R Superior Parietal Lobule BA 7*	36	-55	64	3.46
R Cuneus BA 7*	6	-70	31	3.44
R Middle Frontal Gyrus BA 46*	48	17	25	3.38
R Precuneus BA 7*	6	-73	37	3.31
L Cingulate Gyrus BA 24	-12	-5	40	3.26
L Precentral Gyrus BA 6	-36	-4	40	3.22
L Inferior Frontal Gyrus BA 9	-48	-1	25	3.14

*, Areas that remained significant after masking by Familial FTLD-TDP unknown genetic defect (UGD) versus Controls

Correction for atrophy

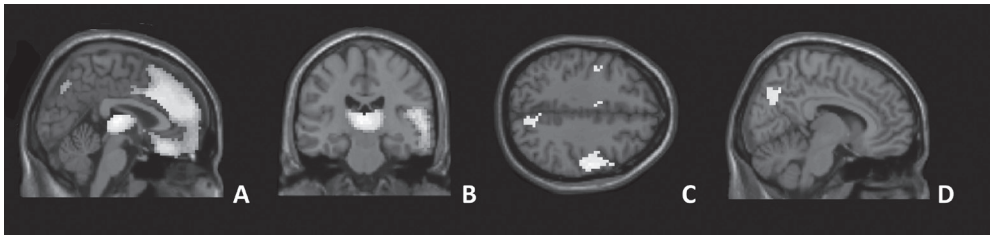
To show that these findings were not just a reproduction of atrophy, we entered the rate of atrophy of the frontal, temporal and parietal lobes as confounding covariate in the design matrix. After accounting for lobar atrophy, hypoperfusion of the right precuneus, cingulate gyrus, precentral gyrus, inferior and middle frontal gyri remained significant in the familial FTLD-TDP group compared to *MAPT* group. In the opposite analysis, relative hypoperfusion of the rectal and medial frontal gyri was found in the *MAPT* compared to familial FTLD-TDP, while the relative hypoperfusion of the left temporal lobe was not present anymore after accounting for atrophy.

After accounting for atrophy in the post-hoc analyses, familial FTLD-TDP UGD compared to *MAPT*, and familial FTLD-TDP UGD compared to *GRN* and vice versa, all previous found regions remained significantly hypoperfused.

Correlation between SPECT findings and clinical data

Memory deficits were significantly more often present in familial FTLD-TDP, whereas obsessive-compulsive behaviour was significantly more present in *MAPT*. We entered the score on memory deficits and obsessive-compulsive behaviour of the 29 patients in two different matrices, with age and gender as nuisance variables.

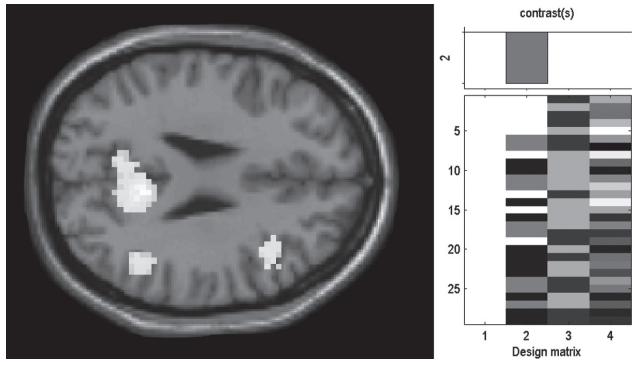
Figure 3 Familial FTLD-TDP UGD compared to controls and *MAPT*



Significant relative hypoperfusion superimposed on a SPM8 canonical single subject template, with a threshold p<.001. Familial FTLD-TDP UGD versus controls **(A and B)**. Familial FTLD-TDP UGD compared to *MAPT*, masked by familial FTLD-TDP UGD compared to controls **(C and D)**.

Memory deficits were negatively correlated with relative hypoperfusion of the posterior cingulate and precuneus, right middle frontal gyrus, right inferior parietal lobule, right cerebellum and right midbrain (Figure 4). This means that a greater memory deficit was associated with a lower perfusion in these areas. Obsessive-compulsive behaviour did not correlate with any brain region.

Figure 4 Correlation with memory performance in 29 FTLD patients



Correlation of memory deficits (p<.001) with perfusion levels in posterior cingulate, precuneus, right inferior parietal lobule and right middle frontal gyrus projected on a SPM8 canonical single subject template.

Discussion

The present functional imaging study comparing familial FTLD-TDP and *MAPT* has demonstrated relative hypoperfusion in the right frontal and posterior cortical regions in familial FTLD-TDP patients compared to *MAPT*, whereas relative hypoperfusion was found in the left lateral temporal lobe and medial frontal gyrus in *MAPT* mutations.

The observation of relative hypoperfusion in the posterior cortical regions, predominantly right-sided, in familial FTLD-TDP (including six patients with *GRN* mutations) is in line with earlier observations in patients with *GRN* gene mutations,(10-11, 14) although most of the present patients with familial FTLD-TDP had no *GRN* mutations. However, parietal lobe and posterior cingulate atrophy on MRI, has also been reported in patients with clinical bvFTD and different underlying pathologies,(22) as well as in patients with FTLD-MND linked to chromosome 9 (Family VSM-20).(23)
In addition, VBM-studies in pathological-proven FTLD cases only did not find differences between patients with FTLD-tau and FTLD-TDP.(24-28) Therefore it will be important in future imaging studies to perform group analyses based on clinical, genetic (sporadic and familial) and pathological status to get explicit information on hypoperfusion and atrophy patterns of FTLD subgroups.

A higher frequency of memory deficits and lower scores on immediate recall memory task were found in familial FTLD-TDP compared to controls, and a non-significant difference was found compared with *MAPT* mutations possibly due to the small sample size of *MAPT* patients. These memory deficits correlated with precuneus hypoperfusion, as this cortical region together with the posterior cingulate is comprised in Papez' circuit and related to the episodic memory functioning.(29) Atrophy and hypoperfusion of these cortical regions are frequently described in mild cognitive impairment (MCI) and early stage of Alzheimer's disease (AD),(30-31) and in patients with *GRN* gene mutations.(10-11). Our results showed that the occurrence of memory problems also applies for FTLD-TDP with unknown genetic defect.

Another novel finding in the present study is the relative hypoperfusion of the precentral gyrus in familial FTLD-TDP compared to controls and *MAPT*, which might be explained by the frequent occurrence of clinical MND in this form.(32-33) Sparing of the precentral gyrus in *MAPT* is in line with the absence of MND in FTLD-tau.(8) It is still unclear why in familial FTLD-MND some family members develop FTD and others MND or both.(18) There might be a certain threshold of precentral hypoperfusion associated with the development of clinical MND, and this hypoperfusion might be an early biomarker in familial FTD-MND.

The finding of predominantly left-sided bifrontotemporal hypoperfusion in *MAPT* mutations corresponds with the clinical and neuropsychological profile of bvFTD with naming deficits in these patients.(10-11, 34) We confirmed in our study the suggestion that *MAPT* mutations not affecting the splicing of exon 10 have anterolateral temporal and frontal atrophy, as the majority of our patients had a P301L mutation.(34)

Furthermore, Whitwell et al. found that *MAPT* mutations that affect exon 10 splicing and thus influence the alternative splicing of tau pre-messenger RNA were found to have a more medial temporal gray matter loss, which suggests a possible association between mutation function and atrophy in *MAPT*.(34)

Newer tools in functional brain imaging may help to differentiate FTLD subtypes in the future, especially the development of specific binding tracers in PET. The Pittsburgh Compound-B (PIB) tracer enables to differentiate between FTLD and Alzheimer's disease,(35) but to date there are no specific tracers available to diagnose FTLD or differentiate its pathological subtypes during life with more certainty. Future studies will focus on the earliest changes in these patients, and which functional molecular networks are affected in the pathogenesis of FTLD-TDP and FTLD-tau.(36) These studies, including PET and functional MRI, will hopefully become sensitive enough to detect the earliest changes in presymptomatic mutation carriers to serve as biomarkers for disease progression in future therapeutic studies. Whether the outcomes of familial FTLD cases can be implemented for sporadic FTLD has yet to be elucidated.

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Chapter 2.2

Survival Profiles of Patients with Frontotemporal Dementia and Motor Neuron Disease

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Abstract

Frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) are neurodegenerative diseases associated with ubiquitin- and TDP-43-immunoreactive pathology. Here we analyse if survival is influenced by symptom of onset in patients with FTD and ALS. This study is a retrospective review of patients with both cognitive impairment and motor neuron disease consecutively evaluated at four academic medical centers in two countries. A total of 87 patients were identified, including 60 who developed cognitive symptoms first, 19 who developed motor symptoms first, and 8 who had simultaneous onset of cognitive and motor symptoms. Among the 59 deceased patients, we identified two distinct subgroups of patients according to survival. Long-survivors had cognitive-onset and delayed emergence of motor symptoms after a long monosymptomatic phase, and significantly longer survival than the typical survivors (mean, 67.5 months vs. 28.2 months, respectively; $p<.001$). Typical-survivors can have simultaneous or discrete onset of cognitive and motor symptoms, and the simultaneous-onset patients had shorter survival (mean, 19.2 months) than those with distinct cognitive or motor onset (mean, 28.6 months, $p<.005$). Distinct patterns of survival profiles exist in patients with FTD and motor neuron disease, and overall survival may depend on the relative timing of secondary symptoms emergence.

Introduction

Frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) form a clinicopathologic continuum. Clinically, features of motor neuron disease (MND) such as ALS or primary lateral sclerosis may develop in patients with a behavioural or language variant of FTD, and prominent behavioural and language deficits can emerge in the course of ALS.(1-4) Pathologically, tau- and alpha-synuclein-negative cases of FTD have characteristic lesions immunoreactive to TAR DNA binding protein of 43 kDa (TDP-43), and TDP-43 immunoreactive (TDP-ir) inclusions are also the characteristic lesions in the spinal cord and motor cortex of ALS.(5-7) The overlapping clinical and pathologic features in frontotemporal lobar degeneration with ubiquitin/TDP-43 positive inclusions (FTLD-TDP) and ALS suggest that the two disorders represent a TDP-43 proteinopathy spectrum despite the differences in initial clinical presentation.(8-9) Patients who develop FTD followed by symptoms of MND are commonly given the diagnosis of FTD-MND,(10-11) and those who develop ALS before dementia are frequently diagnosed as having ALS-D.(12)

One feature common to FTD-MND and ALS-D is short survival: the development of MND in FTD or cognitive symptoms in ALS is significantly associated with poor prognosis. Patients diagnosed with FTD-MND have significantly shorter survival than patients with FTLD with ubiquitin-positive inclusions without MND,(13) and cognitively impaired patients with ALS patients also have shorter survival than cognitively normal patients with ALS.(14) It is unclear how survival in FTD-MND (cognitive onset) compares with survival in ALS-D (motor onset). Here we review the history of a large series of patients with both FTD and MND from four academic centers. We analysed the survival of patients with initial symptoms of FTD or ALS, and evaluated whether survival is influenced by initial symptom type, the relative timing of cognitive and motor symptom onset, the type of cognitive symptom (behavioural vs. motor), and the site of motor neuron disease onset (bulbar vs. limb).

Methods

Patient data were collected from the Cognitive/Dementia Clinics and ALS Clinics at the Mayo Clinic (Rochester, Minnesota), University of Pennsylvania Medical Center (Philadelphia, Pennsylvania), Erasmus University Medical Center (Rotterdam, Netherlands) and Radboud University Nijmegen Medical Center (Nijmegen, Netherlands). The clinical FTD phenotype was determined by using consensus criteria for FTD,(15-16) including both behavioural and language variants. Patients were given the diagnosis of dementia if their cognitive symptoms were sufficiently severe to impair their daily functioning, including interference with interpersonal relationship, job performance, instrumental activities of daily living, or activities of daily living; and if the evaluating clinicians considered the cognitive deficits to be sufficiently severe to cause prominent deficits despite the motor symptoms. Patients were diagnosed with motor neuron disease if they had clinical and laboratory evidence of upper and/or lower motor neuron dysfunction consistent with the diagnosis of ALS according to revised El Escorial criteria(17) or primary lateral sclerosis/upper motor neuron dominant motor neuron disease according to proposed criteria.

(18-19) Patients with primary lateral sclerosis(20) were included if there was no family history to suggest hereditary spastic paraparesis or if they transitioned to amyotrophic lateral sclerosis based on clinical and electrophysiologic criteria during the disease course. Only two patients (one each from MN and NL) were diagnosed with PLS in the setting of cognitive-onset FTD-MND. Throughout the rest of the paper, we refer to these patients and patients with ALS collectively as having ALS.

Forty patients from the Mayo Clinic (MN) were identified by searching the Mayo Clinic electronic medical record system from January 1st, 1998 to December 31st, 2007, for patients with FTD-MND, ALS-D, and ALS with cognitive impairment sufficiently severe for dementia who were evaluated at the Dementia or ALS Clinic. Twenty-three patients were recruited from University of Pennsylvania Medical Center (PA) during prospective evaluation for dementia and MND from January 1st, 1998 to July 31st, 2008. Patients from Erasmus University Medical Center and Radboud University Nijmegen Medical Center (NL) were recruited and evaluated prospectively for cognitive impairment and MND from January 1, 1995 to July 31st, 2008, and 31 patients were identified to have dementia and MND. Medical records were reviewed for historical information including gender, age of cognitive symptom onset, age of motor symptom onset, age of evaluation, age of last follow-up, age of death (if available), cognitive complaints, location of initial motor symptoms (bulbar- vs. limb-onset), and neurological examination findings. Patients were only included if they were evaluated by cognitive/behavioural neurologists with experience in FTD and/or motor neuron disease specialists with expertise in ALS. Patients were excluded if there was insufficient historical information on the cognitive or motor symptom: in 5 patients from Mayo Clinic and 2 patients from University of Pennsylvania, cognitive symptoms were found incidentally during evaluation for motor neuron disease or motor symptoms were found incidentally during evaluation for cognitive symptoms without timing or history of symptomatic progression. These patients were older when they eventually presented for evaluation (mean 74.3 yr, $p<0.001$), but were otherwise similar in gender, FTD phenotype, and site of MND onset. To analyse the relative timing of cognitive and motor symptom onset, a normalized timing of cognitive symptom onset relative to motor symptom onset was calculated by assigning negative or positive value to the absolute time interval between onset of cognitive and motor symptoms. This normalized timing was considered negative if cognitive symptoms preceded motor symptoms ("cognitive-onset"), and positive if cognitive symptoms followed motor symptoms ("motor-onset"). Patients were considered to have "simultaneous-onset" if they had concurrent onset of cognitive and motor symptoms.

Statistical Analysis

All statistical analyses were performed using SPSS version 12.0 (SPSS, Chicago, IL). Descriptive statistics were used to characterize the entire cohort, as well as each clinical subgroup. Student's t-test was used to analyse differences in continuous variables. Chi-square was used to investigate differences in dichotomous variables (including gender, bulbar vs. limb-onset, social/executive vs. language subtype) between groups. Kaplan-Meier survival analysis was used to compare disease duration of patients with

FTD and MND according to onset symptom types. K-means cluster analysis was used to group all patients with FTD and MND into clusters using gender, age of symptomatic onset, type of onset symptom, time interval between symptoms, duration of disease, FTD phenotype (social/executive disorder, language disorder, both), and MND phenotype (bulbar-onset or limb-onset). Davies-Bouldin validity index(21) was used to determine the optimal number of clusters. Cox proportional hazards regression was used to analyse factors associated with survival.

Results

A total of 87 patients were identified to have symptoms of FTD and MND (Table 1). Patients were similar in gender, FTD phenotype, age of onset, age of death, and survival across the three sites. 59 patients (68%) had died, and 20 of these patients (34%) had autopsies confirming TDP-43 pathology. Nineteen patients had pathologic findings of TDP-ir lesions affecting frontal neocortex, hippocampus, and hypoglossal nuclei and/or anterior horn cells, and one previously characterized patient with familial FTD-MND had TDP-ir lesions affecting the hippocampus, hypoglossal nuclei, and anterior horn cells but sparing the frontal neocortex.(22)

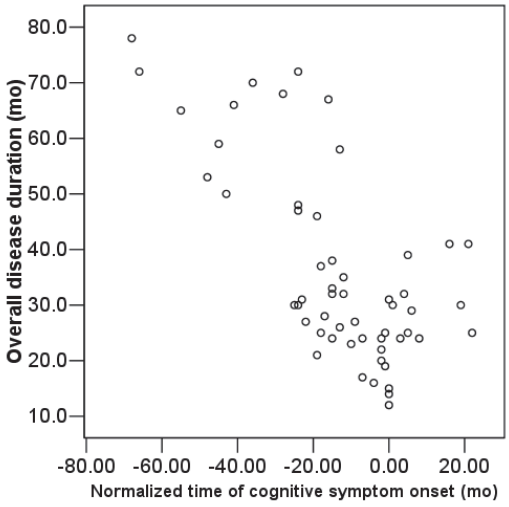
In this combined cohort, we analysed whether survival was associated with the relative timing of cognitive and motor symptom onset. We limited this analysis to the 58 deceased patients after excluding one outlier with cognitive onset followed by ALS symptoms. In this deceased cohort, normalized timing of cognitive symptom onset was inversely correlated with survival ($r = 0.694$, Figure 1): survival was longer as cognitive symptoms appeared earlier relative to motor symptoms. However, cases also appeared to form distinct clusters. K-means cluster analysis was used to identify cluster membership, and an optimal number of two clusters was determined by Davis-Bouldin index. Cluster one contained 15 patients who all had FTD as their onset symptom (cognitive-onset), and Cluster 2 contained 43 patients including both cognitive-onset and motor-onset patients. Patients in Cluster 1 had significantly longer survival (mean 67.5 mo) than patients with a more typical survival in Cluster 2 (mean 28.2 mo, $p < 0.0001$). "Long survivors" had delayed emergence of secondary symptoms (mean 43.0 mo) relative to "typical survivors" (11.2 mo, $p< 0.001$). The long-survivors in Cluster 1 were significantly more likely to have limb-onset MND than typical survivors (80% vs. 26%, $p < 0.001$). Long survivors otherwise were similar to typical survivors in age of onset, gender, and FTD phenotype (Table 2). Only one long survivor had PLS eight months before death. Five of the long survivors went on to autopsy, and all had TDP-ir pathologic changes.

Table 1 Basic characteristics of patients with FTD and MND from the three participating medical centers

	Mayo clinic (n=35)	Philadelphia (n=21)	The Netherlands (n=31)*	Overall (n=87)
Female, No. (%)	14 (40)	10 (48)	10 (32)	34 (39)
Bulbar-onset MND, No. (%)	18 (51)	10 (48)	18 (58)	46 (53)
FTD subtype				
Social/executive dysfunction	21 (60)	14 (67)	22 (71)	57 (66)
Language	6 (17)	4 (19)	4 (13)	14 (16)
Both	3 (9)	2 (9)	5 (16)	10 (11)
Alzheimer disease-like	5 (14)	1 (4)	0	6 (7)
EMG performed, No (%)	26 (74)	21 (100)	18 (58)	65 (75)
Follow-up after second symptom, mean (median), mo	7.5 (7)	11.8 (10)	19 (18)	11.2 (10)
Death/Autopsy	16/6	15/3	28/11	59/20
Cognitive before motor	26	11	23	60
Age of onset, mean (median, yr	59.7 (60.5)	56.8 (57)	57.3 (55)	58.3 (57.5)
Duration until motor symptoms, mean (median), mo	20.3 (18.5)	32.3 (25)	28.5 (18)	25.7 (19)
Death	15 (58%)	8 (73%)	20 (87%)	43 (72)
Overall disease duration, mean (median), mo	34.6 (30)	48.8 (47)	51.7 (43)	44.0 (34.5)
Disease duration after motor symptom, mean (median), mo	14.3 (13)	16.5 (14)	23.9 (21)	18.3 (14)
Simultaneous onset	3	2	3	8
Age of onset, mean (median), yr	63 (63)	61 (68)	65 (73)	63 (67.5)
Death	1 (33%)	0	3 (100%)	4 (50)
Overall disease duration, mean (median), mo	23.7 (24)	21 (21)	13.7 (14)	19.3 (17.5)†
Motor before cognitive	6	8	5	19
Age of onset, mean (median), yr	58.3 (57.5)	57.4 (56.5)	55.8 (51)	57.3 (57)
Duration until cognitive symptoms, mean (median), mo	14.7 (9)	11 (8)	13.2 (5)	12.7 (8)
Died, No (%)	0	7 (88)	5 (100)	12 (63)
Overall disease duration, mean (median), mo	33.3 (27)	31.9 (29.5)	37.4 (39)	34.3 (31)
Disease duration after cognitive symptom onset, mean (median), mo	18.6 (18)	21.9 (21)	24.2 (30)	21.4 (20)

* Erasmus University Medical Center, Rotterdam and Radboud University Nijmegen Medical Center together. † Significantly different from patients with cognitive onset (p<.001) and motor-onset (p=.02).

Figure 1 Relationship between normalized time of cognitive symptom onset and overall disease survival among deceased patients. Normalized time of cognitive symptom onset was derived by subtracting date of cognitive symptom onset from date of motor symptom onset. A negative value means cognitive onset, and a positive value means motor onset.



We extended the analysis to include patients still living at last follow-up. Nine patients still living at last follow-up had similarly long disease duration (mean disease duration 77.1 mo, range 60-119) and represented additional long-survivors. Long-survivors were more likely to have cognitive-onset than typical-survivors (88.0% vs. 59.7%, p=0.02). Among the typical-survivors, 27.4% had motor-onset, and 8 patients (12.9%) had simultaneous onset of cognitive and motor symptoms within the same month (Chi-square = 10.8, p<0.005). There was no difference in survival between patients who began with cognitive- or motor-symptoms (Figure 2). However, patients with simultaneous-onset had the shortest overall disease duration compared to patients with discrete cognitive or motor onset by Kaplan-Meier analysis (mean 19.3 mo vs. 28.6 mo, p=0.005),

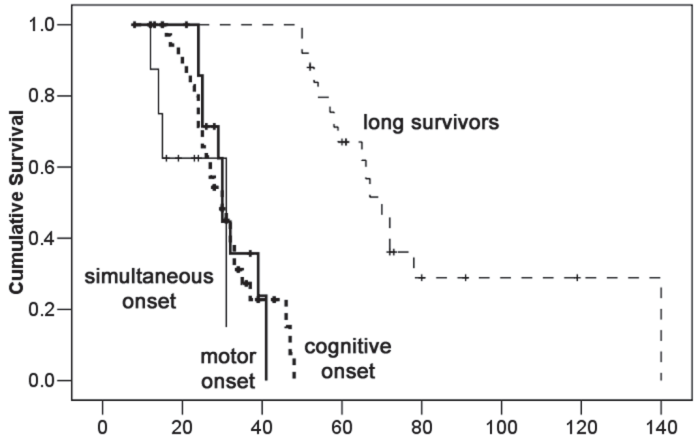
To further determine the effect of inter-symptom duration on survival, we performed a multivariate Cox proportional hazards regression analysis using all patients. This analysis also included age of onset, gender, pattern of symptom onset (cognitive, motor, or simultaneous), and site of motor neuron symptom onset. In the group as a whole, prognosis was better in those with longer inter-symptom duration (H.R.=0.939/mo, 95% CI 0.919-0.959, p<0.001) and worse in those with bulbar-onset ALS (H.R.=1.751, 95% CI 1.006-3.050, p=0.048). When typical- and long-survivors were analysed separately, inter-symptom duration continued to be a strong predictor of survival within each subgroup (H.R.=0.925/mo, p <0.001 for typical-survivors and H.R.=0.951/mo, p=0.003 for long-survivors). However, older age of onset (H.R.=0.913/yr, 95% CI 0.843 – 0.988, p=0.025) was associated with better prognosis only in the long-survivors, and there was only a trend that bulbar-onset ALS was associated with worse outcome in the typical-survivors (p=0.114).

Table 2 Characteristics of patients according to survival patterns

	Typical Survivors			
	Simultaneous Onset (n=8)	Cognitive or Motor Onset (n=54)	Long-term Survivors (n=25)	p-value
Female, No. (%)	8 (63)	54 (39)	25 (32)	.31
Died, No (%)	4 (50)	39 (72)	16 (64)	.40
Age of onset, mean (SD), yrs	63.0 (12.9)	58.7 (10.2)	58.5 (10.3)	.28
Cognitive Onset, No. (%)	0	37 (69)	22 (88)	<.001
Bulbar Onset, N. (%)	5 (63)	34 (63)	7 (28)	.01
Language dominant cognitive symptoms, No. (%)	3 (38)	16 (30)	7 (28)	.88
Overall disease duration, mean (SD), mo	19.3 (6.4)	28.6 (8.8)	69.3 (21.3)	<.001
Disease duration until secondary symptom onset, mean (SD), mo	0	12.4 (7.9)	44.5 (21.6)	<.001
Disease duration after secondary symptom, mean (SD), mo	19.3 (6.4)	15.1 (7.4)	24.1 (15.5)	.01

Chi-square and one way analysis of variance were used to determine differences between dichotomous and continuous variables, respectively.

Figure 2 Kaplan-Meier survival analysis of patients with FTD-MND according to survival grouping.



Discussion

By combining data from four referral centers, we report the largest clinical series of patients who developed motor symptoms in the setting of FTD and dementia symptoms in the setting of ALS. In this large cohort, we were able to identify two unique subgroups of patients according to survival patterns. In addition to typical-survivors, we discovered a group of patients with long survival characterized by mostly cognitive-onset and delayed emergence of secondary symptoms with limb-onset ALS. We discuss these findings below.

In our large cohort, FTD-MND/ALS-D patients have mean and median survival periods in keeping with previously reported values from smaller clinical(11, 14) and clinicopathologic series.(13, 23) We found that the relative timing of secondary symptom onset was a strong predictor of prognosis in our study among both typical- and long-survivors. The timing of secondary symptom onset may thus reflect in part the rate of stage-wise disease progression regardless of cognitive- or motor-onset, as progression rate is known to influence survival in ALS.(24) Furthermore, survival in long-survivors was inversely correlated with age of onset. It is not known if age of onset is associated with prognosis in clinical FTD with TDP-ir pathology, but an older age of onset is associated with better survival in ALS without dementia.(25) Thus, the similarities in prognostic factors between long-survivors, typical-survivors, and typical ALS without dementia offer additional support that they form a clinicopathologic continuum.

The power of the large number of patients included in this study enabled us to identify the long-survivors. One potential explanation for this distinct subgroup may be the differential anatomic involvement by pathology. In typical-survivors, disease involving TDP-ir changes may be evident throughout pre-frontal regions and the pyramidal system. The relative delay in onset between cognitive- and motor-systems is minimal, and the initial symptom at presentation is proportional to the volume of these regions within the frontal lobe. In contrast to the distributed-model of TDP-ir pathology in typical-survivors, long-survivors may have focal onset of TDP-ir changes in pre-frontal regions with subsequent involvement of the pyramidal system only following a long delay. Differences between these two groups were not detectable on autopsy as pathologic changes were insensitive to the relative timing of regional involvement, but future biomarkers of region-specific involvement of TDP-ir pathology may provide some indication of biological differences between typical- and long-survivors. Other differences between these groups may also account for the longer survival, such as progranulin haplotypes, and should be ascertained in future prospective studies of FTD-MND/ALS-D.

This study has a number of limitations. First, our observations are retrospective in nature. More patients had cognitive than motor onset from centers with long established interests in dementing illnesses, although patients were equally likely to be seen at Cognitive/Dementia or ALS Clinics. Ascertainment bias may account for the longer range of inter-symptom durations in cognitive-onset patients as dementia may delay subjective motor complaints, and the smaller number of motor-onset patients

also may be due in part to the recent recognition of dementia in ALS. Survival data was incomplete in some cases as patients were lost in follow-up. Only a proportion of cases had autopsy confirmation. The combination of FTD and MND nearly always correlated with TDP-ir pathology, although rare exceptions with a tau-positive disorder have been reported.(8, 26) Lastly, there may exist referral bias in that most patients were referred by primary neurologists or primary care providers. However, with the low prevalence of FTD-MND and ALS-D in general clinical practice, this represents the largest multi-center study to-date on the clinical features of patients with cognitive and motor TDP-43 involvement. ALS patients with clinical or subclinical cognitive impairment but without dementia are beyond the scope of the current study, although more careful cognitive assessments should be included in future studies so that the severity of cognitive impairment can be correlated with prognosis. With these caveats in mind, we propose two groups of patients with combined FTD and ALS - typical-survivors and long-survivors. Survival is influenced by the inter-symptom duration between initial and secondary symptoms, the symptom of onset, and site of ALS onset. Patients with cognitive-onset and significantly delayed emergence of motor symptoms should be identified for their atypical prognosis, and patients with simultaneous onset of cognitive and motor symptoms also may have an atypical prognosis. Future studies will be necessary to assess biological factors associated with prolonged disease duration for potential therapeutic strategies.

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Chapter 3

Genetic Forms of Frontotemporal Dementia

Chapter 3.1

Progranulin Mutations in Dutch Familial Frontotemporal Dementia

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Abstract

Mutations in the *progranulin* (*GRN*) gene have been recently identified in frontotemporal lobar degeneration with ubiquitin-positive inclusions linked to chromosome 17q21. We report here the finding of two novel frameshift mutations and three possible pathogenetic missense mutations in the GRN gene. Furthermore, we determined the relative frequency of *GRN* mutations in familial cases recruited from a large population-based study of frontotemporal lobar degeneration carried out in The Netherlands.

Introduction

The term frontotemporal lobar degeneration (FTLD) refers to a heterogeneous group of neurodegenerative disorders clinically characterized by progressive behavioural changes and cognitive dysfunctions, including executive and language functions.(1) Language impairment can be the initial symptom in two language variants of FTLD, named progressive non-fluent aphasia and semantic dementia. Additionally, the clinical picture can be complicated by motor disorders such as motor neuron disease (MND) or parkinsonism.(2)

Two main pathological FTLD subtypes are recognized based on the presence of tau-positive inclusions (tauopathies) or tau-negative ubiquitin-positive neuronal inclusions (FTLD-U).(3) Characteristically the ubiquitin immunoreactive inclusions (ub-i) are observed in the dentate gyrus of the hippocampus and in the superficial layers of the frontal and temporal cortex.(4)

A positive family history is found in approximately 40 percent of FTLD cases, and linkage studies have shown that FTLD is genetically heterogeneous with loci and genes identified on chromosomes 3 (FTD3),(5) 9p,(6) 9q(7) and 17q (FTDP-17(8) and FTDU-17.(9-10) Recently, mutations in the *progranulin* gene (*GRN*) were found in several families with FTDU-17.(9-10)) *GRN* encodes a biologically active precursor glycoprotein previously described as a multifunctional growth factor involved in development, inflammation and wound repair.(11)

In the present study, we report the finding of two novel frameshift mutations and three possible pathogenetic missense mutations in the GRN gene. In addition, we describe genetic contribution of *GRN* to FTLD in a series of familial cases recruited from a large cohort of FTLD patients.

Material and Methods

Patients

Three hundred and thirty-eight patients with FTLD (182 females and 156 males) with mean age at onset of 57.4 ± 9.3 years were identified in a genetic-epidemiological study in the Netherlands. The clinical diagnosis in all patients was established according to the International Consensus Criteria.(12) Clinical family history was positive in 166 patients (59%) and among them DNA was available in 137 cases. Eighty-seven of these 137 patients came from independent families: ten families presented *MAPT* mutations,(13) two large families showed FTLD-U with definite linkage to chromosome 17q21-22, six smaller families had multiple (> 2) affecteds, and 69 had two affecteds.

DNA study

The 13 exons of *GRN* including intron/exons boundaries were amplified from genomic DNA by PCR and directly sequenced in both strands. Novel sequence variants were analysed in a minimum of 380 chromosomes from healthy individuals of matched ethnicity.

Immunohistochemistry

Immunohistochemistry experiments were performed on eight available brains as previously described.(14)

Results

To determine the possible involvement of the newly found *GRN* gene in our cohort, we systematically screened for mutations in 77 cases with positive family history of dementia consistent with autosomal dominant pattern of inheritance and with no *MAPT* and *CHMP2B* mutations. The mean age at onset in this group was 59.3 ± 9.1 years.

We identified two novel frameshift mutations Ser81Valfs174X and Val411Sfr1X (Table1) predicted to cause premature termination of the coding sequence likely leading to loss of functional GRN protein similar to previous reports. One nonsense mutation (Gln125X) was also observed in an independently ascertained member of the 1083 FTLD-U family already described.(10) Furthermore we identified 5 novel coding sequence variants (three missense and two silent mutations) and two intronic sequence changes in intron 2 and 7 and the two previously reported missense mutation Gly414Val.(15) The frameshift mutations and the GGG93GGA, Thr182Met, Pro233His, CAC447CAT and Trp541Cys mutations were not in controls; in contrast two intronic variants were also present in individuals suggesting they are not pathogenetic. Moreover, the GGG93GGA silent mutation was detected in co-occurrence with the Pro233His.

Table 1 *GRN* mutations identified in FTLD patients and healthy control individuals

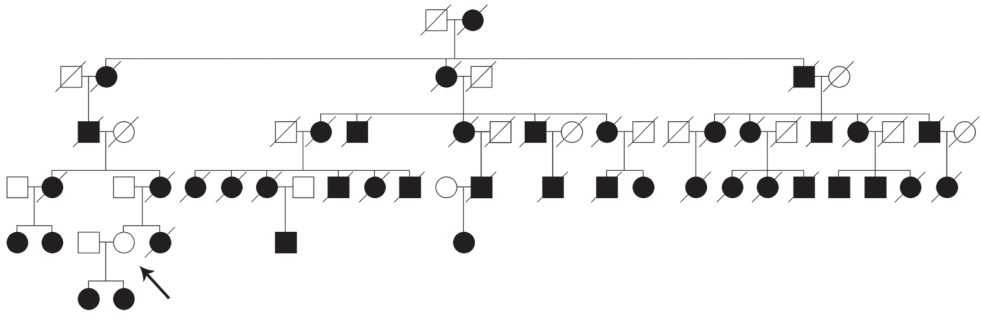
Location	Genomic[a]	Predicted cDNA[b]	Protein[c]	Rs number	Patients N	Controls N
Exon 2	g.4407delC	c.243delC	Ser82ValfsX174		HFTD3	-
Intron 2	g.4436G>A				1	4
Intron 2	g.4445G>A			rs9897526	19	35
Exon 3	g.4559G>A	c.279G>A	Gly93Gly		1	-
Intron3	g.4661G>C				-	1
Exon 4	g.5129C>T	c.592C>T	Gln125X		1	-
Exon 5	g.5402C>T	c.545C>T	Thr182Met		1	-
Exon 6	g.5667>A	c.698C>A	Pro233His		1	-
Intron 7	g.6048G>A				22	18
Exon 10	g.6944_6945 delGT	c.1231_1232delGT	Val411SerfsX1		1	-
Exon10	g.6954G>T	c.1241G>T	Gly414Val		1	1
Exon10	g.7054C>T	c.1341C>T	His447His		1	-
Exon10	g.6966G>A	c.1253G>A	Arg418Gln		-	2
Exon11	g.7428G>C	c.1623G>C	Trp541Cys		1	-

A: numbering relative to NC_000017.9 Genbank accession number and starting at nucleotide 1
B: numbering relative to NM_002087.2 starting at the ATG
C: numbering relative to NP_002087.1

The Ser81Valfs174X mutation was found in a 69-year old woman, member of the HFTD3 family previously linked to 17q21-22(4) (Figure 1). A large variation in age at onset (between 45 – 75 years) and in age at death (45 – 83 years) was observed between affected individual from this family. In addition the 72-years old mother of two affected sisters (age 45 and 46 respectively) who carried the mutation did not have any cognitive complaints and behavioural changes as confirmed by reports of other family members and by neurological examination.

Sequencing of 13 additional DNA samples from affected family members showed completely segregation of the mutation with the disease. The clinical symptoms in this family consisted of apathy, loss of initiative and interest, roaming behaviour and word finding difficulties. Two patients developed parkinsonism early in the course of the disease, which moderately responded to levodopa treatment. Signs of motor neuron disease were not observed. Extensive neuropsychological testing in 6 patients revealed impaired naming with normal comprehension of language.

Figure 1 Pedigree of family HFTD3; only affected individuals are shown. This large consists of 42 affected and 102 unaffected members. Arrow indicates a healthy carrier: a 72-year old mother of two affected sisters who carried the mutation and did not have any cognitive complaints and behavioural changes as confirmed by reports of other family members and by neurological examination.



The Val411Serfr1X mutation was identified in a 66 years-old woman, who presented with speech, and writing errors, and word finding difficulties in the final three years. The patient showed social inappropriate behaviour and emotional bluntness. Magnetic resonance imaging showed asymmetric right-sided frontotemporal atrophy. The patient developed loss of initiative, and died from bronchopneumonia six years later. Her mother and grandmother suffered from identical symptoms, whereas her uncle and a nephew were diagnosed as Pick's disease.

The Gln125X mutation was found in a 60 years old woman, who came from the family 1083, previously described.(10) She presented with memory problems and word findings difficulties.

Neuropathological examination showed ubiquitin-positive in dentate gyrus, neocortex and/or striatum. Ubiquitin inclusions were also present in additional brains from FTLD with no *GRN* mutations, with the distinct morphology and distribution pattern characteristic of FTLD-U type 2,(16) including intranuclear ubiquitin-positive inclusions in frontal cortex in one brain.

Discussion

The present study report the identification of three (Val411Serfr1X and Ser81Valfs174X as novel) *GRN* mutations that account for approximately four percent of the independent familial FTLD cases.

Similar to previous studies the two novel mutations determine a frameshift which results in the generation of premature termination codons. Eukaryotic cells are capable to detect and degrade transcripts harbouring premature signals for the termination of translation through the nonsense-mediated mRNA decay (NMD) pathway. Degradation of mutant mRNAs results in null alleles (9, 15) with loss of functional GRN.

Several rare missense and silent mutations with unknown pathological significance were detected in patients and not in controls. Segregation studies could not be performed in these cases, as DNA from affected family members was not available.

Although it can not be excluded that these changes are benign variants as they are located in granulin domain each composed of 7,5 tandem repeats of highly conserved motifs of 12 cystein residues suggested to be functional redundant,(15) several studies have shown that separate repeats may have alternative binding capacities and therefore different functions,(17) highlighting the possibility that these variants are pathogenetic. The Pro233 and the Trp541 in particular, are highly conserved among species and in the granulin domains. Furthermore, previous reports have suggested that these amino acids are essential for the proper folding of the protein. (18-20) The Trp residue is likely involved in the hydrophobic packaging of the beta-sheet and substitution with the cys residue, which has the ability to from disulfide bridges, might affect GRN 3D structure. The Pro residue is part of an antiparallel beta-sheet, and might be important for stacking multiple repeats, necessary for the proper protein conformation. Therefore, his substitution with the His residue may also change GRN 3D structure with consequences at functional level. The effect of the Thr182Met mutation is less clear since it is just outside the granulin motif. This amino acid is conserved between mammal and was not detected in controls.

Consistent with other *GRN* studies, the clinical presentation in patients carrying the pathogenic mutations is characterized by a large variation in age at onset and by occurrence of symptoms of non-fluent aphasia whereas semantic deficits were more often seen in patients with missense and intronic *MAPT* mutations within the same cohort.(21) The HTFD3 family, in particular, shows a large variation in age at onset varying between 45 and 75 years, with a maximum change of 25 years between consecutive generations. The high variability is further confirmed by the presence of a 72 years old healthy carrier. Our and other findings show that a significant proportion of patients remain unaffected until old age suggesting therefore an interplay of several genetic and/or environmental factors in the disease development.

The percentage of *GRN* mutations detected in our familial FTLD cohort (up to seven percent by including the two highly conserved missense mutations) is lower compared to the much higher frequency observed in other studies where *GRN* mutations explain up to approximately 25 percent of familial FTLD.(10, 15) The lower frequency of *GRN* mutations in our group might reflect differences in patients recruitment methods, as the *MAPT* mutations in the Belgian cohort account for only to seven percent of

all familial cases compared to 14 percent detected in this cohort.(9-10, 14) In addition in the studies by Cruts *et al*(10) and Baker *et al*(9) a strong founder effect among probands carrying the IVS0+5G>C and Arg493X was observed, while we estimated mutation frequency only in independent patients.
In addition, geographical differences may also play a role, as seen in *MAPT* studies, and they cannot be ruled out until more reports will allow a better estimate of *GRN* mutation frequency in familial FTLD.
In summary mutations in *GRN* explain only part of FTLD in our cohort and they are absent in 81 percent of cases including familial FTLD-MND as well as FTLD-U without MND strongly suggesting that we are only beginning to unravel the molecular pathways leading to FTLD and that additional genes contribute to the disease pathogenesis.

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Chapter 3.2

Distinct Genetic Forms of Frontotemporal Dementia

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Abstract

Frontotemporal dementia (FTD) is the second most common type of presenile dementia and can be distinguished into various clinical variants. The identification of *MAPT* and *GRN* defects and the discovery of the TDP-43 protein in FTD have led to the classification of pathological and genetic subtypes. In addition to these genetic subtypes, there still exist familial forms of FTD with an unknown genetic defect(s). We investigated the frequency, demographic and clinical data of FTD patients with a positive family history in our prospective cohort of 364 patients. Genetic analyses of genes associated with FTD were performed on all patients with a positive family history. Immunohistochemical studies were carried out with a panel of antibodies (tau, ubiquitin, TDP-43) in brains collected at autopsy. In our total cohort of 364 patients, 27 percent had a positive family history suggestive for an autosomal mode of inheritance, including *MAPT* (11%) and *GRN* (6%) mutations. We identified a new Gln300X *GRN* mutation in a patient with a sporadic FTD. The mean age at onset in *GRN* patients (61.8 ± 9.9 years) was higher than *MAPT* patients (52.4 ± 5.9 years). In the remaining 10 percent of patients with suggestive autosomal dominant inheritance, the genetic defect has still to be identified. Neuropathologically, this group can be distinguished into familial FTLD-MND and familial FTLD-U with hippocampal sclerosis. Future genetic studies have to identify genetic defects in at least two distinct familial forms of FTD with unknown genetic defect(s): FTLD-U+HS and FTLD-MND.

Introduction

Frontotemporal dementia (FTD) is the second most common type of presenile dementia, and is characterized by behavioural changes and cognitive dysfunctions. Different clinical variants within the spectrum of FTD are defined according to the International Consensus criteria,(1) including the behavioural variant of FTD (bvFTD), progressive non-fluent aphasia (PNFA), semantic dementia (SD) and FTD with motor neuron disease (FTD-MND). Two main neuropathological subtypes of frontotemporal lobe degeneration (FTLD) can be distinguished: those with tau-positive pathology (FTLD-tau) and those with tau-negative, ubiquitin-positive inclusions (FTLD-U).(2) Hippocampal sclerosis, defined as severe neuronal loss and gliosis in cornu ammonis field 1 (CA1) and subiculum is a frequent finding in FTLD-U, but less often FTLD-MND.(3-4) TAR-DNA binding protein 43 (TDP-43) has been identified as major constituent of ubiquitin-positive inclusions in both FTD and ALS,(5-7) and this TDP-43 pathology has been further classified into four subtypes.(2, 6, 8)

FTD has shown a strong familial component with a positive family history in 30 – 50 percent of the patients.(9-11) Mutations in the *MAPT* gene are associated with hereditary FTD with neuronal and glial tau inclusions.(12-13) Mutations in the *Progranulin* (*GRN*) gene have been identified in families with ubiquitin- and TDP-43 positive inclusions.(14-15) The genetic heterogeneity of FTD has been further emphasized by genetic defects in the *VCP* and *CHMP2B* genes, and significant linkage to chromosome 9.(16-18). However, there still exist familial forms of FTD with unknown genetic defect(s).

In the present study, we investigated the frequency, the clinical and pathological features of FTD patients with a positive family history in our prospective cohort. With genetic screening of the latter group, we aimed to identify and characterize familial forms with unknown genetic defect(s).

Methods

Clinical data

Patients with FTD were ascertained in an ongoing genetic-epidemiological study conducted in the Netherlands since 1994,(9) after referral to the out-clinic department of the Erasmus Medical Center, or after visiting nursing homes and psychogeriatric hospitals by the research physician. Detailed clinical history was obtained from the spouses and first-degree relatives using a checklist of behavioural and cognitive changes, and motor symptoms.

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. Ambulant patients, who visited our outpatient clinic underwent neuropsychological evaluation and neuroimaging (MRI, CT, or SPECT with 99mTc-hexamethyl propyleneamine oxime (HMPAO)). Patients with muscle weakness or other signs suggestive of lower motor neuron disease underwent electromyography. For patients visited in nursing homes by the research physician, data collection was limited to detailed clinical history, neurological examination, and clinical data available from medical records, including hard copies of neuroimaging. 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 disease.(9)

Neuropsychological evaluation carried out in 147 ambulant patients consisted of several (language) tests (e.g. Boston Naming Test, the Dutch revised version of the Semantic Association Test,(19) word fluency), memory functions (Rey auditory verbal learning test, 15-Word test),(20) attention and concentration, executive functions (Trail Making test, Stroop, modified Wisconsin Card Sorting Task, Similarities, and proverbs), and visuospatial abilities (Clock drawing, Block Design of the WAIS).

The FTD diagnosis was made according to the International Consensus Criteria.(1) Only patients with a definite or probable FTD were included in this study. SD was defined as fluent speech with marked anomia, loss of word meaning and impaired word comprehension or semantic paraphasias, whereas PNFA was defined as disrupted, non-fluent speech output, with agrammatism, phonemic paraphasias, or anomia. Patients with dementia and bulbar symptoms, muscle atrophy, fasciculations or electromyographic evidence of motor neuron disease (MND) were classified as FTD-MND. Parkinsonism was defined present if at least two of four clinical cardinal signs (rigidity, tremor, bradykinesia, and postural instability) were present. The topographic distribution and severity of cerebral atrophy on CT or MRI was evaluated, as described previously.(21)

Data on family history were obtained from a structured questionnaire as described before.(22) Two classes of positive family history were distinguished; 1) an autosomal dominant mode of inheritance, if at least three individuals over two or more generations were affected 2) a positive family history with, in addition to the proband, one or more affected individuals with dementia, parkinsonism, or MND within one generation or different branches of the same family, not sufficient for an autosomal dominant mode of inheritance. Two levels of information about affected relatives were distinguished: 1) data obtained from medical record 2) family report of progressive cognitive dysfunction.

The study was approved by the Medical Ethical Committee of the Erasmus Medical Center of Rotterdam. For each patient, a spouse or first-degree relative of the patient gave written informed consent. Genomic DNA was extracted from peripheral lymphocytes according to standard procedures.(23) All patients were followed up, either by visits to our outpatient department or by telephone interview with relatives.

Genetic analysis

Mutation screening was performed of all exons and exon-intron regions of *MAPT*, *GRN* and *CHMP2B* genes in all patients with a positive family history, and in a subset of patients with sporadic FTD (n=72).(22, 24-25) *VCP*, *angiogenin* (*ANG*), and *Dynactin* (*DCTN1*) gene mutation analysis was performed in patients with familial FTD-MND as described before.(22) The SNP rs9897526 was genotyped by direct sequencing. Primer sequences to amplify rs9897526 were: aaatggcccacaacactgagc (forward), ggcagggccctttttatctgc (reversed); amplicon size: 212 bp. Direct sequencing of both strands was performed using Big Dye Terminator chemistry ver.3.1 (Applied Biosystems) on an ABI3100 automated sequencer and analysedusing DNA Sequencing Analysis (ver.3.7) and SeqScape (ver.2.1) software (Applied Biosystems).

Pathological examination

Brain autopsy was carried out within four hours of death according to the Legal and Ethical Code of Conduct of the Netherlands Brain Bank. Tissue blocks taken from all cortical areas, hippocampus, amygdala, basal ganglia, substantia nigra, pons, medulla oblongata, cerebellum, and cervical spinal cord and were embedded in paraffin blocks, and underwent routine staining with haematoxylin-eosin, Bodian, methenamine-silver, and Congo red. The severity of neuron loss was scored into absent, mild and moderate to severe. Hippocampal sclerosis (HS) defined by severe neuronal loss and gliosis in CA1 and subiculum, occasionally spreading to the entorhinal areas and amygdala.

Immunohistochemistry was performed using the following primary antibodies against hyperphosphorylated tau (AT8, Innogenetics, Ghent, Belgium; 1:40), ubiquitin (anti-ubiquitin, DAKO, Glostrup, Denmark; 1:500, following 80˚C antigen retrieval), β-amyloid protein (anti-beta amyloid, DAKO, Glostrup, Denmark; 1:100, following formic acid pre-treatment), α-synuclein (anti-α-synuclein, Zymed Laboratories, San Francisco, California, USA; undiluted, following formic acid pre-treatment), p62 (BD Biosciences Pharmingen, San Diego, CA, USA; 1:200, following 80˚C antigen retrieval), and TDP-43 (Biotech, Chicago, IL, USA; 1:100, following pressure-cooking), and stained as previously described.(22) Primary antibodies were incubated overnight at 4ºC. Endogenous peroxidase activity was inhibited by incubation in PBS-hydrogen peroxide-sodiumazide solution (100ml 0,1 M PBS + 2 ml 30% H_2O_2 + 1 ml natriumazide) for 30 minutes. The Histostain-Plus broad-spectrum kit DAB (Zymed, San Francisco, California, USA) was used, and slides were counterstained with Mayer's haematoxylin and mounted in entellan. The pathological diagnosis was made by one of two neuropathologists (W.K., J.M.R.). The pattern of FTLD-U pathology was classified into four different subtypes according to the morphology and laminar distribution of neuronal inclusions as proposed by Cairns et al.(2)

Statistical analysis

SPSS 11.0 for Windows (SPSS, Chicago, Ill. U.S.A.) was used for statistical analysis. Data of age at onset were analysedusing independent sample t-test, whereas data of age at death and duration of illness were analysedusing univariate analysis of variance, with gender and age at onset as covariables. A paired sample t-test was used to compare age at onset in affected parent versus affected offspring with *GRN* mutation. In addition, we analysedthe change in age at onset in parent-offspring pairs with *GRN* versus those with *MAPT* (P301L) mutation. A significance level of $p<0.05$ was used.

Results

Demographic data and clinical variants

The present cohort consisted of 364 patients with FTD (174 men, 190 women). The mean age at onset was 57.8. ± 9.1 years. The behavioural variant (bvFTD) was the most common clinical subtype (68%), followed by SD (15%), PNFA (10%), and FTD-MND (7%). Detailed demographic and neuroimaging data of these four subtypes are summarized in Table 1. Early parkinsonism was seen in 16 percent of the total cohort.

Family history was positive in 170 patients (47%) and negative in 194 patients (53%). An autosomal dominant mode of inheritance was found in 27 percent of all patients, including 11 percent with *MAPT* gene mutations, 6 percent with *GRN* gene mutations, and 10 percent with an unknown genetic defect. The remaining 20 percent with a positive family history had, in addition to the proband, one of more affected family members not sufficient for an autosomal dominant pattern of inheritance.

Table 1 Demographic and neuroimaging data of four clinical subtypes

	bvFTD n = 246	SD n = 56	PNFA n = 37	FTD-MND n = 25	Total Group n = 364
Female, %	58	39	49	32	52
Died during follow-up	160	24	15	17	216
Age at onset, years (sd)	57.0 (9.2)	59.3 (7.2)	61.2 (8.5)	57.8 (10.7)	57.8 (9.1)
Age at death, years (sd)	65.1 (9.7)	69.2 (6.8)	68.1 (9.7)	59.1 (10.2)	65.3 (9.7)
Duration of illness, years (sd)	9.0 (4.1)	9.6 (3.1)	7.6 (2.8)	3.4 (1.5)	8.6 (4.1)
Neuroimaging					
Frontotemporal, %	75	11	32	80	61
Predominant temporal, %	18	89	65	16	33
Generalized/no atrophy, %	7	-	3	4	6
Asymmetric, %	34	80	82	20	45
Family history					
Negative family history (%)	118 (48)	40 (71)	20 (54)	16 (64)	193 (53)
Autosomal dominant (%)	88 (36)	2 (4)	3 (8)	5 (20)	99(27)
Other familial (%)	40 (16)	14 (25)	14 (38)	4 (16)	72 (20)
Parkinsonism	45 (18)	6 (11)	5 (14)	1 (4)	57 (16)

Values are percentages or unadjusted means (sd)

Autosomal dominant mode of inheritance

MAPT mutations

Six different *MAPT* gene mutations (G272V, ΔK280, P301L, L315R, S320F, R406W) were identified in 11 percent (n=39; 18 male, 21 female) coming from 10 families. Demographic data are summarized in Table 2. The mean age at onset was 52.4 ± 5.9 years (range 42-65 years) (Figure 1). Semantic deficits occurred in 44 percent of the patients. Parkinsonism developed early in five with P301L mutation, and two patients with R406W mutation, whereas none has developed MND.

GRN gene mutations

Three different *GRN* mutations (Val411SerfrX1, Ser82ValfsX174, Gln125X) were identified in 6 percent (n=23).(25) A novel fourth mutation, Gln300X (c.592C>T), mutation was detected in a single, sporadic patient with language problems at the age of 60, and followed by behavioural changes two years later. She died in a nursing home aged 66.

The Ser82ValfsX174 mutation was identified in 14 patients from one four-generation family. The mean age at onset was 61.8 ± 9.9 years (range 45-79 years) (Figure 1), was higher than those with *MAPT* mutations (p<0.001), whereas the duration of illness was shorter (Table 2).

Figure 1 Variability in age at onset between patients with *MAPT* and *GRN* gene mutations

The age at onset in eight affected parent–offspring pairs showed a mean lowering in age at onset in the next generation of 13.5 years (p=0.018), which was significantly different from 25 affected-offspring pairs with the *MAPT* (P301L) gene mutation (p=0.032). SNP analysis showed that the mean age at onset in patients with a GG genotype of the SNP rs9897526 (mean age at onset 62 years, range 45-75 years) did not differ from those with a GA genotype (mean age at onset 64 years, range 48-79 years). Two patients with the Ser82ValfsX174 and the A allele on the wild-type allele had an onset age of 48 and 54 years, whereas the mean onset age was 62 years for the remaining patients with the G allele from this family.

Memory problems had led to the erroneous initial diagnosis of Alzheimer disease in eight patients. Reduced fluency and naming impairment developed in 56 percent, whereas semantic impairment was seen in only 17 percent. Parkinsonism was found in five, with asymmetric rigidity and dystonia in two patients, whereas none has developed MND.

Autosomal dominant form with unknown genetic defect

The mean age at onset, age at death, and the duration of illness of the 10 percent (36 patients from 31 families) with unknown genetic defect were in between those of patients with *MAPT* mutations and *GRN* mutations (Table 2).

Patients presented with the behavioural variant of FTD (n=27), progressive aphasia (n=4), or FTD - MND (n=5). Co-occurrence of memory problems has frequently led to the initial misdiagnosis Alzheimer's

disease (n=12). Information about affected relatives (onset age was <65 years in 84% of the families) was obtained from family reports (37%) or medical records (63%). Sixteen families had >3 affected family members. Parkinsonism was reported in five, and FTD-MND in seven families.

Other patients with a positive family history

Demographic data of the remaining 20 percent (n=72, 67 families) with a positive family history are summarized in Table 2. Information on dementia, parkinsonism, or MND in an affected first-degree relative (onset age <65 years in 43%) was obtained from family report in 18 percent, and from medical records in 82 percent. Three families had >2 affected family members. Four families showed FTD-MND.

Pathology

MAPT mutations

Of the 15 brains available from patients with *MAPT* gene mutations, moderate to severe neuron loss and gliosis was seen in the frontal cortex (n=3), temporal cortex (n=4), or both (n=7), whereas the neocortex showed only mild changes in one brain. Striatum was severely involved in one, and substantia nigra in four brains. Tau pathology present in all brains consisted of neuronal inclusions (pretangles, NFT, Pick bodies) with or without tau-positive glial inclusions, as described before.(13, 26-29) Hippocampal sclerosis (HS) was found in five patients. Six brains showed amyloid plaques (stage A in 3, B in 2 and C in one brain).

GRN mutations

Moderate to severe neuronal loss and gliosis was seen in the frontal, temporal or both cortices of all three available brains. Striatum and substantia nigra were severely involved in one brain each. Numerous ubiquitin- and TDP-43 positive NCIs, DNs and neuronal intranuclear inclusions (NIIs) were visible in the superficial layers of the neocortex, dentate gyrus and caudate nucleus, according to type-3 pathology. HS was seen in two brains. None of the three brains showed Alzheimer changes.

Autosomal dominant form with unknown genetic defect

Of the eight available brains, three showed moderate to severe neuronal loss and gliosis in the frontal, temporal or both cortices, and the other five brains showed only mild changes in the neocortex. Motor neuron loss was found in the hypoglossal nuclei (n=2) and in the anterior horns of the spinal cord (n=3) in cases with clinical FTD-MND.

Seven brains had ubiquitin/TDP-43 type-2 pathology and the eighth brain showed type-1 pathology. NIIs were observed in three brains with FTLD-U+HS, and in two brains from patients with familial FTLD-MND. Hippocampal sclerosis was present in four brains, all of them without motor neuron loss. Three of the four patients with HS (age at onset 60.0 ± 7.5 years) have been initially diagnosed as Alzheimer disease during life; none of the four patients neither their affected relatives have developed MND during life (Figure 2, family 1 to 4). One brain with HS and three brains with FTLD-MND showed Alzheimer changes (Braak stage 1, Amyloid A).

Table 2 Demographic, clinical and pathological features of patients with *MAPT*, *GRN* gene mutations, autosomal dominant and other familial forms without *MAPT* or *GRN* gene mutations

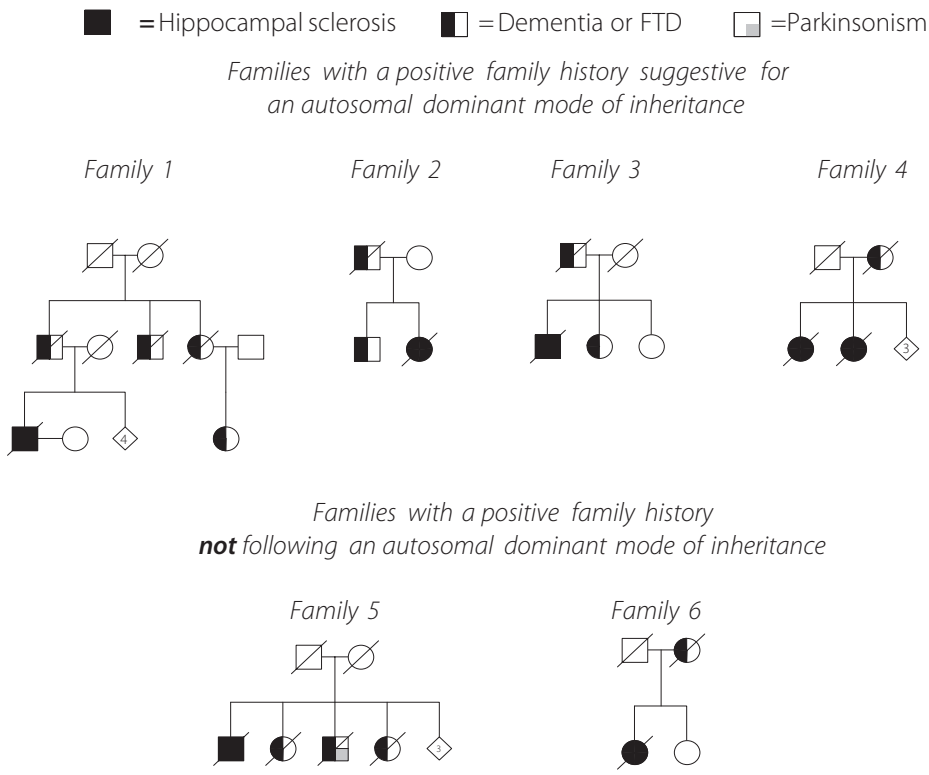
	MAPT* gene mutations	***GRN* gene mutations†**	**Aut.Dom, *MAPT-,GRN-***	**Other familial *MAPT-,GRN-***
Number of patients	39	24	36	72
Number of families	10	4‡	31	67**
Number of families with affected relative <65 years	10	4‡	31	28
Female, %	49	71	47	53
Died during follow-up	34	14	26	34
Age at onset, years (sd)	52.4 (5.9)	61.8 (9.9)	59.5 (8.0)	58.9 (9.2)
Age at death, years (sd)	61.9 (7.5)	71.2 (9.6)	66.9 (6.4)	64.5 (10.9)
Duration of illness, years (sd)	9.4 (4.6)	8.1 (2.7)	7.5 (3.8)	7.3 (3.4)
Clinical subtypes				
bvFTD (%)	38 (97)	23 (96)	27 (75)	40 (56)
SD (%)	1 (3)	-	1 (3)	14 (19)
PNFA (%)	-	1 (4)	3 (8)	14 (19)
FTD-MND (%)	-	-	5 (14)	4 (6)
Parkinsonism (%)	7 (18)	4 (17)	6 (17)	10 (14)
Pathology	15	3	8	9
Mean brain weight (sd)	1035 (166)	1051 (170)	1198 (167)	1100 (120)
Tau	15	-	-	1
TDP-43 type-1 ††	-	-	1	3
TDP-43 type-2 ††	-	-	7	5
TDP-43 type-3 ††	-	3	-	-
Hippocampal sclerosis	5	2	4	2

Values are unadjusted means (sd) or percentages; AO = age at onset; *, G272V, **Δ**K280, P301L, L315R, S320F, R406W mutations; † Val411SerfsX1, Ser82ValfsX174, Gln125X, Gln300X (sporadic) mutations; ‡, one sporadic patient; **, 63 families with one affected relative, and four families with two or more affected first – or second-degree relatives in one generation; ††, according to classification of Cairns et al.(2)

Other patients with a positive family history

Of the nine brain specimens, six showed moderate to severe neuronal loss and gliosis in frontal, temporal or both cortices, whereas the neocortex showed only mild changes in three brains. Striatum was severely involved in one, and substantia nigra in two brains. TDP-43 type-1 pathology was found in three, type-2 in five brains, and one brain showed tau pathology. Hippocampal sclerosis was found in two brains with type-2 pathology, none of them with motor neuron loss. One patient with HS had three sibs with dementia, whereas another patient had a mother died from Pick's disease at age 62 (Figure 2, family 5 and 6). Both brains with HS had Alzheimer changes (Braak stage 1-2, Amyloid stage A-B).

Figure 2 Pedigrees of patients with familial hippocampal sclerosis



Discussion

Our study of the Dutch FTD cohort showed the existence of autosomal dominant forms with unknown genetic defect(s), apart from *MAPT* and *GRN* mutations. The mean age at onset of patients with *GRN* mutations was significantly higher than those with *MAPT* mutations, and its variation could not be explained by the A/G allele of the rs9897526. We suggest, that familial forms with unknown genetic defect(s) can be neuropathologically distinguished into familial FTLD-U with hippocampal sclerosis and familial FTLD-MND.

The frequency of an autosomal dominant form in the present cohort is similar to that in other studies.(11, 30) After the identification of *GRN* mutations, there still remains a group of 10 percent with an autosomal dominant mode of inheritance, and an unknown genetic defect. This latter percentage will probably be higher after the identification of a new genetic defect, as other patients with a positive family history had frequently one or more affected first-degree relative with presenile dementia.

The low frequency of *GRN* gene mutations of 6 percent differs slightly from other studies,(31-32) and might be underestimated as quantitative analysis to identify exon deletions or duplications were not performed in the present study. Although we found a younger onset age in consecutive generations of

the largest family, we could not confirm the effect A allele of rs9897526 on a delayed onset, as reported by Rademakers et al.(33) This phenomenon has probably got to be explained by other genetic or environmental factors. The new *GRN* gene mutation in a sporadic patient with pathological-proven FTD, together with the wide range in age at onset of other *GRN* mutations, have important implications for genetic counselling. In our view, the *GRN* screening should not be restricted to patients with a family history strongly suggestive for an autosomal dominant inheritance.

Hippocampal sclerosis has been associated with other neurodegenerative diseases, like tauopathies and FTLD-U.(4, 34-37) The present study confirms this association by a high percentage of HS in *MAPT* mutations, FTLD-U with *GRN* mutations, and FTLD-U without MND. The most interesting observation is the suggestion of hippocampal sclerosis in patients with an autosomal dominant FTD with an unknown genetic defect. The absence of HS in patients with FTLD-MND might merely due to a shorter survival in these patients. However, the fact that none of the affected relatives of patients with HS have developed MND, might be an argument for a distinct disease entity.(4) The presence of memory problems in most patients with HS has also been reported in other studies.(3, 38-39) The fact that the familial form of FTLD-U+HS has not been recognized might be explained due to lack of data on family history in other studies.(4, 8, 39)

The familial FTLD-MND with phenotypical variation within the present families emphasizes the idea of a disease continuum.(22, 40-41) The occurrence of pure FTD within the present families was much higher than in the chromosome 9p linked families with similar TDP-43 type-2 pathology.(16, 42) As the present families were too small for linkage studies, future identification of the responsible gene defect(s) on chromosome 9 has to be awaited.

How the TDP-43 protein becomes aggregated in the four disease-specific TDP-43 subtypes, is yet unknown. It also remains unclear how the functional loss of GRN protein as a result of *GRN* mutations leads to the aggregation of the TDP-43 protein.(43) Whereas GRN protein as a growth factor is involved in inflammation, wound repair and tumor genesis, TDP-43 protein plays a role as a transcription factor in the nucleus.(44) Future biochemical studies of the different TDP-43 subtypes have to elucidate whether they reflect different pathophysiological mechanisms or only reflect some posttranslational modifications of the TDP-43 protein.

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Chapter 4

Neuropathology in Frontotemporal Dementia

Chapter 4.1

TDP-43 Pathology in Familial Frontotemporal Dementia and Motor Neuron Disease Without *Progranulin* Mutations

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Abstract

Frontotemporal dementia is accompanied by motor neuron disease (FTD-MND) in approximately 10 percent of cases. There is accumulating evidence for a clinicopathological overlap between FTD and MND based on observations of familial aggregation and neuropathological findings of ubiquitin-positive neuronal cytoplasmatic inclusions (NCI) in lower motor neurons, hippocampus and neocortex in both conditions. Several familial forms exist with different genetic loci and defects. We investigated the familial aggregation and clinical presentation of FTD-MND cases in a large cohort of 368 FTD patients in the Netherlands. Immunohistochemistry of available brain tissue of deceased patients was investigated using a panel of antibodies including ubiquitin, p62 and TDP-43 antibodies. A total of eight patients coming from six families had a family history positive for FTD-MND (mean age at onset 53.2 ± 8.4 years). Five patients presented with behavioural changes and cognitive changes followed by motor neuron disease, whereas symptoms of motor neuron disease were the presenting features in the remaining three patients . Other affected relatives in these families showed dementia/FTD, MND or FTD-MND reflecting the clinical intrafamilial variation. No mutations were identified in any of the candidate genes, including *SOD1, Dynactin, Angiogenin, MAPT, Valosin-Containing Protein* and *Progranulin* gene. Available brain tissue of five patients with familial FTD-MND showed NCI in hippocampus, neocortex and spinal cord in all, and neuronal intranuclear inclusions (NII) in two brains. TDP-43 antibody showed robust staining of neuronal inclusions similar in distribution and morphology to NCI and NII. Additionally, TDP-43 antibody also stained ubiquitin-negative glial inclusions in the basal striatum of one case. In conclusion, there exists considerable clinical variation within families with FTD-MND, which may be determined by other genetic or environmental factors. NII could also be found in familial FTD-MND without *Progranulin* mutations. The pathophysiological significance of glial TDP-43 positive inclusions in one brain has to be determined.

Introduction

Frontotemporal dementia (FTD) is clinically, pathologically and genetically a heterogeneous disorder characterized by behavioural changes and cognitive decline. FTD has often, but not always, a presenile onset.(1) Semantic dementia and non-fluent progressive aphasia are clinical variants of FTD. In 5 – 10 percent of cases, FTD is preceded, accompanied or followed by signs of motor neuron disease (FTD-MND).(1-3) Several studies have shown that survival in patients with FTD-MND is significantly shorter than in FTD patients without features of motor neuron disease.(4-6)

There is accumulating evidence supporting a clinical overlap between FTD and MND by observations, as cognitive dysfunction is present in 30 – 50 percent of patients with amyotrophic lateral sclerosis (ALS).(7-10) Further arguments for this overlap are the occurrence of primary progressive aphasia in MND patients,(11-12) and the presence of frontotemporal atrophy in patients with MND.(13-15) From an epidemiological perspective, the significantly higher risk of dementia in relatives of MND patients compared to controls suggests a shared susceptibility.(16)

Pathologically, FTD-MND is characterized by neuronal loss in brainstem nuclei and anterior horns of the spinal cord, with or without corticospinal tract degeneration.(17) The presence of ubiquitin-positive (ub-positive) inclusions in lower motor neurons of the spinal cord and granular cells of the hippocampus have originally been reported in FTD-MND,(18-20) and has been confirmed in other pathological series (19-24). Ub-positive inclusions are also found in approximately 40-60 percent of FTD cases without MND,(2, 25) in hereditary FTD with and without *Progranulin* (*GRN*) mutations (26-28) and in semantic dementia.(29) These observations have further strengthened the concept that FTD and MND are part of a spectrum.(30) The recent identification of TAR DNA-binding protein of 43kDa (TDP-43) as constituent of ub-positive inclusions in both FTD and sporadic ALS is another argument for an overlap in pathology between these two entities.(31)

Genetic factors play an important role in FTD and MND. Families with an autosomal dominant form of FTD-MND have been described in previous reports.(32-34) Several genetic defects have been identified, including *chromatin-modifying protein 2b* gene (*CHMP2B*),(35-36) and *Dynactin* gene (*DCTN1*).(37) Mutations in the *Valosin-containing protein* gene are associated with FTD, Paget's disease and myopathy. (38-39) Other genetic loci for FTD-MND are found on chromosome 9p), (39-41) 9q,(42) and 17q.(43)

In this study, we investigated the familial aggregation, clinical features and genetic defect of FTD-MND in a large Dutch cohort of 368 FTD patients. Additionally, immunohistochemistry of available brain tissue was carried out by means of a panel of antibodies, including ubiquitin-, tau, p62 and TDP-43-antibodies to determine the pathological phenotype.

Material and Methods

Clinical data

Since 1994, three hundred and sixty-eight patients have been recruited in the Dutch prospective FTD cohort, as previously described by Rosso and Stevens.(1, 44) The cohort study includes a detailed clinical

history regarding the onset and course of the disease obtained from the spouses and first-degree relatives by using a checklist of behavioural and cognitive changes and motor symptoms. The age at onset was defined as the age at which the first symptom compatible with the diagnosis FTD or MND was observed by a close relative or caretaker.

All patients underwent a routine neurological examination with special attention for the presence of extrapyramidal and upper and lower motor signs. The clinical diagnosis MND was based on the presence of the following abnormalities at neurological examination: swallowing problems and dysarthria, muscular wasting or weakness, and fasciculations in tongue and extremities (see Table 2). Ambulant patients, who visited our outpatient clinic, underwent neuropsychological evaluation and neuroimaging (magnetic resonance imaging (MRI) or single photon emission computed tomography (SPECT) with 99mTc-hexamethyl propyleneamine oxime (HMPAO), or both). Neuropsychological evaluation included testing of intelligence, language functions (Boston Naming test), attention and concentration, executive and visuospatial functions. Electromyography was carried out in ambulant patients visiting our outpatient clinic with muscle weakness or other signs suggestive of lower motor neuron disease. For patients visited in nursing homes by the research physician, data collection was limited to detailed clinical history and neurological examination, whereas clinical, neuropsychological and neurophysiological data, as well as hard copies of neuroimaging, already available from medical records, were reviewed. The diagnosis FTD was based on the criteria of Lund and Manchester (45-46) and included (1) a progressive behavioral 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. 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. 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.(47) Left-right asymmetry was considered to be present if there was at least one grade difference.

Data on family history were obtained by a structured questionnaire provided by spouse or first-degree relative. The family history was defined as positive if there was at least one first-degree relative with dementia, parkinsonism, or motor neuron disease before the age of 80. Family history was to be considered as suggestive for an autosomal dominant pattern of inheritance if at least three individuals over two or more generations were affected. All patients were followed up by visits to our outpatient department or by telephone interview of relatives. The duration of the disease was determined in all patients who died during the course of the study. Each patient, spouse or first-degree relative of the patient gave written informed consent for blood sampling for extracting genomic DNA from peripheral lymphocytes according to standard procedures.

From this cohort, we selected all patients with familial FTD-MND, defined as: 1) patients with FTD-MND and a family history positive for dementia or MND, 2) FTD patients with a family history positive for MND. The clinical diagnosis of MND in FTD patients was defined by the presence of upper or lower (bulbar and

spinal) motor neuron symptoms, or both, documented by neurological examination with or without electromyographic testing. The clinical diagnosis in affected family members was based on clinical data obtained from relatives, available data from medical records and/or autopsy reports.

Genetic analysis

DNA was isolated from peripheral blood cells according to standard procedure. *MAPT*, *CHMP2B* and *GRN* genes were sequenced in all patients with familial FTD-MND; PCR and sequencing conditions for all coding exons of these genes have been previously described (26, 35, 48). Novel sequence variants were analysed in a minimum of 380 chromosomes from healthy individuals of matched ethnicity and gender. Control DNA was screened by PCR amplification of the specific exon followed by direct sequencing. The 13 exons of GRN gene including intron/exons boundaries were amplified from genomic DNA by PCR and directly sequenced in both strands.

Additionally, patients with familial FTD-MND were separately screened for mutations in *Superoxide dismutase* (*SOD1*), *Angiogenin* (*ANG*), *valosin-contaning protein (VCP)* gene and *dynactin (DCTN1)* gene. Exons of the *ANG*, and *DCTN1* genes including intron/exons boundaries were amplified from genomic DNA by PCR and directly sequenced on both strands. PCR reactions were performed in 25 µl containing 1x Invitrogen PCR buffer, 1,5 mM MgCl2, 250 µM of each dNTP, 0,5U Platinum Taq polymerase and 0,4µM of primers. 10% DMSO was added to amplify exon 1 and exon 2 of the *ANG* gene. The annealing temperature for all primer pairs was 58˚C. Direct sequencing of both strands was performed using the Big Dye Terminator chemistry version 3.1 and loaded on an ABI 3730 automated sequencer.

Immunohistochemistry

Brain autopsy was carried out by the Netherlands Brain Bank within four hours after death according to Legal and Ethical Code of Conduct of the Netherlands Brain Bank. After macroscopic review, tissue blocks were taken from the frontal, temporal, parietal and occipital cortex, hippocampus, striatum, thalamus, substantia nigra, locus coeruleus and pons, medulla and cerebellum and frozen at –80˚C. Half of the brain was fixed in 10% buffered-formalin solution for four weeks. Eight µm paraffin-embedded sections of the same brain regions underwent routine staining with hematoxylin-eosin, Bodian, methenamine-silver, and Congo red.

Primary antibodies were used for recognition of the following proteins: hyperphosphorylated tau (AT8, Innogenetics, Ghent, Belgium; 1:40), PHF1 (donated by P. Davies, Albert Einstein College of Medicine, New York, USA; 1:100), antibodies directed against ubiquitin (anti-ubiquitin, DAKO, Glostrup, Denmark; 1:500, following 80˚C antigen retrieval), β-amyloid protein (anti-beta amyloid, DAKO, Glostrup, Denmark; 1:100, following formic acid pre-treatment), α-synuclein (anti-α-synuclein, Zymed Laboratories, San Francisco, California, USA; undiluted, following formic acid pre-treatment), CD68 (DAKO, Glostrup, Denmark; 1:200, following 80˚C antigen retrieval), p62 (BD Biosciences Pharmingen, San Diego, CA, USA; 1:200, following 80˚C antigen retrieval) , TDP-43 (Biotech, Chicago, IL, USA; 1:100, following pressure-cooking), glial fibrillary acidic protein (GFAP; DAKO, Glostrup, Denmark; 1:500, following 80˚C antigen retrieval), neurofilament (SMI-32, Sternberger Monoclonals, Lutherville, MD, USA; 1:7000, following 80˚C

antigen retrieval) and small ubiquitin modifier-1 (SUMO-1, Santa Cruz Biotechnology Inc., Santa Cruz, California, USA; 1:100, following 80˚C antigen retrieval) was performed on sections with NII.
Additional immunohistochemistry of neuronal intranuclear inclusions with antibodies against nuclear proteins, including promyelocytic leukaemia protein (PML, Santa Cruz Biotechnology Inc., Santa Cruz, California, USA; 1:50, following pressure-cooking), and small ubiquitin modifier-1 (SUMO-1, Santa Cruz Biotechnology Inc., Santa Cruz, California, USA; 1:100, following 80˚C antigen retrieval) was performed on sections with NII.
Antigen retrieval was done either for 30 minutes in 0.1 M sodium citrate buffer at 80˚C and pH 7.7 or using pressure-cooking in 0.1 M sodium citrate buffer (pH 6) for 5 minutes. Pre-treatment with 99% formic acid was done for 5 minutes (α-synuclein) or 20 seconds (β-amyloid).
Primary antibodies were incubated overnight at 4ºC. Endogenous peroxidase activity was inhibited by 30 minutes incubation in PBS-hydrogen peroxide-sodiumazide solution (100ml 0,1 M PBS + 2 ml 30% H_2O_2 + 1 ml natriumazide) and immunohistochemistry performed as described for paraffin-embedded sections. The Histostain-Plus broad-spectrum kit DAB (Zymed, San Francisco, California, USA) was used as a detection system. Slides were counterstained with Mayer's hematoxylin and mounted in entellan.

Results

Demographic and clinical features

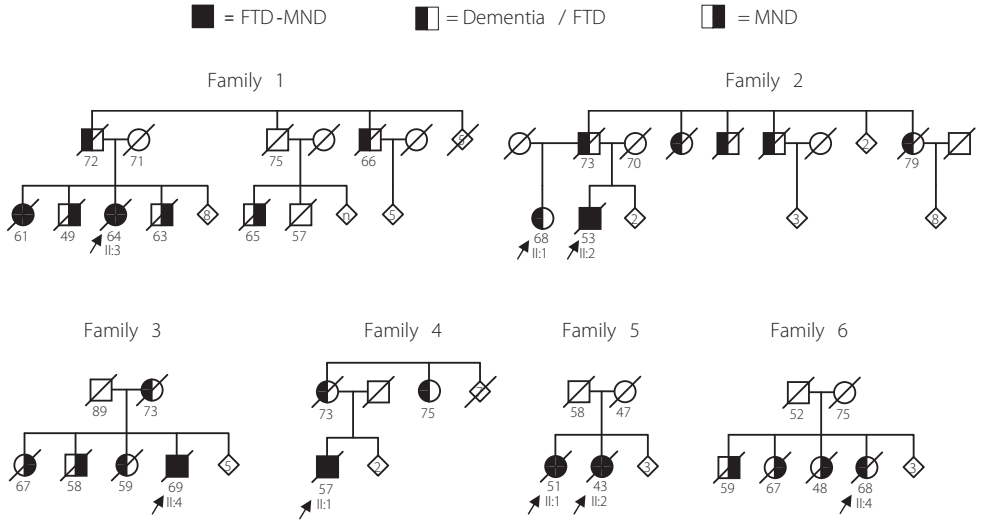
The Dutch FTD cohort consisted of 368 patients with FTD (mean age at onset 57.8 ± 9.2 years). Of 368 patients, 10 patients had sporadic FTD-MND and eight patients had a positive family history for dementia/FTD, MND or both (44.4 %). The mean age at onset in the FTD-MND group (56.0 ± 9.2 years) was similar to that of the FTD group (57.9 ± 9.2 years), whereas survival in the first group (3.4 ± 1.6 years) was significantly shorter than in the latter (see Table 1). From the group of FTD patients, available brain tissue of 54 cases showed tauopathy in 28 cases, 14 FTDP-17T associated with MAPT mutations and 14 sporadic tauopathies (six Pick's disease). Twenty-five of the remaining 26 brains had FTLD-U was in 25, and showed neither tau- nor ub-positive inclusions (dementia lacking distinctive histology, DLDH). Neuropathological examination in six FTD-MND patients (four familial, two sporadic) was consistent with the clinical diagnosis FTD-MND (see below).

Table 1 Demographic data of the 350 patients with FTD and 18 patients with FTD-MND

	Total FTD cohort (n = 368)		
	FTD (n=350)	**FTD-MND (n=18)**	**P-value**
Male : Female	165 : 185	10 : 8	> 0.05
Onset (years)	57.9 ± 9.2	56.0 ± 9.2	> 0.05
Family history	162/350 (46.3 %)	8/18 (44.4 %)	> 0.05
Death (years)	66.4 ± 9.4 (n=205)	56.9 ± 8.5 (n=15)*	< 0.001
Survival (years)	9.0 ± 3.9 (n=205)	3.4 ± 1.6 (n=15)*	< 0.001

* Three patients with FTD-MND were still alive.

Figure 1 Familial FTD-MND cases



Numbers are age of death or current age. Arrows indicate index patients.

The group of eight patients with familial FTD-MND (mean age at onset 53.2 ± 8.4 years), came from six families with FTD-MND (See Figure 1). The mean age of onset and of death in eight patients with familial FTD-MND did not differ from those with sporadic FTD-MND. Five index patients presented with behavioural changes or memory problems followed by motor signs in three (interval 18.0 ± 8.5 months). The remaining three index patients presented with slurred, speech, swallowing difficulties, and weakness of extremities followed by dementia (interval 6.0 ± 3.0 months). One of the two patients with only FTD was still alive without any sign of motor neuron sign five years after onset (Family 2, II:1). The other patient with FTD (Family 6, II:4) died from bronchopneumonia after disease duration of four years, and neuropathological examination showed features consistent with FTD-MND (see below).

Neurological examination revealed muscle wasting in interossei of the hands (n=4), thighs (n=2), or tongue (n=4) and fasciculations in arms (n=6), hands (n=3), thighs (n=4), or tongue (n=5). EMG showed muscle denervation (fibrillations, positive waves and fasciculations), without the evidence of conduction block in the three investigated patients. Deep tendon reflexes and extensor reflexes in upper and lower extremities were increased in three patients. One of these patients had clinical FTD but no lower motor neuron signs. Extrapyramidal signs were absent in all patients at ascertainment and follow-up, although none of the patients were examined in the final stages of the disease.

Table 2 Clinical features of index patients

	Family 1	Family 2		Family 3	Family 4	Family 5		Family 6
	II:3	II:1	II:2	II:4	II:1	II:1	II:2	II:4
Sex	Female	Female	Male	Male	Male	Female	Female	Female
Age at onset (years)	61	63	51	63	54	48	40	64
Age at death (years)	64	alive	53	69	57	51	43	68
Onset disease	MND	FTD	FTD	FTD	MND	MND	FTD	FTD
Presenting symptoms			,					
Language dysfunction	-	-	+	+	-	-	++	++
Executive impairment	-	++	++	-	-	-	++	+
Behavioural problems	-	+	++	++	-	-	++	++
Memory loss	-	+	+	+	+	-	+	+
Weakness, dysarthria	+	-	-	-	+	+	-	-
Neurological Examination								
Tongue atrophy	+	-	-	+	-	+	+	-
Tongue fasciculations	+	-	+	+	-	+	+	-
Muscular atrophy	+	-	+	+	+	+	-	-
Muscular weakness	+	-	+	+	+	+	+	-
Fasciculations extremities	+	-	+	+	+	+	+	-
Pyramidal signs	+	-	+	-	-	-	-	+
EMG	+	-	+	n.a.	+	n.a.	n.a.	-
MRI/CT	FT+	F+T+	FT+	F++	FT+	n.a.	F+	F+T++
SPECT	FT+	n.a.	FT+	n.a.	n.a.	FT+	F+	n.a.

+,mild/moderate; ++, severe; F, frontal; T, temporal; n.a., not available

Language deficits developed with disease progression in seven patients, which consisted of impaired comprehension (n=6), reduced spontaneous speech (n=5), word finding difficulties/impaired naming (n=3), perseverations (n=2) and paraphasia (n=2). Impaired attention and executive functions (Wisconsin, Trailmaking, Stroop, Mazes), decreased word fluency, and perseverations were found, whereas memory and visuoconstructive functions were relatively preserved. Neuroimaging (MRI/CT) showed mild symmetrical frontotemporal atrophy (n=5) or mild frontal atrophy (n=2). SPECT performed in four patients revealed frontal with or without temporal hypoperfusion in all (see Table 2).

Intrafamilial clinical variation

The occurrence of FTD-MND in four families was suggestive for an autosomal dominant form, whereas the mode of inheritance was uncertain in the remaining two families with affecteds in one generation. The clinical diagnosis was established on available data from medical records in eight affected relatives (with neuropathological reports in two), whereas the diagnosis in the remaining 12 affected relatives was based on clinical data obtained from family members.

The six families showed considerable intrafamilial variation in age at onset and clinical presentation. Of the two ascertained patients from a single family (Family 2), patient II:2 presented with FTD-MND, whereas his sister did not have clinical features nor EMG signs of MND five years after onset of FTD. Additionally, of 20 affected relatives from the six families, 11 had died from dementia/FTD, eight from MND and one from MND with dementia (see Figure 1).

Neuropathology

Brain autopsy became available in five patients with familial FTD-MND, four with clinical FTD-MND (Family 1, II:3; Family 2, II:2; Family 4, II:1; Family 5, II:2) and one with clinical FTD (Family 6, II:4) ; the two brains of sporadic FTD-MND are excluded from this analysis. The mean brain weight was 1214 grams ± 163 gram (not recorded in one). Macroscopical inspection showed mild atrophy of frontal lobes (n=4) and severe atrophy of the temporal cortex (n=1). On coronal sections, the lateral ventricles were dilated in three and normal in two patients. Depigmentation of the substantia nigra was seen in two patients.

Mild neuronal loss, gliosis and spongiosis were seen in the superficial layers of the frontal cortex (n=4), and of the temporal cortex (n=3). Neuronal loss was also present in the substantia nigra (n=3) and in hypoglossal nuclei (n=3), whereas brainstems at the level of hypoglossal nuclei were not available for evaluation in the remaining two brains (see Table 3). The spinal cord showed loss of lower motor neurons (n=3) and degeneration of corticospinal tracts with occasionally axonal torpedoes/spheroids and foamy cells (n=2).

Immunohistochemistry with tau antibodies stained a few neurofibrillary tangles (NFT) in hippocampus (n=3) and temporal cortex (n=2). Staining with ubiquitin antibody showed ub-positive neuronal cytoplasmatic inclusions (NCI) in the granular cells of the dentate gyrus in all cases (see Figure 2a). The frontal and temporal cortex showed many ub-positive NCI and dystrophic neurites in four brains (see Figure 2c). Many ub-positive NCI in the striatum were seen in one brain (see Figure 3a), and some in the other brains. Ub-positive NCI were not found in hypoglossal nuclei or other brainstem nuclei in the three cases where the brainstem at this level was available. Two brains showed a low number of neuronal intranuclear inclusions (NII) with lentiform or cat-eye shape in the frontal cortex, the dentate gyrus and striatum. Ub-positive (skein-like) inclusions were present in the spinal cord in all patients (see Figure 2e), including the patient with clinical FTD during life (Family 6, II:4, see above). The above-mentioned ubiquitin pathology is consistent with type 2 of the classification by Sampathu et al. and with type 3 of the classification by MacKenzie et al.(49-50)

Table 3 Pathology and TDP-43 staining

	Family 1 II:3	Family 2 II:2	Family 4 II:1	Family 5 II:2	Family 6 II:4
Gross findings					
Weight (grams)	1076	1260	1425	1096	n.a.
Atrophy					
Frontal	+	+	-	+	-
Temporal	-	+	-	-	++
Substantia Nigra	-	+	-	-	+
Microscopy/Immunohistochemistry					
Frontal					
Neuronal loss/Gliosis	+	+	-	+	+
NCI	+	++	-	+	+
NII	-	-	-	-	+
Temporal					
Neuronal loss/Gliosis	-	+	-	+	++
NCI	++	+	-	++	+
NII	-	-	-	-	-
Hippocampus					
Neuronal loss/Gliosis	-	-	-	+	-
NCI	++	++	+	+	-
NII	+	-	-	-	-
Striatum					
Neuronal loss/Gliosis	+	-	-	-	-
NCI	++	+	+	++	+
NII	+	-	-	-	+
Glial inclusions*	-	-	-	+	-
Substantia nigra					
Neuronal loss/Gliosis	++	++	+	++	-
NCI	-	+	+	+	+
NII	-	-	-	-	-
Spinal cord/ lower motor neurons					
Neuronal loss/Gliosis	+	+	+	-	-
NCI	+	+	+	+	+
NII	-	-	-	-	-
Nucleus hypoglossus					
Neuronal loss/Gliosis	++	+	n.a.	+	n.a.
NCI	-	-	-	-	-
NII	-	-	-	-	-

NCI, neuronal cytoplasmatic inclusions; NII, neuronal intranuclear inclusions; n.a., not available; -, none; +, mild/moderate; ++, severe.
* denotes TDP-43 positive, ub-negative glial inclusions

Staining with the TDP-43 antibody showed a robust staining of NCI in the dentate gyrus, frontal and temporal cortex, and spinal cord (see Table 3 and Figure 2b, d, f). The morphology of TDP-43 inclusions resembled ub-positive NCI. TDP-43 antibody also stained NII found in the two cases with ub-positive NII (see Figure 3c, d). Nuclei of unaffected neurons showed TDP-43 staining, which was not observed in neurons positive for TDP-43 with NCI. Neurons in the olivary nucleus showed strong nuclear TDP-43 staining in one brain. Positive TDP-43 staining of ub-negative glial inclusions was seen in the basal striatum of a single brain (see Figure 3b), whereas ub-, TDP-43 positive NCI in the striatum were seen in all five brains (see Figure 3a). TDP-43 positive glial cells had small dark-blue nuclei and were often located within the white matter. In addition, serial 4µ sections of the striatum with glial inclusions stained with TDP-43 and NeuN antibodies, respectively, clearly distinguished TDP-43 positive glial cells from small neurons. The p62 antibody stained both ub-positive and TDP-43 positive NCI and TDP43-positive, ub-negative glial inclusions. The SUMO-1 antibody stained NII in the two brains, whereas PML staining of NII were negative in both cases. Staining with β-amyloid showed a few senile plaques in the temporal cortex of two patients. The corticopsinal tracts in the spinal cord of three patients showed many CD68-positive cells. A few neurons with Lewy bodies and few Lewy neurites in the substantia nigra of two brains were visualized with α-synuclein staining.

DNA- Analysis

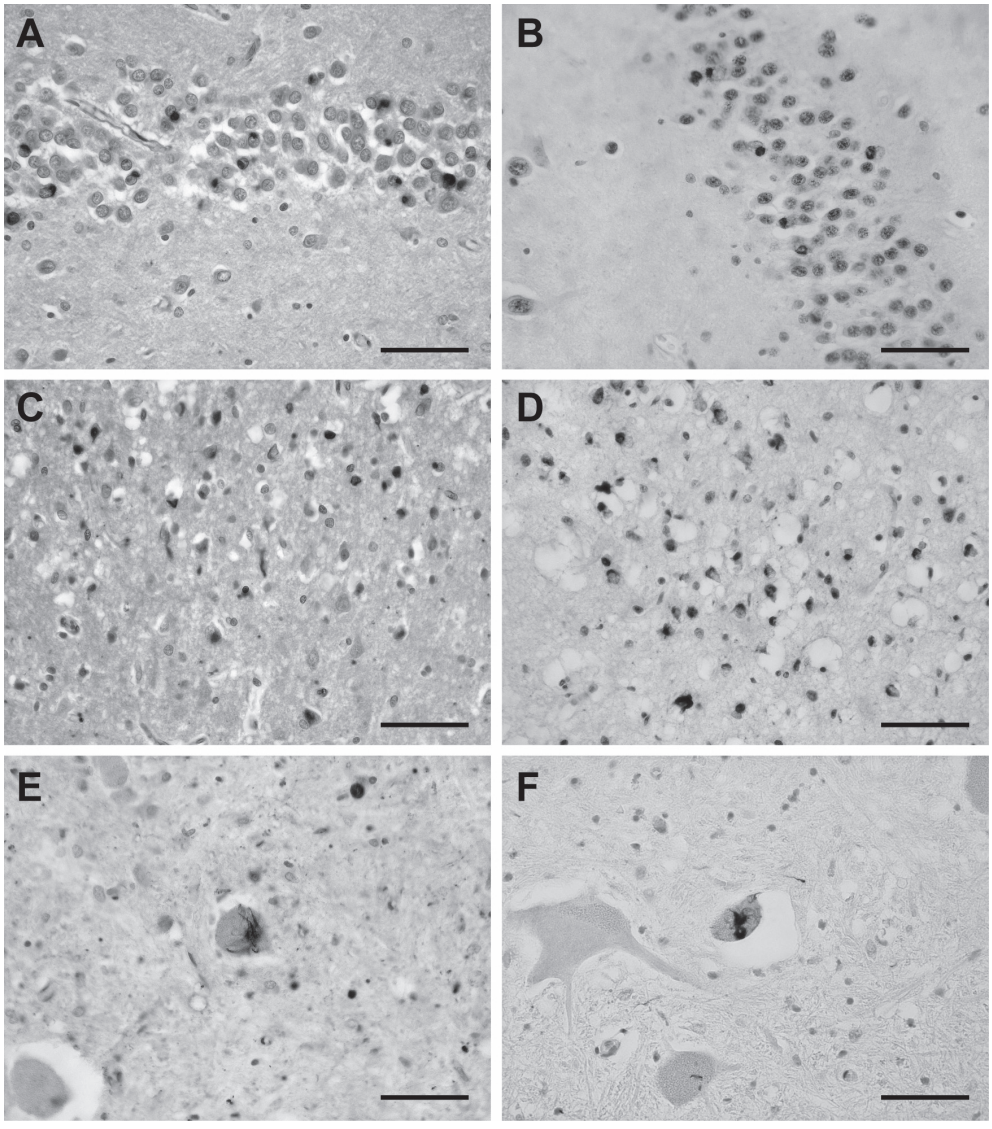
Genetic analysis did not show any mutations in the *MAPT* and *GRN* genes in the eight patients with familial FTD-MND, whereas their frequency in the total FTD group was 10.3 and 5.7 percent respectively. No mutations were found in *CHMP2B*, *SOD1*, *ANG, VCP* or *DCTN1* genes.

Discussion

The present study described the occurrence of FTD-MND in 4.3 percent of a large FTD cohort in the Netherlands. Familial FTD-MND found in 44.4 percent of cases showed considerable intrafamilial clinical variation. TDP-43 antibody showed robust staining of neuronal cytoplasmatic ub-positive inclusions in five available brains, and also of ub-negative glial inclusions in one brain. Rare neuronal intranuclear inclusions were found in neocortex, hippocampus and striatum of two brains, despite the absence of mutations in the *Progranulin* gene.

Our observation that 44.4 percent of the FTD-MND cases in our cohort had a positive family history, is similar to that reported in other studies.(3, 51) The considerable clinical variation within the present families occurred between generations as well as within the same generation, as reported in familial FTD-MND with unknown genetic locus or linked to chromosome 9 and 17.(32, 34, 43, 51) Some affected individuals presented with behavioural changes and executive dysfunctions,(52) localized frontal atrophy on MRI scan,(53-58) whereas other patients had muscle wasting and dysarthria as the initial symptoms.(17, 55-56) In our view, the phenomenon of clinical variation within families with FTD-MND warrants the combined analysis of patients with FTD, MND or FTD-MND.

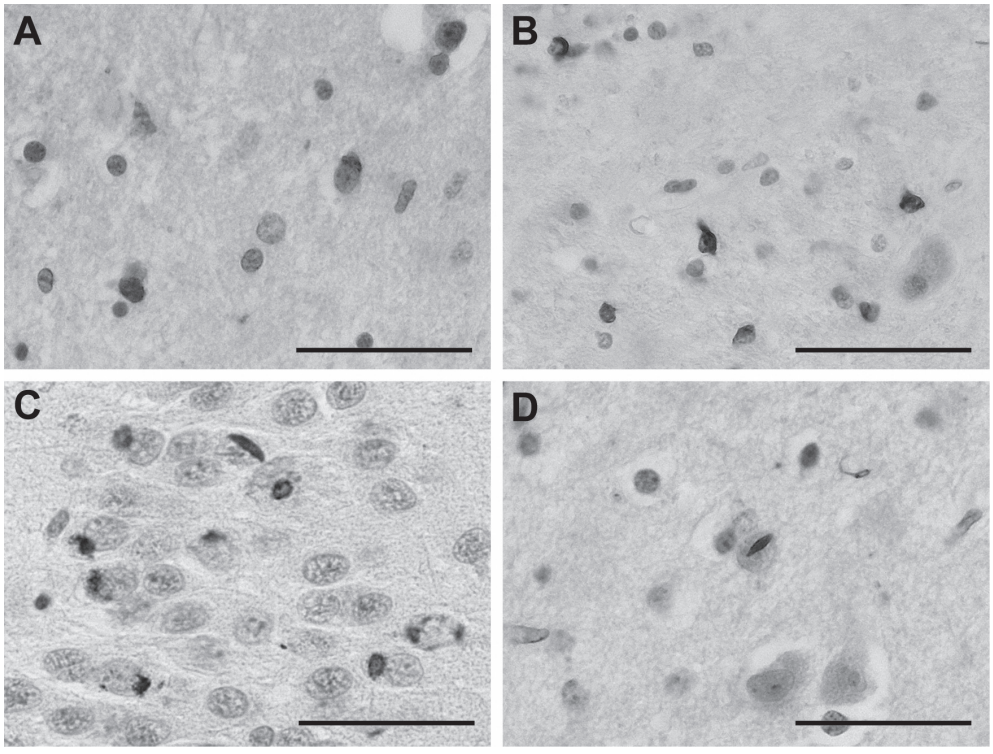
Figure 2



Positive staining with anti-ubiquitin and TDP-43 antibody neuronal cytoplasmatic inclusions in the granular cells of the dentate gyrus were seen in the hippocampus in all cases with ubiquitin (A) and TDP-43 (B). NCI in the neocortex with ubiquitin (C) and TDP43 (D) and (skein-like) inclusions were seen in all spinal cords with both ubiquitin (E) and TDP-43 (F). Scale bar = 100 mm.

The robust TDP-43 staining of neuronal cytoplasmatic ub-positive inclusions in the present study strongly supports the hypothesis that the TDP-43 protein is substantial constituent of ub-positive inclusions.(31, 59) These TDP-43 inclusions showed a morphology similar to that of ub-positive inclusions and their pattern consistent with ubiquitin pathology type 2, as reported by Sampathu (49) or type 3 reported

Figure 3



Ub-positive neuronal cytoplasmatic inclusions were found in the caudatus **(A)** and the glial cells stained positive with TDP-43 **(B)**. Lentiform or cat-eye shaped NII in the dentate gyrus **(C)** and neocortex (**D)** also stained positive with TDP-43. Scale bar = 100 μm.

by Mackenzie.(50) In our view, the presence of a few NII in two of our FTD-MND cases should not result into a reclassification. In contrast to the more granular aspect of neuronal inclusions in the dentate gyrus of cases with *GRN* mutations,(60) the ub-, and TDP-43 positive inclusions in our brains had a more solid consistency. Skein-like inclusions in motor neurons of the spinal cord were not only found in patients with clinical FTD-MND,(17, 61) but also in a FTD patients without signs of MND confirming earlier observations.(17, 55) An interesting phenomenon is the negative TDP-43 nuclear staining of ub-containing neurons in contrast to positive nuclear staining of normal neurons.(31) The question is whether the TDP-43 protein moved from the nucleus to the cytoplasm during the disease process or that modified TDP-43 isoforms in the cytoplasm of specific neurons never entered the nucleus, as has recently been proposed by Davidson et al.(62) In both situations, a loss of TDP-43 nuclear function could be responsible for the disease. As TDP-43 is involved in splicing of pre-mRNA, future studies should attempt to identify target mRNAs of TDP-43 in order to elucidate the pathophysiological mechanisms.

The most interesting observation is the presence of rare neuronal intranuclear inclusions in two of the present familial FTD-MND cases without *GRN* mutations, although large deletions could not

be excluded by the use of conventional sequencing techniques in this study. Previous studies have reported these NII in FTD families without MND (28, 63-65). After the identification *GRN* gene mutations in these families,(26-27) their presence was considered to be specific for these gene defects (60). In a few studies however, they were also found in familial FTD-MND cases, although at that time the genetic defect was still unknown.(6, 21, 63) Our observation supports the idea that the presence of intranuclear inclusions are not exclusively restricted to cases with *GRN* mutations.(60) The positive SUMO-1 of NII in the present two cases confirms the observations by Mackenzie et al. and had also been found in other neurodegenerative disorders.(65-66) This ubiquitin-like protein, SUMO-1, appears to be involved in the nuclear proteasomal degradation.(66) The negative PML-staining of NII has to be further investigated in other series, as previous studies have shown inconclusive results.(65-66)

Another very interesting finding was the presence of TDP-43-positive, ub-negative glial cytoplasmatic inclusions in the striatum of a single brain. Very recently, the polyclonal TDP-43 antibody have shown positive staining of glial inclusions in the gray matter and spinal cord of FTD-MND.(59) This suggests that glial cells may be involved in the pathophysiological process of the disease. Future immunohistochemical and biochemical studies in FTD and FTD-MND cases are needed to investigate the significance of glial pathology.

The pyramidal tract degeneration in one of the present patients indicates that the neurodegeneration in FTD may extend to upper and lower motor neuron system in patients living long enough, as suggested earlier.(17, 55-56, 67) Neuron loss of the substantia nigra and striatum in several of the present cases also shows that the pathological process is more widespread than the motor system alone, (6, 13, 15, 21, 55-56, 61, 68) although the presence of extrapyramidal signs is an uncommon feature in the present and other studies.(69-70)

Whereas a genetic locus on chromosome 9p have found for several families with an autosomal dominant form of FTD, MND or FTD-MND in affected sibs, the present families were too small for significant linkage. Studies with a genome-wide scan using single nucleotide polymorphisms in these small families will be performed in the future.

In conclusion, there exists considerable intrafamilial variation in FTD-MND, which should be explained by yet unidentified genetic and environmental factors. The significance of neuronal intranuclear inclusions and TDP-43 glial inclusions in some of the present FTD-MND cases has to be determined in future studies. To elucidate the role of the TDP-43 protein in the pathophysiology might hopefully explain the considerable variation in clinical phenotype.

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Chapter 4.2

Frequency of Ubiquitin and FUS-positive, TDP-43-negative Frontotemporal Lobar Degeneration

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Abstract

Frontotemporal lobar degeneration (FTLD) is a clinically, genetically and pathologically heterogeneous disorder. Within FTLD with ubiquitin-positive inclusions (FTLD-U), a new pathological subtype named FTLD-FUS was recently found with FUS positive, TDP-43-negative inclusions, and striking atrophy of the caudate nucleus. The aim of this study was to determine the frequency of FTLD-FUS in our pathological FTLD series, and to describe the clinical, neuroimaging, especially caudate atrophy, and neuropathological features of FTLD-FUS.

Demographic and clinical data collected prospectively from 387 patients with frontotemporal dementia (FTD) yielded 74 brain specimens. Immunostaining was carried out using a panel of antibodies, including AT-8, ubiquitin, p62, FUS, and TDP-43. Cortical and caudate atrophy on MRI (n=136) was rated as normal, mild-moderate or severe.

Of the 37 FTLD-U cases, 33 were reclassified as FTLD-TDP and four (0.11, 95%: 0.00 – 0.21) as FTLD-FUS, with ubiquitin and FUS-positive, p62 and TDP-43-negative neuronal intranuclear inclusions (NII). All four FTLD-FUS cases had a negative family history, behavioural variant FTD (bvFTD), and three had an age at onset ≤ 40 years. MRI revealed mild-moderate or severe caudate atrophy in all, with a mean duration from onset till MRI of 63 months (range 16 – 119 months). In our total clinical FTD cohort, we found 11 patients (0.03; 95% CI: 0.01 – 0.05) with bvFTD, negative family history, and age at onset ≤ 40 years. Caudate atrophy was present in 10 out of 136 MRIs, and included all four FUS-cases.

The newly identified FTLD-FUS has a frequency of 11 percent in FTLD-U, and an estimated frequency of three percent in our clinical FTD cohort. The existence of this pathological subtype can be predicted with reasonable certainty by age at onset ≤ 40 years, negative family history, bvFTD and caudate atrophy on MRI.

Introduction

Frontotemporal dementia (FTD) is the second most common type of presenile dementia, and a clinically, genetically and pathologically heterogeneous disorder.(1-2) Behavioural changes and cognitive dysfunctions, especially language, are core clinical features. Four clinical subtypes can be distinguished: the behavioural variant of FTD (bvFTD), semantic dementia (SD), progressive non-fluent aphasia (PNFA), and FTD with motor neuron disease (FTD-MND).

Frontotemporal lobar degeneration (FTLD) is the common underlying pathology of all four clinical variants, and can be divided in FTLD with tau-positive immunoreactive inclusions (FTLD-tau) and FTLD with ubiquitin-positive immunoreactive inclusions (FTLD-U). After the TDP-43 protein was found to be the major constituent of ubiquitin-positive inclusions,(3) the term has been changed in FTLD-TDP. However, the identification of TDP-43-negative inclusions in some FTLD-U cases has resulted into the designation of a new neuropathological subtype, atypical FTLD-U.(4-6) Very recently, the term of this subtype has been changed into FTLD-FUS by the observation of positive staining of these inclusions with antibody against FUS (fused in sarcoma) protein.(7)

An early-onset FTD and severe progressive psychobehavioural changes, a negative family history, and striking atrophy of the striatum at neuropathological examination are the characteristic features of FTLD-FUS.(4-7) Its exact frequency is yet unknown, and neuroimaging features may differentiate it from other subtypes during life. The aim of this study was to determine the frequency of FTLD-FUS in our FTLD-U cases, and to describe the clinical, neuroimaging and neuropathological features of FTLD-FUS. Furthermore, we estimated the prevalence of FTLD-FUS in our clinical cohort of FTD on the basis of a combination of specific clinical features.

Methods

Patients with FTD were ascertained in an ongoing genetic-epidemiologic study conducted in the Netherlands since 1994, (1, 8-9) after referral to the out-clinic department of the Erasmus Medical Center, or after a visit in nursing homes and psychogeriatric hospitals by the research physician. Detailed clinical history was obtained from the spouses and first-degree relatives using a checklist of behavioural and cognitive changes, and motor neuron signs.

The age at onset was defined as the age at which the first symptom, compatible with FTD diagnosis, was observed by a close relative or caretaker.(1, 9) During the neurological examination carried out in all patients, special attention was paid to the presence of extrapyramidal and motor neuron disease signs. Data on family history were obtained using a structured questionnaire provided by a spouse or first-degree relative. Family history was defined as positive if patients had at least one first-degree relative with dementia, parkinsonism, or MND, irrespective of their age of onset.

Neuropsychological evaluation consisted of tests for memory functions (Rey figure, 15-Word test(10)), attention and concentration, executive functions (Stroop, Trailmaking A and B, Wisconsin Card Sorting Test, WAIS subtest substitution), language (Boston Naming Test, the Dutch revised version of the

Semantic association test(11)), and visuoconstructive and visuospatial skills (Clock drawing, Block design of the WAIS), and was performed in a subset of patients at our out-clinic department. Due to the variation in test batteries a detailed comparison between subgroups was not possible. Severity of dementia at ascertainment was assessed using the Clinical Dementia Rating scale.(12)

MRI scans performed between 1990 and 2008 were available for evaluation in 136 out of 387 patients. Available T1-weighted MR, T2-weighted MR, Proton-density (PD) weighted MR, and fluid attenuated inversion recovery (FLAIR) MR images were used for evaluation.

The severity of the cortical and caudate atrophy on MRI was evaluated and semi-quantitatively rated according to a three-point scale: 0) normal caudate nucleus; 1) mild or moderate atrophy of the caudate nucleus, either still bulging into the lateral ventricle or with a flat contour; 2) severe atrophy with no visible caudate nucleus. The presence of caudate atrophy was evaluated by a radiologist and neurologist blinded to clinical and pathological findings. Disagreement was solved by consensus.

Mutation screening of all exons and exon-intron regions of *MAPT*, *GRN* and *CHMP2B* genes was performed in all patients with a positive family history, and in a subset of patients with sporadic FTD (n=72) as previously described.(1, 9)

Two hundred thirty-two patients died during a follow up period of 14 years, of which 74 patients underwent brain autopsy. Brain autopsy was carried out within four hours of death according to the Legal and Ethical Code of Conduct of the Netherlands Brain Bank. Macroscopic inspection of the brain included the frontal, temporal, parietal, and occipital lobes, cerebellum, hippocampus, basal ganglia and substantia nigra.

Tissue blocks were taken from all cortical areas, hippocampus, amygdala, basal ganglia, substantia nigra, pons, medulla oblongata, cerebellum, and cervical spinal cord. They were embedded in paraffin blocks and subjected to routine staining with haematoxylin-eosin, Bodian, methenamine-silver, and Congo red. The severity of neuron loss was scored as absent, mild, or moderate-severe.

Immunohistochemistry was performed with antibodies directed against: hyperphosphorylated tau (AT-8, Innogenetics, Ghent, Belgium; 1:400); ubiquitin (anti-ubiquitin, DAKO, Glostrup, Denmark; 1:500, following 80˚C antigen retrieval); β-amyloid protein (anti-beta amyloid, DAKO, Glostrup, Denmark; 1:100, following formic acid pre-treatment); α-synuclein (anti-α-synuclein, Zymed Laboratories, San Francisco, California, USA; undiluted, following formic acid pre-treatment); poli-ubiquitin-binding protein p62 (BD Biosciences Pharmingen, San Diego, CA, USA; 1:200, following pressure cooking); TDP-43 (Biotech, Chicago, IL, USA; 1:100, following pressure cooking); and α–internexin (anti-alpha-internexin, Invitrogen, Camarillo , CA , USA, 1:100, following pressure-cooking), FUS (Sigma-Aldrich anti-FUS; 1:25–1:200 with initial overnight incubation at room temperature, following pressure cooking).

Antigen retrieval was done either for 30 minutes in 0.1 M sodium citrate buffer at 80˚C and pH 7.7 or using pressure cooking in 0.1 M sodium citrate buffer (pH 6) for 5 minutes. Pre-treatment with 70% formic acid was done for 5 minutes (α-synuclein and β-amyloid).

Primary antibodies were incubated overnight at 4ºC. Endogenous peroxidase activity was inhibited by incubation for 30 minutes in PBS-hydrogen peroxide-sodiumazide solution (100ml 0,1 M PBS + 2 ml

30% H2O2 + 1 ml natriumazide). The Histostain-Plus broad-spectrum kit DAB (Zymed, San Francisco, California, USA) was used and slides were counterstained with Mayer's haematoxylin and mounted in entellan.

Neuropathological examination of brains from 74 autopsied patients with clinical FTD revealed 70 brains with FTLD (FTLD-U in 37, FTLD-tau in 32 and FTLD with no inclusions (FTLD-ni) in one), whereas familial Creutzfeldt-Jakob disease (fCJD) was diagnosed in two brains, and Alzheimer's disease (AD) in the remaining two.

The study was approved by the Medical Ethical Committee at the Erasmus MC – University Medical Center Rotterdam. For each patient, a spouse or first-degree relative of the patient gave written informed consent.

Results

Thirty-three out of 37 FTLD-U cases (0.89; 95% CI: 0.79 – 1.00) showed positive staining with the TDP-43 antibody and were reclassified as FTLD-TDP, whereas four cases had ubiquitin-positive, TDP-43-negative inclusions. All four FTLD-U patients with TDP-43 negative inclusions showed positive staining with the FUS antibody, and were designated as FTLD-FUS. Inclusions of FTLD-TDP cases did not stain with the FUS antibody. FTLD-FUS was present in 4 out of all 70 FTLD brains (0.06; 95% CI: 0.00 – 0.11), and in four out of 37 FTLD-U cases (0.11; 95% CI: 0.00 – 0.21).

Demographic and clinical data FTLD-FUS

Demographic and clinical data of the four patients with FTLD-FUS are presented in Table 1. All four patients had clinical bvFTD and a negative family history, and three out of four patients had an age at onset before the age of 40. The presenting symptoms varied among these four cases. Two patients presented with obsessive-compulsive behaviour, in particular showing extreme parsimonious behaviour. Case 1 and Case 3 showed sexual disinhibited behaviour (Case 1 frequently visited prostitutes, while married). Three out of four patients shoplifted several shops, especially the two patients who presented with obsessive-compulsive behaviour. Hyperorality has developed in all four patients, and was the presenting symptom in three patients. Visual hallucinations of deformed paintings were mentioned in one patient, and persecution delusions in another. All patients developed language problems later in the course of the disease, with economy of utterances in all, perseverations (n=3), echolalia (n=2), stereotypy (n=2) and eventually mutism in all. Neurological examination was unremarkable without signs of motor neuron disease, pyramidal or extrapyramidal dysfunction, except for mild left-sided cogwheeling in one patient. Neuropsychological testing revealed impaired attention and concentration, and executive deficits, with normal orientation, memory and visuoconstructive functions in all four patients, except for evident memory problems in one.

MRI showed severe frontotemporal atrophy in three patients, and mild to moderate in. All four patients had mild-moderate (n=1), or severe caudate atrophy (n=3), at a mean duration of illness of 63 months

(range 16 – 119 months) (Figure 1). Genetic screening revealed no mutations in *MAPT* or *GRN* in all four patients, and in *CHMP2B* genes in three patients. DNA was not available anymore in Case 1 to screen for *CHMP2B* gene mutations.

Table 1 Demographic, clinical and MRI features of FTLD-FUS cases

	Case 1	Case 2	Case 3	Case 4
Gender	Male	Female	Female	Female
Onset (years)	49	30	32	35
Death (years)	59	46	41	45
Family history	Negative	Negative	Negative	Negative
FTD subtype	BvFTD	BvFTD	BvFTD	BvFTD
Prominent symptoms	Sexual Disinhibition Visual hallucinations Hyperorality	Obsessive-compulsive Persecution delusions Extreme parsimonious Shoplifting Hyperorality	Apathy Sexual disinhibition Shoplifting Hyperorality	Obsessive-compulsive Parsimonious Shoplifting
Neurological examination	No abnormality	Mild cogwheeling left	No abnormality	No abnormality
Neuropsychological evaluation	Attention and concentration ↓ Executive functions ↓ Memory ↓ Perseverations	Attention and concentration ↓ Executive functions ↓ Perseverations Impulsive Stereotypical	Attention and concentration↓ Executive functions ↓ Mild memory ↓	Attention and concentration ↓ Executive functions ↓ Impulsive
CDR*	3	3	2	3
MRI	FT++ Cau++	F+T++ Cau++	F+ Cau+	FT++ Cau++

*, Clinical dementia rating scale at ascertainment; F, frontal; T, temporal; Cau, caudate; + mild-moderate atrophy, ++ severe atrophy

Age at onset ≤ 40 years, negative family history, and behavioural variant FTD

The frequency of FTLD-FUS in our clinical cohort was estimated by using negative family history, age at onset ≤ 40 years, and bvFTD as distinctive features for FTLD-FUS patients. We selected patients with a negative family history and bvFTD (n=126). We stratified our patients according to age at onset and made three subgroups; age at onset ≤ 40 years (n=11), > 40 – ≤ 50 years (n=23), and > 50 years (n=92). FTLD-FUS was found in three out of 11 patients (0.27) in the group with age at onset ≤ 40 years, one out of 23 (0.04) in the group of patients with age at onset > 40 – ≤ 50 years, and was absent in the group of 92 patients with age at onset > 50 years. FTLD-FUS is not more frequently found in patients with an age at onset ≤ 40, than in patients with an age at onset > 40 - ≤ 50 years (Fisher's Exact Test, p=0.089). However, FTLD-FUS is more frequently found in patients with an age at onset ≤ 40 years than in patients with an age at onset > 50 years (Fisher's Exact Test, p=0.001).

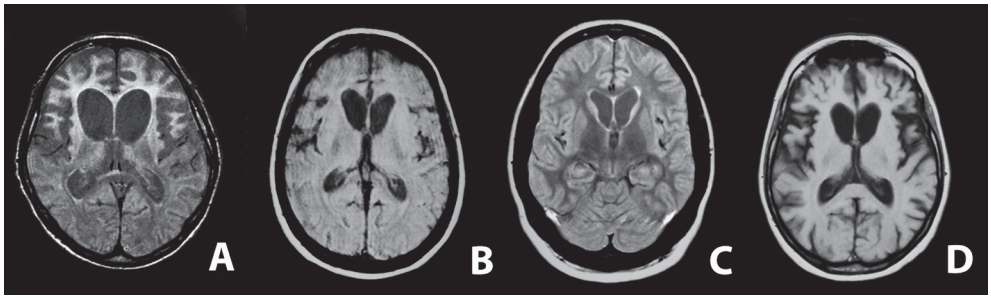
The estimated prevalence of FTLD-FUS for the total clinical FTD group based on family history, age at onset ≤ 40 years, and bvFTD as clinical subtype, is 11 out of 387 patients (0.03; 95% CI: 0.01 – 0.05).

Caudate atrophy

Severe caudate atrophy is one of the pathological hallmarks in FTLD-FUS. We reviewed all available MRIs of all clinical FTD patients (n=136) for the presence of caudate atrophy. Caudate atrophy was present in 10 out of 136 MRIs (0.07; 95% CI: 0.03 – 0.12). Seven of these 10 patients had a negative family history, onset ≤ 40 years, and bvFTD, of whom three had pathological proven FTLD-FUS. Pathology was not available in the other four cases. Of the remaining three cases with caudate atrophy, two had a *GRN* mutation and had an age at onset > 40 years, and the third patient had FTLD-FUS with an age at onset of 49 years. (Case 1). The interval between onset and time of MRI scanning between cases with and without caudate atrophy did not differ (36 months in cases with caudate atrophy as well as in cases without caudate atrophy).

Seven of the eleven patients with a negative family history, onset ≤ 40 years, and bvFTD had caudate atrophy. There were no MRIs available of the other four patients.

Figure 1 MRI scans of FTLD-FUS cases



(A) Patient 1 with severe atrophy (PD-weighted MR image). **(B)** Patient 2 with severe atrophy (PD-weighted MR image). **(C)** Patient 3 with mild-moderate atrophy (FLAIR MR image). **(D)** Patient 4 with severe atrophy (T1-weighted MR image).

Pathology of FTLD-FUS

Brain weight, gross atrophy and depigmentation of the substantia nigra are summarized in Table 2. The most characteristic feature was the striking atrophy of the caudate in all.

All four brains showed severe neuronal loss of CA1 and subiculum consistent with hippocampal sclerosis, as well as severe neuronal loss of the caudate nucleus. The frontal and/or temporal cortices showed moderate-to-severe neuronal loss in all, whereas the parietal cortex was mildly affected in only one brain. The substantia nigra was severely affected in all four brains. There was no involvement of the pons, medulla oblongata, cerebellum, or spinal cord.

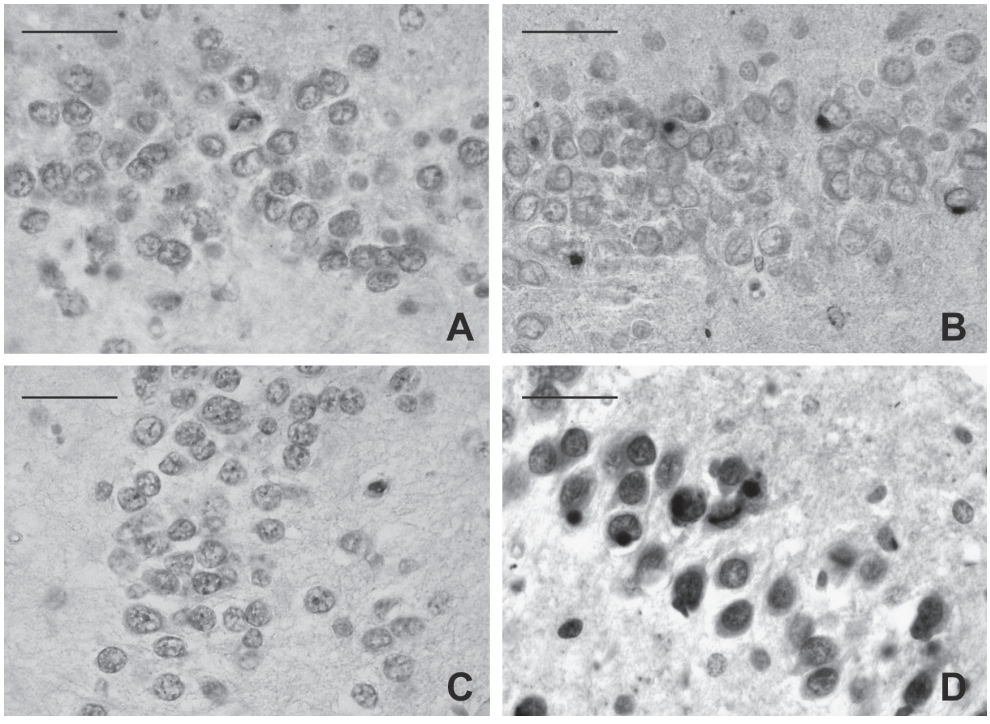
Table 2 Anatomical distribution and severity of degeneration in FTLD-FUS cases

	Case 1	Case 2	Case 3	Case 4
Brain weight (g)	1171	868	1040	870
Gross atrophy/ depigmentation	FT++ Hipp++ Str++	FT++ Hipp++ Str++ SN +	FT++ Hipp++ Str++ SN ++	FT++ Hipp++ Str ++ SN +
Frontal				
Degeneration	++	+	++	++
NCI / DN	+	+	+	+
NII	-	-	+	-
Temporal				
Degeneration	++	++	++	++
NCI / DN	+	+	+	
NII	-	-	-	-
Hippocampus				
Degeneration	++	++	++	++
NCI	++	++	++	+
NII	+	+	+	+
Striatum				
Degeneration	++	++	++	++
NCI/DN	+	+	+	+
NII	-	-	-	-
Substantia nigra				
Degeneration	++	++	++	++
NCI	-	-	-	-

F, frontal; T, temporal; Hipp, hippocampus; Str, striatum; SN, substantia nigra; NCI, neuronal cytoplasmatic inclusions; NII, neuronal intranuclear inclusions; DN, dystrophic neuritis. Grading: -, absent; +, mild: ++, moderate-severe. NCI and DNs stained positive for ubiquitin, p62, and FUS immunohistochemistry only. NII stained positive for ubiquitin, and FUS immunohistochemistry only.

Immunohistochemistry revealed many ubiquitin, p62, and FUS-positive, TDP-43-negative neuronal cytoplasmatic inclusions (NCI) in the granular cells of the dentate gyrus of the hippocampus, whereas the number of NCI and dystrophic neurites (DN) in the frontotemporal cortex and caudate nucleus was low. Several ubiquitin and FUS-positive, p62 and TDP-43-negative neuronal intranuclear inclusions (NII) of variable shape (straight, worm-like appearance, C-shaped, or ring-like structured) were found in the granular cells of the dentate gyrus in all four brains (Figure 2 A,C,D); two brains also had NII in the CA4 pyramidal neurons, including one with NII in CA3 (Case 1). Case 3 had NCI and NII in the medulla oblongata. The antibody against the ubiquitin-binding protein p62 gave positive staining of NCI and DN, but not with NII (Figure 2B). There were no inclusions found in the substantia nigra, pons, medulla, or cerebellum of any of the four brains. Immunostaining with AT-8, α-synuclein, and α-internexin was negative in all cases.

Figure 2 Immunohistochemistry of the FTLD-FUS cases.



Ubiquitin-positive neuronal intranuclear inclusions (NII) in the granular cells of the dentate gyrus, which are worm-like **(A)**. Only the ubiquitin-positive neuronal cytoplasmatic inclusions (NCI) stained positive with p62 **(B)**, and NII not. TDP-43-immunohistochemistry only stained normal nuclei, and did neither stain ubiquitin-positive NCI nor NII **(C)**. The NCI as well as NII stained positive with FUS antibody **(D)**. Scale bar = 50 µm

Discussion

This study showed a frequency of FTLD-FUS of six percent of all our pathologically proven FTLD cases, and 11 percent of the FTLD-U cases. We estimated a frequency of FTLD-FUS of three percent in the total series of patients with clinical FTD, based on the clinical variables age at onset, family history, and clinical subtype. All four FTLD-FUS cases presented with severe behavioural changes, young age at onset, negative family history, and caudate atrophy on MRI. FTLD-FUS cases were pathologically characterized by striking atrophy of the caudate nucleus, ubiquitin and FUS-positive, p62 and TDP-43-negative NII with remarkable morphology.

The present observation of three percent of FTLD-FUS in a large clinical cohort of FTD patients gives a frequency estimation of this subtype for the first time. Of the four FTLD-FUS patients, three had an age at onset ≤ 40 years, negative family history, and the clinical presentation of bvFTD. As 11 patients within the age group ≤ 40 years of our cohort meet the combination of these features, this suggests an even higher

frequency in this age group. Our observed age distribution is in line with that of the other reported series of FTLD with TDP-43 negative inclusions (28 till 63 years). (4-6) Delusions and hallucinations in the present cases may be discriminative features of this type of FTLD, as their occurrence is uncommon for the total FTD group.(13) The initial diagnosis of a primary psychiatric disorder has been considered in one of the present patients,(14) as well as in several other recently reported cases.(4-5) The occurrence of disinhibition and obsessive compulsive behaviour was reported in the present cases, while aggressive behaviour in others.(4)

Although we did not have pathological verification of four other patients, the combination of age at onset ≤ 40 years, negative family history, presence of severe behavioural changes, and caudate atrophy is highly suggestive for FTLD-FUS. Our suggested prevalence of three percent could be an underestimation, as ~30 percent of all cases described so far had an age at onset above 40 years, and not all patients in our cohort had an available MRI to semi-quantitatively score the caudate atrophy.(4-6) The observation of caudate atrophy on MRI in our FTLD-FUS cases during life has not been reported so far, but is in line with the presence of caudate atrophy and severe neuron loss at neuropathological examination. Neuroimaging features during life were not explicitly mentioned in three pathological studies on TDP-43-negative FTLD-U cases (FTLD-FUS in the new nomenclature).(15-17) Although smaller caudate volumes may be seen in the end-stage of FTD,(18-19) severe caudate atrophy was already detectable in the early disease stage of the present FTLD-FUS cases. Prominent caudate atrophy is characteristic for Huntington's disease, but chorea or other extrapyramidal signs were absent in the present cases.

The occurrence of FTLD-FUS in 11 percent of our FTLD-U cases is in-between the frequencies that are reported in other series,(4-6) and emphasized the idea that alternative pathophysiological mechanisms exist in the clinicopathological spectrum of FTLD and motor neuron disease. The FUS protein containing 526 amino-acids has structural similarities with TDP-43 and is involved in DNA repair and the regulation of transcription, RNA splicing, and export to cytoplasm.(7, 20-21). Mutations in the *FUS* gene located on chromosome 16 have recently been identified in familial ALS with ubiquitin and FUS-positive, TDP-43-negative inclusions.(20-21)

The TDP-43-negative, FUS-positive cytoplasmatic inclusions have also been found in neuronal intermediate filament inclusion disease (NIFID) cases,(22) whereas their presence has yet to be investigated in other related disorders: FTLD caused by *CHMP2B* mutations, basophilic inclusion body disease (BIBD), and ALS due to *SOD1* mutations.(23-26)

The remarkable vermiform or C-shaped morphology of the NII reported in all cases seems to be pathognomic for FTLD-FUS and differs from the lentiform or cat-eye shaped NII in *GRN* mutations.(4-6, 27-29). Moreover, NII of *GRN* mutations shows a different immunohistochemical pattern with TDP-43 positive and FUS negative staining.

The relevance of exclusive p62-positive staining of the cytoplasmatic inclusions (and not of NII) has still to be determined. The question is whether the p62 protein is merely entrapped in these inclusions, or plays a pathophysiological role in these disorders. The recent finding of accumulation of

hyperphosphorylated tau and neurodegeneration in mice with genetic inactivation of the *p62* gene supports the latter hypothesis.(30)

A major drawback of the present study is the absence of a quantitative assessment of caudate atrophy on MRI.(18) Future prospective follow-up studies with voxel-based morphometry of the caudate nucleus on MRI are needed to determine the progression of caudate atrophy over time in FTLD-FUS.

In conclusion, FTLD-FUS is estimated to account for three percent of our series of clinical FTD. Age at onset ≤ 40 years, negative family history, bvFTD and caudate atrophy on MRI could be useful clinical predictors of FTLD-FUS as underlying pathology in patients with clinical FTD. Larger samples of FTLD-FUS cases are needed to verify and to add new predictors for this clinicopathological subtype.

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Chapter 5

General Discussion

Frontotemporal dementia (FTD) is the second most common form of early-onset dementia and is clinically characterized by progressive behavioural changes and frontal executive deficits and/or selective language dysfunctions. Three clinical syndromes encompass the clinical spectrum of FTD: behavioural variant of FTD (bvFTD), and the language variants semantic dementia (SD) and progressive non-fluent aphasia (PNFA).(1-3)
A positive family history is found in 30 – 50 percent of patients with FTD, with an autosomal dominant mode of inheritance in 10 – 27 percent.(4-5) Two major gene defects are found in familial FTD: mutations in the *microtubule-associated protein tau* (*MAPT*) and *progranulin* (*GRN*) genes.(6-8) However, there are one or more familial forms of FTD with unknown genetic defect, in particular familial FTD with motor neuron disease (FTD-MND).(4-5) However, there still are one or more familial forms of FTD with unknown genetic defect, in particular familial FTD with motor neuron disease (FTD-MND).(4-5)
The identification of the TAR DNA binding protein of 43 kDa as major constituent of ubiquitin-positive inclusions was a major breakthrough in the frontotemporal lobar degeneration (FTLD) research field.(9) FTLD-TDP can be classified in four subtypes based on distribution and morphology of the inclusions. A relatively small number of cases show ubiquitin- and FUS-positive, but TDP-43-negative inclusions (FTLD-FUS).(10) Due to the rapid changes in the neuropathology, new nomenclature and nosology for neuropathological subtypes of FTLD were required.(11)

The focus of my thesis was to describe and determine the clinical, genetic and pathological heterogeneity of frontotemporal dementia (FTD).
In my opinion, there are four key topics for further research: 1) to improve the early detection and monitoring of the disease; 2) to develop reliable biomarkers in predicting the underlying pathology 3) to discover the genetic defect in chromosome 9p linked families and other families with unknown genetic defect 4) to unravel the pathophysiology of all FTLD subtypes in order to develop strategies preventing or delaying the disease process.
In this chapter I will discuss the studies described in this thesis and give a review of all recent developments on research in clinical, genetic and pathological aspects of FTD, and present suggestions for future research.

Clinical heterogeneity

For patient care and future therapeutic intervention it is important to diagnose FTD early in the course of the disease. All variants of FTD have major influences on daily life for patients' caregivers. The caregiver burden in FTD is greater than in AD, and the behavioural changes rather than level of disability seem to be correlated with caregiver distress.(12-14) Early diagnosis and improved understanding of the symptoms, as well as advice in dealing with the behavioural symptoms are important in reducing caregiver burden. Furthermore, future therapeutic options will probably be most effective at early stages of the disease.
Revised clinical criteria are on the way to improve the sensitivity of the diagnosis in the early phase of

the disease. Major revisions are the incorporation of neuroimaging and genetic data in a set of simplified criteria of bvFTD.(15) Revised criteria on primary progressive aphasia (PPA) are also on the way, and include SD, PNFA and a novel entity logopenic variant of PPA (LPA). Differentiating between these PPA subtypes is important, as the underlying pathology differs: TDP-43 pathology is found in the majority of the SD cases, PNFA is associated with tau-pathology, whereas LPA is associated with Alzheimer's disease.(16) Standard neuropsychological examination cannot reliably differentiate bvFTD from Alzheimer's disease.(17-19) Therefore, the utility of emotional processing and social recognition has been explored in the clinical diagnosis of FTD. It will be interesting to investigate whether social recognition is a very early feature of FTD by following presymptomatic *MAPT* or *GRN* mutation carriers.

Disease progression in individual patients can be monitored clinically, as the FTLD modified clinical dementia rating scale (FTLD-CDR)(20) and FTD rating scale (FRS)(21) have become available. In particular the FRS characterizes the features of the severity stages and can aid in staging and determining progression.(21) Furthermore, the FRS could be used to monitor disease progression in future clinical trials.

FTD-MND

FTD is accompanied by motor neuron disease (FTD-MND) in 5 – 15 percent of the cases.(22-24) On the other hand, 30 – 50 percent of the patients with amyotrophic lateral sclerosis (ALS) have cognitive dysfunctions.(25) FTD-MND patients have a mean survival of three years after onset which is significantly shorter than other forms of FTD.(23-24) Furthermore, ALS patients with cognitive deficits have a shorter survival than cognitively normal ALS patients.(26)

In **chapter 2.2**, we investigated whether survival is influenced by symptom onset in patients with FTD-MND. We retrospectively reviewed 87 FTD-MND patients from four academic medical centers and found that survival was longer when cognitive symptoms appeared earlier than motor symptoms. The timing of the secondary symptom onset may thus reflect in part the rate of stage wise disease progression regardless of cognitive or motor onset. In the so-called 'long-term survivors', older age at onset was inversely correlated with survival. An older age at onset is also associated with a better survival in ALS without dementia.(27) Timing of the secondary symptom s probably depends on distribution of TDP-43-positive inclusions. It has to be investigated whether genetic factors like SNPs in *GRN* or *TMEM106B* or *Sortilin-1 (SORT1)* genes may play a role in the survival of FTD-MND.

Biomarkers

Neuroimaging

Neuroimaging plays a major role in the differentiation of neurodegenerative disorders. In **chapter 2.1**, we investigated whether clinical features and neuroimaging using SPECT scans may help to differentiate pathological subtypes in a cohort of 29 patients. The group of 29 patients consisted of 10 patients with *MAPT* gene mutations, and 19 with familial FTLD-TDP of whom six had *GRN* gene mutations, and 13 patients with familial FTLD-TDP and an unknown genetic defect. We found that at clinical presentation

MAPT patients had more naming deficits and obsessive-compulsive behaviour, whereas familial FTLD-TDP more frequently presented with memory deficits. When we compared perfusion patterns on SPECT of the two patient groups, *MAPT* revealed more left temporal lobe and orbitofrontal hypoperfusion, whereas familial FTLD-TDP showed more asymmetrical right frontoparietal lobe hypoperfusion. We found the same results when we compared *MAPT* with patients with FTLD-TDP with unknown genetic defect. Clinical presentation and neuroimaging will help to differentiate patients with distinct familial forms. In the revised clinical criteria frontal and/or anterior temporal atrophy on structural imaging or frontal hypoperfusion or hypometabolism on SPECT or PET will be included.(15) This will not only help to differentiate between bvFTD and Alzheimer's disease, but also between bvFTD and phenocopies named 'non-progressive' or 'benign' bvFTD.(28-29) It is still unclear whether this last group is really part of the FTLD spectrum as these patients seldom come to autopsy.(28-29)

Recent FTLD imaging studies using voxel-based morphometry (VBM) on MRI have focused on differentiating FTLD-tau from FTLD-TDP. Probably due to the (clinical) heterogeneity within these subgroups, no differences in atrophy patterns have been found in these analyses.(30-33) However, in FTLD-TDP three different subtypes (type 1-3) were associated with distinct patterns of atrophy.(34-35) Patients with *MAPT* or *GRN* gene mutations are good candidates for neuroimaging studies, as these are two relatively homogenous groups of patients with distinct underlying pathologies. Furthermore, autopsy is not required in patients with gene mutations, as the underlying pathology is known, which expands the patient numbers in these studies.

A consistent finding is that *MAPT* mutations are associated with relatively symmetrical anterotemporal and orbitofrontal atrophy, whereas *GRN* mutations are associated with asymmetrical frontal, temporal, and parietal pattern of atrophy.(36-37) An interesting finding is the possible association between mutation function and atrophy in *MAPT*.(38) Mutations that affect exon 10 splicing and thus influence the alternative splicing of tau pre-mRNA showed medial temporal atrophy. Mutations that do not effect exon splicing but affect protein structure showed more atrophy of the lateral temporal lobe.(38) In our study we could confirm the latter finding, as the majority of our *MAPT* group consisted of P301L patients with anterolateral temporal and frontal hypoperfusion (**chapter 2.1**). However, it remains unclear how these different disease mechanisms lead to distinct atrophy patterns.(38) Furthermore, it is unknown how a single mutation (in either *MAPT* or *GRN*) may lead to different clinical phenotypes, and affect different brain regions or even hemispheres within the same family.(37, 39)

Patients with familial FTLD-TDP with unknown gene defect are a new focus of interest. We showed in chapter 2.1 that familial FTLD-TDP with unknown genetic defect is associated with asymmetrical frontal and also parietal, precuneus and cuneus hypoperfusion. These findings suggest that posterior cortical involvement is not specific for *GRN* mutations only. Parietal and occipital atrophy has also been described in familial FTD-MND linked to chromosome 9p.(40) A major drawback of our study is that the patient group of familial FTLD-TDP with unknown genetic defect probably consists of two or more genetic defects. Also the number of cases was relatively small. Further neuroimaging studies are needed to confirm whether parietal lobe hypoperfusion or atrophy is a specific marker for familial FTLD-TDP with unknown genetic defect.

Future neuroimaging studies

One of the more novel concepts that emerged from recent neuroimaging studies on FTD using the technique of resting state functional MRI (RS-fMRI), is the idea that FTD is caused by neurodegeneration within specific intrinsic functional connectivity networks that are selectively vulnerable to FTLD pathologies.(41-42)

Consistent with earlier VBM findings in structural MRI studies of bvFTD, RS-fMRI studies show attenuated connectivity within an anterior 'salience' network, which is anchored by the dorsal anterior cingulate and frontoinsular cortices and is involved in processing emotional stimuli.(42) In contrast, there appears to be enhanced connectivity in the more posterior 'default mode' network which has shown to be affected in Alzheimer's disease.(42) Future RSfMRI studies will need to find out whether specific genetic and pathological subtypes can be linked to distinct neural network changes. Presymptomatic *MAPT* and *GRN* gene carriers are an interesting study population for this purpose.

A different focus is the development of protein-specific tracers in PET. Amyloid molecular imaging (eg, PIB-PET) has shown to be a reliable tool to differentiate between Alzheimer's disease and FTD.(43) However, to date it is not possible to define the underlying substrate in FTD. Development of tau or TDP-43 tracers would be helpful in predicting underlying pathology, and for the use of future specific therapeutical options.

Cerebrospinal fluid and plasma biomarkers

Cerebrospinal fluid and plasma biomarkers studies are not included in this thesis. However, they will play an important role in the future diagnostic work-up of FTD. Currently CSF markers have limited ability to identify FTD. Only decreased levels of progranulin protein are found in CSF, plasma and serum by ELISA, and reliably differentiate *GRN* mutation carriers from non carriers.(44-47) However, *GRN* mutations account for the minority of all FTD cases. Plasma phosphorylated TDP-43 levels have been found to be correlated with the extent of TDP-43 pathology in FTLD, although it remains to be investigated if measurement of plasma or CSF levels of TDP-43 is useful diagnostically.(48-49)

Furthermore, the CSF and plasma biomarkers could be useful in detecting the underlying pathology (tau, TDP-43 or FUS). Recent biomarker studies on CSF are using multiplex platforms which allow simultaneous measurement of multiple analytes (multianalyte profiling) to derive novel biomarkers for neurodegenerative disorders.(50-51) Some promising neuropeptides (agouti-related peptide (AgRP), adrenocorticotrophic hormone (ACTH), IL-17 and IL-23 and Fas) have been identified, which may be useful in distinguishing FTLD-TDP from FTLD-tau.(50-51) Large international collaborations are needed to find new biomarkers in patients with pathologically or genetically proven FTLD.

Genetic heterogeneity

Progranulin and genetic risk factors

A major breakthrough in 2006 was the identification of mutations in the *Progranulin* (*GRN*) gene, 1.7 Mb centromeric to the *MAPT* gene, causing tau-negative and ubiquitin-positive FTLD linked to chromosome 17q21. In **chapter 3.1** we screened our previously described family with ubiquitin-positive inclusions linked to chromosome 17q21 (family HFTD3)(52) for *GRN* mutations, and we identified a novel frameshift mutation Ser82ValfsX174 in this family. In addition, we described the genetic contribution of *GRN* in our familial cases from our FTD cohort. This revealed another novel frameshift mutation Val411Serfr1X, and one nonsense mutation Gln125X in an independently ascertained member of the 1083 family already reported in the original *GRN* paper.(7) Furthermore, three possible pathogenetic missense mutations were found.

In **chapter 3.2** we found *GRN* mutations to be present in six percent of all our FTD cases, which is in line with other studies where *GRN* mutations account for 5 – 10 percent of all FTD cases, and up to 22 percent of familial FTD.(7-8, 53-55) The frequency found in our cohort could be an underestimation as quantitative analysis to identify exon deletions or duplications was not performed in our studies. We also identified a Gln300X *GRN* gene mutation in a patient with sporadic bvFTD. Although sporadic *GRN* mutations are occasionally reported in other series, it is unknown whether this is due to a low penetrance or a spontaneous mutation. To date more than 60 pathogenetic mutations are found in the *GRN* gene.(56)

The age at onset is remarkably variable in *GRN* patients and even within families with a mean age at onset of around 60 years, and a range of 35 – 89 years.(56) The penetrance is 90 percent by the age of 70 years.(53) This in contrast to *MAPT* patients, who have a younger mean age at onset of 55 years and usually a smaller intrafamilial variation ranging between 45 and 65 years. It is still unclear what causes the intrafamilial variation in age at onset in *GRN* families. We could not confirm the previously reported effect of A allele of rs9897526 on a delayed onset in *GRN* families.(57)

A large genome wide association study (GWAS) with 550 pathologically confirmed FTLD-TDP cases revealed three SNPs within the *TMEM106B* gene on chromosome 7p21 that were significantly associated with FTLD-TDP.(58) The association of *TMEM106B* SNPs is even more significant in patients with *GRN* gene mutations.(59) Homozygosity of the minor allele of rs1990622 and rs6966915 has been found to be protective, i.e. associated with a later age at onset, in patients with *GRN* mutations.(59) Although this may be a promising finding for future therapeutic interventions, this finding needs replication in other cohorts.

Recent studies over the last months have used an unbiased genetic approach to identify genes and proteins involved in the regulation of *GRN*, and have identified *Soritilin-1* (*SORT1*) as a major regulator of extracellular GRN levels.(60-61) Sortilin is able to rapidly endocytose and deliver GRN to lysosomes.(60-61) Interestingly, the 50 percent GRN protein levels in $GRN^{+/-}$ mice can be normalized by *SORT1* ablation.(60) The sortilin-mediated GRN endocytosis could be a potential therapeutic target in *GRN*

patients.(60-61) Two main questions on *GRN* gene mutations remain unsolved and are of interest for future research: 1) what is the causative role of GRN haploinsuffiency, and 2) how does loss of GRN protein leads to accumulation of TDP-43.

Familial FTLD with unknown genetic defect

As *MAPT* and *GRN* gene mutations do not explain the total of hereditary FTD, we investigated in **chapter 3.2** the occurrence and phenotype of patients of hereditary FTD with unknown genetic defect. In our cohort of 364 patients, 10 percent had an autosomal dominant mode of inheritance with unknown genetic defect. Interestingly, all these patients with unknown genetic defect had FTLD-TDP pathology and could be divided in familial FTLD-MND and familial FTLD-TDP with hippocampal sclerosis. Three of the four patients with FTLD-TDP and hippocampal sclerosis presented with memory deficits and were initially diagnosed with Alzheimer's disease. We suggest that future genetic studies need to identify at least two genetic defects in familial forms of FTD.

Familial FTD-MND is a major focus of genetic studies in FTLD-TDP with unknown genetic defect. Several FTD-MND families have been linked to chromosome 9p.(40, 62-68) Our FTD-MND families reported in **chapter 4.1** were too small for significant linkage. However, the neuropathological findings of FTLD-TDP type 2 as proposed by Sampathu et al. (69) in our families are compatible with that of other chromosome 9p linked families.

A common gene defect probably causes FTD or MND in these families, as linkage to chromosome 9p has been found in several independent families in Europe and US. (40, 62-68) However, all groups to date have failed to identify non-polymorphic exonic mutations in this region, suggesting a non-protein coding pathogenetic mechanism.(68)

Detecting the genetic defect on chromosome 9p will not solve all of the remaining familial FTD-TDP with unknown genetic defect, as we suggested a second distinct neuropathological form of familial FTLD-TDP also with type 2 pathology but with hippocampal sclerosis. Furthermore, in families with familial FTLD-TDP with hippocampus sclerosis, there were no family members with clinical FTD-MND or MND. Arguments that these are not two distinct disease entities could be: 1) the absence of hippocampal sclerosis in familial FTLD-MND might merely be due to the shorter survival in these patients; 2) subclinical MND could be present in FTLD-TDP with hippocampal sclerosis; 3) our finding could be a serendipitous dissociation due to the small sample of patients.

Besides the two possible distinct entities within FTLD-TDP type 2, several groups have reported *GRN* FTLD-TDP type 3 pathology to be present in familial FTLD-TDP cases without *GRN* mutations.(5, 70-71) This is another argument that there are several genes which contribute to the genetic heterogeneity of familial FTLD-TDP.

Future genetic studies

The focus of genetic studies will be the detection of the genetic defect of familial FTD-MND linked to chromosome 9p. Linkage in families with FTLD-TDP type 3 pathology is lacking to date and will probably reveals novel loci. Whole exome sequencing as innovative genetic technique might reveal new genetic defects in small families with FTD and for pathologically well-characterized FTLD subtypes (like FTLD-FUS). Identification of novel genetic defect(s) will help to understand the pathophysiology of TDP-43 in hereditary and probably also sporadic FTLD-TDP. A large genome-wide association study of more than 3000 DNA samples is currently underway and will hopefully reveal additional genetic factors with a small effect on the disease process.

Pathological heterogeneity

TDP-43

The pathology of FTLD has evolved rapidly over the last few years. Formerly, FTLD was divided in patients with tau-positive inclusions (FTLD-tau), ubiquitin-positive and tau negative inclusions (FTLD-U), or dementia lacking distinctive histopathology (DLDH). In 2006, the identification of TAR DNA binding protein of 43 kDA (TDP-43) as major constituent of ubiquitin-positive inclusions was a major breakthrough in the FTD field (FTLD-TDP), and it was thought that the enigma concerning the nature of the ubiquitinated inclusions in FTLD-U was ended.(9) Furthermore, ubiquitin-and TDP-43 positive inclusions were also found in ALS, which supports that these two clinically distinct disorders are part of the same clinicopathological spectrum.(9)

In **chapter 4.1** we described the clinical presentation, familial aggregation and occurrence of TDP-43 positive inclusions in familial FTD-MND. In our cohort of 368 patients, we found eight patients from six independent FTD-MND families with variable clinical presentation (FTD/dementia, MND or FTD-MND). Brain specimens were available from five patients and showed TDP-43 positive neuronal cytoplasmatic inclusions (NCI) in hippocampus, neocortex and spinal cord in all, and neuronal intranuclear inclusions (NII) in two patients. Additionally, we found ubiquitin-negative TDP-43 positive glial inclusions in the striatum in one case. TDP-43 positive glial inclusions found in all patients of the VSM-20 FTLD-MND family,(40) which suggests a role of glia in the pathophysiological process of the disease.

The TDP-43 protein is encoded by the *TARDBP* gene on chromosome 1p36.2 and consists of five coding and one non-coding exons. However, mutations in the *TARDBP* gene are rarely found in FTD or FTD-MND.(72-73) TDP-43 shuttles between the nucleus and cytoplasm, and plays a role in transcription and splicing regulation, and microRNA processing, mRNA stabilization, cell division and apoptosis.(74-75) It is suggested that transient redistribution to cytoplasm may be a normal response to neuronal injury. TDP-43 may also play a role in the regulation or neuronal plasticity and maintenance of dendritic integrity.(74-75) It remains unclear how mutations in the *TARDBP* as well as in *GRN* and *valosin containing protein* (*VCP*) genes lead to the accumulation of TDP-43 in the brain. However, the morphology and distribution of TDP-43 accumulation are different between these gene defects and suggest distinct pathophysiology.

FTLD-TDP is further classified into four different subtypes according to the morphology and distribution of the inclusions (69, 76-77), and can be predicted by genetic status and to some extent by the clinical picture: SD is strongly associated with abundant dystrophic neurites (type 1), FTD-MND with or without linkage to chromosome 9p with numerous neuronal cytoplasmatic inclusions in both superficial and deep cortical laminae (type 2), pathology of *GRN* mutations is characterized by numerous cytoplasmatic inclusions, dystrophic neurites and neuronal intranuclear inclusions (type 3), and *VCP* mutations are characterized by numerous intranuclear and an infrequent number of neuronal cytoplasmatic inclusions and dystrophic neurites (type 4).(69, 77) It remains unclear what differences in underlying pathophysiology determine the distinction between these TDP-43 subtypes.

FTLD-FUS

TDP-43 was the major constituent of ubiquitin-postive inclusions in most cases. However, a small number of cases had ubiquitin-positive, but TDP-43 negative inclusions. The identification of mutations in the in *fusion in sarcoma* (*FUS*) gene, also known as *translocated liposarcoma* (*TLS*) gene, on chromosome 16 in familial ALS cases, provided new insights in the TDP-43 negative ubiquitinopathies.(78-79) FUS protein was also identified in cases with TDP-43 negative FTLD-U pathology, who presented with a young age at onset (most cases <40 years) and had striking caudate atrophy at neuropathological examination without mutations in the *FUS* gene.(10)

In **chapter 4.2** we reported a frequency of FTLD-FUS in 11 percent of our FTLD-U cases and four percent of all pathologically confirmed FTLD cases in our cohort. This is in accordance with frequencies reported by other groups.(10, 80-81) Three of our four FTLD-FUS cases had an age at onset before 40 years and had a negative family history. The striking caudate atrophy at neuropathological examination was also present in our four FTLD-FUS cases, and this was already present at MRI early in the course of the disease. We combined the clinical parameters (young onset, bvFTD, negative family history and caudate atrophy on MRI) and found an estimated frequency of FTLD-FUS in our clinical FTD cohort of three percent. This percentage could be an underestimation as approximately 30 percent of all FTLD-FUS cases have an age at onset above 40 years, (10, 80-81) and not all patients in our FTD cohort had an available MRI to semiquantitatively score caudate atrophy. A major drawback is the absence of a quantitative assessment of caudate atrophy on MRI. However, other studies using VBM supported our finding and even suggested that caudate atrophy distinguishes FTLD-FUS from FTLD-TDP and FTLD-tau.(82-83)

FUS protein shows striking structural and functional similarities with TDP-43.(74, 84) FUS protein is encoded by the *FUS* gene, and shuttles between the nucleus and cytoplasm and is implicated in cell proliferation, DNA repair, transcription regulation, RNA and microRNA processing. (74, 84)

FUS pathology accounts for 92 percent of TDP-43-negative FTLD-U cases.(85) Screening for *FUS* gene mutations in a larger series of pathologically proven FTLD-FUS did not reveal pathogenetic mutations. (85) In clinical series, one patient with clinical FTD-MND had a p.Arg521His mutation, which is known to be pathogenetic in patients with pure ALS.(86) A novel p.Met254Val *FUS* mutation was described in a patient with sporadic bvFTD, although the pathogenetic nature is unclear and pathological verification

is lacking.(87) Compared to our reported cases (**chapter 4.2**), these two clinical FTD cases with *FUS* mutations were relatively older at onset (71 and 51 years) and showed no caudate atrophy on MRI.(86-87) The absence of caudate atrophy could be due to a short interval between onset and MRI scan in these two patients, or might reflect a distinct pathology between FTLD-FUS patients with or without *FUS* mutations.

There still remains a small number of patients with TDP-43 and FUS negative FTLD-U. This group is designated by the term FTLD-ubiquitin proteasome system (FTLD-UPS), and contains patients with and without *CHMP2B* gene mutations. Further research on FTLD-UPS is needed to elucidate the full complement of FTLD pathologies.

Future research on TDP-43 and FUS

FUS and TDP-43 are both RNA/DNA-binding proteins, and their striking structural and functional similarities imply that abnormal RNA metabolism plays a central role in both FTLD-TDP and FTLD-FUS.(74-75) However, the mechanisms leading to accumulation of TDP-43 and FUS leading in FTLD is currently unknown.(74-75) It seems that TDP-43 and FUS pathology are mutually exclusive, although one study found co-localization of FUS and TDP-43.(88) Future studies have to elucidate the possible interactions of TDP-43 and FUS. It will be important to understand the role of both proteins in normal central nervous system, and then identify disease-relevant post-translational modifications, and brain specific messenger RNA and microRNA targets.(74-75)

Conducting future clinical trials

The ultimate goal is the development of therapeutic options for every FTLD pathological substrate. A major limitation in conducting future therapeutic trials, is the heterogeneity of underlying pathologies in patients with clinical FTD, in particular bvFTD. Hopefully, earlier mentioned developments on biomarkers will help to predict the underlying pathology early in the course of the disease in the nearby future. Another important issue is monitoring the disease progression. FTLD-CDR(20) and FRS(21) together with standard neuropsychological examination could be used for clinical evaluation. MRI scans performed on regular basis (for example every 6 months) are useful as to follow atrophy rate during clinical trials. Perhaps RSfMRI could be used to monitor changes in neuronal network dynamics. An important role in monitoring disease progression in clinical trials is reserved for CSF, plasma and serum biomarkers. However, these biomarkers are currently unavailable.

To date, trials are limited to tau-pathologies progressive supranuclear palsy (PSP), corticobasal syndrome (CBS) and PNFA. Although the vast majority of patients with CBS and PNFA has underlying tau-pathology, some patients will have underlying TDP-43 pathology and might disturb the outcome in tau-related clinical trials. BvFTD patients that could potentially be included in tau-related trials are patients with *MAPT* mutations.

A phase II study on Davunetide 15 mg intranasally twice daily has been conducted recently. Davunetide (NAP) is a drug derived from activity dependent neurotrophic protein that was proved to have beneficial effects on memory and tau phosphorylation in tau mutation transgenic mice.(89) In humans it has already demonstrated benefit on attention and working memory in amnestic MCI (www.allontherapeutics.com).

Another potential therapeutic approach in tauopathies are microtubili(MT)-stabilizing drugs. (90) These drugs, like paclitaxel, have been successfully used in oncology. However, MT-stabilizing drugs have poor blood-brain barrier permeability, which makes them unsuitable for FTLD tauopathies. In a tau transgenic mouse model, Epothilone D, was found to be one of the several compounds that readily enter the brain and improve axonal MT density and integrity in the central nervous system.(91) Whether Epothilone D has effect in human brains has to be elucidated.

TDP-43 trials have not been conducted to date. Potential study populations for TDP-43 trials are patients with SD, FTD-MND or patients with *GRN* mutations, and perhaps patient from families with pathological FTLD-TDP verification in a first degree relative.

Conclusions

Major advances in the field of FTD have led to an impressive increase of our clinical, genetic and pathological knowledge. Novel clinical criteria for bvFTD and PPA will help to detect all variants of FTD in the early phase of the disease. Development of CSF, plasma or serum biomarkers are of major interest for clinicians and will hopefully be implemented in the diagnostic work-up of FTD soon.

An interesting study population are presymptomatic *MAPT* or *GRN* mutation carriers. Clinical and neuromaging studies may reveal the earliest changes on social recognition, MRI and RSfMRI. RSfMRI may detect whether specific pathological subtypes can be linked to distinct neural networks.

Although mutations in the *GRN* gene have been found in patients with familial FTLD-TDP, further research is needed to discover the genetic defect in familial FTD-MND linked to chromosome 9p and other families with TDP-43 pathology with unknown genetic defect. Identification of novel genetic defects and risk factors will help to understand the pathophysiology of TDP-43 in hereditary and hopefully also of the sporadic FTLD-TDP cases. Identification of the FUS protein as a pathological component of neuronal inclusions emphasises the existence of different pathways and will contribute to the further understanding of the underlying pathophysiology.

All these small steps in unravelling the pathophysiology should finally lead to the development of therapeutic interventions for FTD.

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Chapter 6

Summary
Samenvatting

Summary

Frontotemporal dementia (FTD) is a neurodegenerative disorder which presents in most cases before the age of 65 years and is characterized by progressive behavioural disturbances, language deficits and frontal functioning problems. FTD is, after Alzheimer's disease, the most common form of presenile dementia. FTD is part of a clinicopathological spectrum designated with the term frontotemporal lobar degeneration (FTLD).

FTD is a clinical, genetic and pathological heterogeneous disorder. Major developments have been achieved over the past few years. One of them is the discovery of mutations in the *Progranulin* (*GRN*) gene causing familial frontotemporal lobar degeneration (FTLD) with ubiquitin-positive, tau-negative neuronal inclusions in families linked to chromosome 17q21. A second major breakthrough was the finding of TAR DNA binding protein of 43 kDa (TDP-43) a major constituent of ubiquitin-positive inclusions. Later on, it was found that FUS protein was the major constituent in the minority of the ubiquitin-positive, TDP-43 negative cases.

These findings have lead to an increase of our knowledge on the pathophysiology of FTD. However, further research is necessary to unravel the pathophysiology of FTD to achieve the ultimate goal: development of therapeutic options for all forms of FTD.

The aim of this thesis was to describe and determine the relationship between the clinical presentation, genetics and pathology of FTD, with emphasis on the hereditary form of FTD.

Chapter 1 is the introduction to the thesis, where **chapter 1.1** is a general introduction to the thesis and **chapter 1.2** is an extensive overview on clinical presentation, neuroimaging, genetics and pathology of FTD.

Chapter 2 reveals the clinical differences within FTD. In **chapter 2.1** we describe the clinical presentation and perfusion patterns on SPECT of 10 patients with *MAPT* gene mutations and 19 with familial FTD with TDP-43 pathology (FTLD-TDP). The latter group consisted of 6 patients with *GRN* mutations and 13 with unknown genetic defect. Patients with familial FTLD-TDP presented more often with memory deficits with hypoperfusion of the parietal lobe, including precuneus, and precentral gyrus compared to *MAPT*. *MAPT* patients presented more often with naming deficits and obsessive-compulsive behaviour associated with left temporal and medio-frontal hypoperfusion on SPECT compared to familial FTLD-TDP. We suggest that *MAPT* and familial FTLD-TDP could be differentiated by their clinical presentation and perfusion patterns.

Chapter 2.2 described the survival profiles of patients with FTD with motor neuron disease (FTD-MND). We found two distinct profiles, the so-called long-term survivors and typical survivors, with a mean survival of 69 months and 19 months, respectively. De long-term survivors had an onset with cognitive disturbances and delayed emergence of motor neuron symptoms after a long monosymptomatic phase, whereas typical survivors had simultaneous or discrete onset of cognitive and motor-neuron

disease symptoms. The distinct profiles in FTD-MND suggest that overall survival may depend on the relative timing of the emergence of secondary cognitive or motor symptoms.

The hereditary forms of FTD are described in **chapter 3**. In **chapter 3.1** we report *GRN* mutations in our FTD cohort. One of the families was previously linked to chromosome 17q21-22, with neuropathologically ubiquitin-positive, tau-negative neuronal inclusions. We found two novel frameshift mutations and three possible pathogenetic missense mutations in the *GRN* gene in hereditary FTD. *GRN* mutations account for a part of the hereditary FTLD with ubiquitin-positive inclusions.
Chapter 3.2 describes the hereditary forms of FTD with unknown genetic defect. We found an autosomal dominant mode of inheritance in 27 percent of all our FTD cases, of whom 11 percent had *MAPT* gene mutations and 6 percent *GRN* mutations. The remaining 10 percent with unknown genetic defect presented predominantly with behavioural variant of FTD (bvFTD) or FTD-MND. All patients with available brain specimens had FTLD-TDP pathology and could be divided in patients with familial FTLD-MND or patients with familial FTLD-TDP with hippocampal sclerosis. Although both groups had TDP-43 pathology, we suggest that future genetic studies may reveal two or more genetic defects in hereditary FTLD with TDP-43 pathology.

Chapter 4 we focus on the neuropathology of FTD. **Chapter 4.1** described the clinical presentation and pathology of familial FTD-MND. We found a clear intrafamilial clinical variation, where patients or family members developed FTD or dementia, MND or FTD-MND. We did not find mutations in genes involved in FTD or MND. Available brain tissue of five patients revealed ubiquitin- and TDP-43 positive neuronal inclusions in all and intranuclear inclusions in two. Furthermore, we reported ubiquitin-negative, TDP-43 positive glial inclusion in the striatum of one patient. We conclude that there is an intramilial clinical variation in familial FTD-MND with unknown genetic defect and TDP-43 pathology in all.
In **chapter 4.2** we describe the frequency, clinical presentation, neuroimaging and pathology of patients with ubiquitin- and FUS- positive, but TDP-43 negative inclusions. Eleven percent of all ubiquitin-positive brains were also FUS positive (FTLD-FUS). Our four FTLD-FUS cases presented with bvFTD, negative family history, young age at onset before age of 40 years in three, and caudate atrophy on MRI. Using these four clinical parameters, we estimated a prevalence of underlying FUS-pathology in three percent in our clinical FTD cohort. The pathological heterogeneity in FTLD is further emphasized by FUS pathology, although it is the underlying pathology in only a minority of the clinical FTD cases.

Finally, in **chapter 5** we discuss our most important findings of this thesis, the relevant developments on FTD and give suggestions for future research.

Samenvatting

Frontotemporale dementie (FTD) is een neurodegeneratieve aandoening die doorgaans ontstaat voor het 65e levensjaar en gekenmerkt wordt door progressieve gedragsveranderingen, taalproblemen en frontale functiestoornissen. FTD is na de ziekte van Alzheimer de meest voorkomende dementie voor het 65e levensjaar. FTD is onderdeel van een clinicopathologisch spectrum die frontotemporale lobaire degeneratie (FTLD) genoemd wordt.
FTD is een klinisch, genetisch en pathologisch heterogene ziekte. Er hebben de afgelopen jaren belangrijke ontwikkelingen plaatsgevonden. Eén daarvan is de ontdekking van mutaties in het *progranuline* (*GRN*) gen hetgeen familiaire frontotemporale lobaire degeneratie (FTLD) met ubiquitine-positieve, tau-negatieve neuronale inclusies veroorzaakt in families gekoppeld aan chromosoom 17q21. Een tweede belangrijke doorbraak was de bevinding dat het TAR DNA bindend eiwit van 43 kDa (TDP-43) het belangrijkste bestanddeel is van ubiquitine-positieve inclusies. Later werd bekend dat het fused in sarcoma (FUS) eiwit in de minderheid van de gevallen het belangrijkste bestanddeel van de ubiquitine inclusies is.
Deze bevindingen hebben onze kennis vergoot ten aanzien van de pathofysiologie van FTD. Niettemin is er meer onderzoek nodig om de pathofysiologie van FTD te ontrafelen om zo het uiteindelijke doel te bereiken: de ontwikkeling van therapeutische opties voor alle vormen van FTD.

Het doel van dit proefschrift is het beschrijven en bepalen van de relatie tussen de klinische presentatie, de genetica en pathologische kenmerken van FTD, waarbij de nadruk wordt gelegd op de erfelijke vormen van FTD.

Hoofdstuk 1 vormt de inleiding van het proefschrift, waarbij **hoofdstuk 1.1** een algemene inleiding op het proefschrift zelf is. **Hoofdstuk 1.2** betreft een uitgebreide introductie over de kliniek, beeldvorming, genetica en pathologie van FTD.

Hoofdstuk 2 omvat de klinische verschillen tussen subtypes van FTD. In **hoofdstuk 2.1** worden de klinische presentatie en perfusie patronen op SPECT scans van 10 patiënten met een *MAPT* mutatie en 19 patiënten met familiare FTLD met TDP-43 pathologie (FTLD-TDP) beschreven. Deze laatste groep bestond uit 6 patiënten met een *GRN* mutatie en 1 met een onbekend genetisch defect. Familiaire FTLD-TDP patiënten waren in vergelijking met *MAPT* significant ouder, presenteerden zich klinisch vaker met geheugenproblemen en hadden minder perfusie van de parietaal kwab, inclusief precuneus, en precentrale gyrus. *MAPT* patiënten presenteerden zich vaker met benoemproblemen en obsessief-compulsief gedrag en hadden op SPECT minder perfusie links temporaal en medio-frontaal ten opzichte van familiare FTLD-TDP patiënten. Wij suggereren dat *MAPT* en familiaire FTLD-TDP op basis van klinische presentatie en patroon van perfusie onderscheiden kunnen worden.
In **hoofdstuk 2.2** beschrijven we de verschillende overlevingsprofielen van patiënten met FTD met motorisch voorhoornlijden, in de Engelstalige literatuur bekend als motor neuron disease (MND). Er

werden twee verschillende groepen gevonden, FTD-MND patiënten met een relatief lange overleving van 66 maanden, en een groep FTD-MND patiënten met een typische overleving van gemiddeld 19 maanden. FTD-MND patiënten met een lange overleving hadden eerst cognitieve klachten en ontwikkelden relatief laat motorische symptomen, terwijl patiënten met een typische overleving gelijktijdig of met een kort interval cognitieve en motorische verschijnselen ontwikkelden. Dit suggereert dat de overleving afhangt van het tijdstip van ontstaan van de secundaire cognitieve of motorische klachten.

De erfelijke vormen van FTD worden besproken in **hoofdstuk 3**. In **hoofdstuk 3.1** worden mutaties in het *GRN* gen in ons FTD cohort beschreven. De families waren eerder gekoppeld aan chromosoom 17q21-q22, en hadden bij neuropathologisch onderzoek ubiquitine-positieve, maar tau-negatieve neuronale inclusies. Twee nieuwe frameshift mutaties en drie mogelijke pathogene missense mutaties in het *GRN* gen werden bij de familiare FTD patiënten gevonden. *GRN* mutaties verklaren een deel van de familiaire FTLD met ubiquitine-positieve inclusies.
In **hoofdstuk 3.2** worden de frequentie en klinische en pathologische kenmerken van FTD patiënten met een positieve familieanamnese beschreven. In 27 procent van alle FTD-patiënten is er een autosomaal dominant overervingspatroon, waarvan 11 procent een *MAPT* gen mutatie en zes procent een *GRN* gen mutatie had. De resterende 10 procent met een onbekend genetisch defect presenteerden zich klinisch voornamelijk met de gedragsvariant (bvFTD) van FTD of FTD-MND. Alle patiënten met een onbekend genetisch defect hadden bij neuropathologisch onderzoek ubiquitine- en TDP-43-positieve neuronale inclusies (FTLD-TDP), welke verder onder te verdelen was in patiënten met FTLD en motorisch voorhoornlijden en patiënten met FTLD-TDP en hippocampale sclerose. Hoewel beide groepen TDP-43 pathologie hebben, suggereren wij dat toekomstige genetische studies nog twee of meer genetische defecten zullen vinden in erfelijke vormen van FTLD met TDP-43 pathologie.

Hoofdstuk 4 richt zich op de neuropathologische afwijkingen bij FTD waarbij geen genetisch defect wordt gevonden. In **hoofdstuk 4.1** wordt de kliniek en pathologie van familiaire FTD met motorisch voorhoornlijden (FTD-MND) beschreven. Er is een duidelijke klinische intrafamiliaire variabiliteit, waarbij patiënten of familieleden FTD of dementie, MND of FTD-MND ontwikkelden. Bij genetisch onderzoek werd geen genetisch defect gevonden in de bij FTD en MND bekende ziekte veroorzakende genen. Hersenweefsel was beschikbaar van vijf patiënten, waar bij allen ubiquitine- en TDP-43-positieve neuronale inclusies werden gevonden en intranucleaire inclusies in twee. Verder vonden wij ubiquitine-negatieve, maar TDP-43-positieve inclusies in het striatum van één patient. (FTLD-TDP). Concluderend is er bij familiaire FTD-MND sprake van intrafamiliaire klinische variatie, met onbekend genetisch defect die in alle gevallen leidt tot TDP-43 pathologie, met een mogelijke rol van gliale cellen in de pathofysiologie van FTD-MND.
In **Hoofdstuk 4.2** beschrijven we de kliniek, beeldvorming en neuropathologische afwijkingen bij patiënten met ubiquitine- en FUS-positieve, maar TDP-43-negatieve neuronale inclusies. Elf procent van

alle patiënten met FTLD en ubiquitine-positieve inclusies hadden FUS-positieve en TDP-43-negatieve inclusies. Onze vier FTLD-FUS patiënten hadden alle vier bvFTD subtype, een negatieve familieanamnese, een jonge aanvangsleeftijd van de ziekte onder de 40 jaar in drie van de vier patiënten en ernstige caudatus atrofie op de MRI. Met deze klinische parameters hebben wij de frequentie van onderliggend FUS pathologie in ons klinisch FTD cohort op drie procent geschat. De klinische en pathologische heterogeniteit wordt verder benadrukt door FTLD-FUS pathologie, hoewel dit in de minderheid van klinische FTD patiënten de onderliggende pathologie is.

Tot slot worden in **hoofdstuk 5** slot de belangrijkste bevindingen van dit proefschrift besproken en tevens de belangrijkste recente ontwikkelingen en suggesties voor toekomstig onderzoek.

List of abbreviations

aFTLD-U	Atypical frontotemporal lobar degeneration with ubiquitin positive inclusions
ALS	Amyotrophic lateral sclerosis
ALS-D	Amyotrophic lateral sclerosis with dementia
bvFTD	Behavioural variant of FTD
CA	Cornu ammonis
CBD	Corticobasal degeneration
CBS	Corticobasal syndrome
CDR	Clinical dementia rating scale
CSF	Cerebrospinal fluid
CT	Computed tomography
DN	Dystrophic neurites
DNA	Deoxyribonucleic acid
EMG	Electromyography
FTD	Frontotemporal dementia
FTD-MND	Frontotemporal dementia with motor neuron disease
FTLD-FUS	Frontotemporal lobar degeneration with FUS positive inclusions
FTLD-tau	Frontotemporal lobar degeneration with tau positive inclusions
FTLD-TDP	Frontotemporal lobar degeneration with TDP-43 positive inclusions
FTLD-U	Frontotemporal lobar degeneration with ubiquitin positive inclusions
FUS	*fused in sarcoma*
GRN	*Progranulin*
HS	Hippocampal sclerosis
LPA	Logopenic variant of progressive aphasia
MAPT	*microtubule associated protein tau*
MMSE	Mini Mental State examination
MND	Motor neuron disease
MRI	Magnetic resonance imaging
NCI	Neuronal cytoplasmatic inclusions
NII	Neuronal intranuclear inclusions
PSP	Progressive supranuclear palsy
VCP	*Valosin containing protein*
CHMP2B	*Charged multivesicular body protein 2B*
PET	Positron emission tomography
PNFA	Progressive non-fluent aphasia
RNA	Ribonucleic acid
SD	Semantic dementia
SPECT	Single photon emission computed tomography nucleotide
SPM	Statistical parametric mapping
TDP-43	TAR DNA binding protein of 43 kDA
UGD	Unknown genetic defect
VBM	Voxel-based morphometry

About the author

Harro Seelaar was born on September 26th, 1984 in Schiedam, The Netherlands. He attended secondary school at the Stedelijk Gymnasium in Schiedam, from which he graduated in 2002. The same year he started his training in medicine at the Erasmus University Rotterdam. In February 2006 he started his research project on familial frontotemporal dementia with motor neuron disease at the Department of Neurology, Erasmus MC under supervision of Dr. J.C. van Swieten. After obtaining his doctorate in August 2006 he continued as researcher on frontotemporal dementia at the Department of Neurology. In October 2010 he started his internships to obtain his medical degree.

Dankwoord

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List of publications

Seelaar H, Papma JM, Garraux G, de Koning I, Reijs AE, Valkema R, Rozemuller JM, Salmon E, van Swieten JC . Brain SPECT perfusion patterns in familial frontotemporal lobar degeneration. *Submitted.*

Seelaar H, Rohrer JD, Pijnenburg YAL, Fox NC, van Swieten JC. Clinical, genetic and pathological heterogeneity of frontotemporal dementia: a review. *J Neurol Neurosurg Psychiatry, Epub 2010 Oct 22.*

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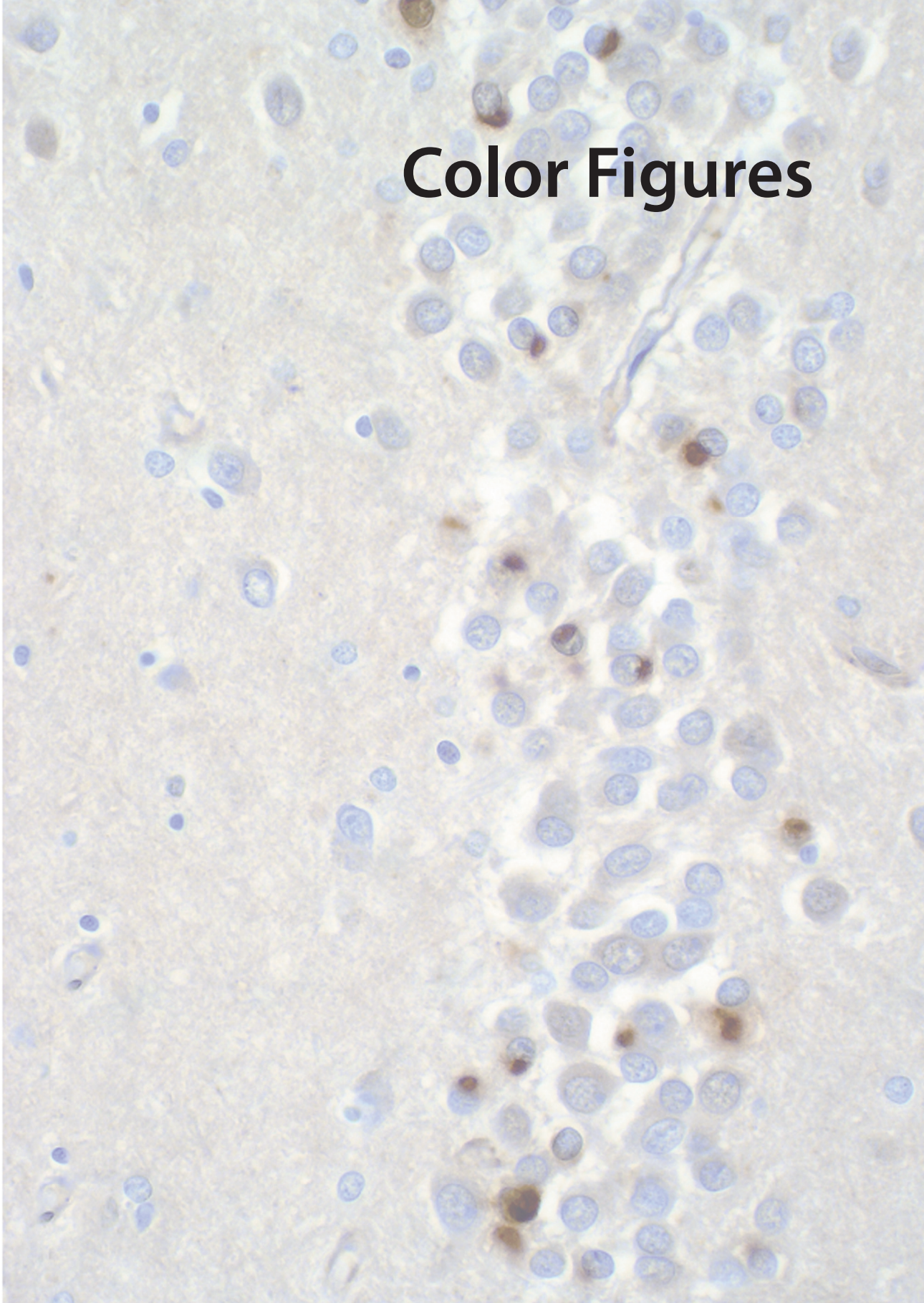
PhD Portfolio

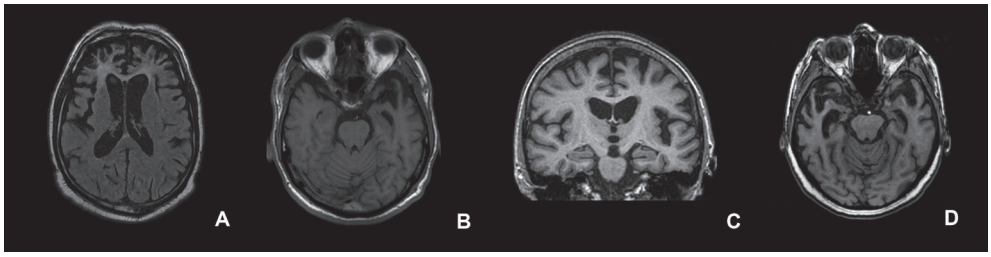
1. PhD training	Year	Workload(ECTS)
General courses		
- Classical Methods for Data-analysis	2007	5.7
- Biomedical English Writing and Communication	2009	4
In-depth courses		
- SNP course V	2008	1.4
Presentations		
- 5th International conference on Frontotemporal dementias, San Francisco, USA, Poster	2006	1
- American Academy of Neurology, Chicago, USA, Poster	2007	1
- 6th International conference on Frontotemporal Dementias, Rotterdam, The Netherlands, Oral presentation	2008	1
- American Academy of Neurology, Seattle, USA, poster		
- 7th International conference on Frontotemporal dementias, Indianapolis, USA, poster	2009	1
	2010	1
International conferences		
- 5th International conference on Frontotemporal dementias, San Francisco, USA	2006	1
- American Academy of Neurology, Chicago, USA	2007	1
- 6th International conference on Frontotemporal dementias, Rotterdam, The Netherlands	2008	1
- American Academy of Neurology, Seattle, USA	2009	1
- 7th International conference on Frontotemporal dementias, Indianapolis, USA	2010	1
Seminars and workshops		
- Workshop update on Dementia, American Academy of Neurology, Seattle, USA	2009	0.2
- Seminar: Neuropathologie belicht – Spotlight on Neurodegeneration, VU MC, Amsterdam	2010	0.2
2. Teaching activities		
Lecturing		
- Landelijke terugkomdag AIO's Ouderengeneeskunde, Utrecht	2009	1
- Frontotemporale dementie: an update, Amstelveen	2010	1
Supervising practicals and excursions		
- High school and first year medical students	2010	0.5
Supervising Master's theses		
- Five students	2006- 2010	12
Other		
- Member of the Dutch FTD expertgroup	2008-2011	1
Total		**37**

Color Figures



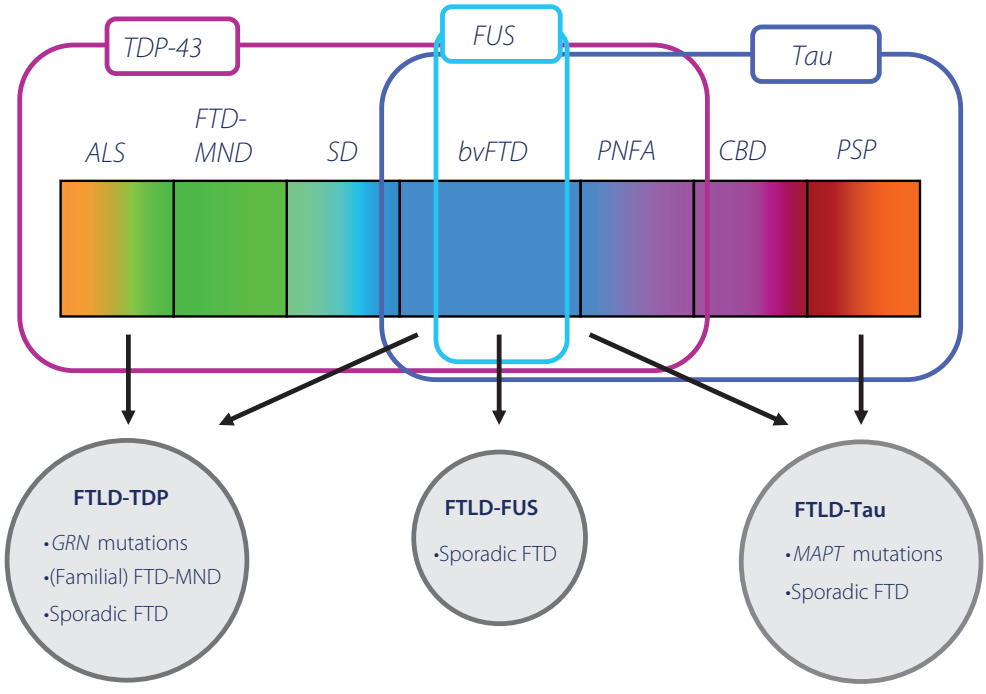
Chapter 1.2

Figure 1 Imaging of FTD subtypes



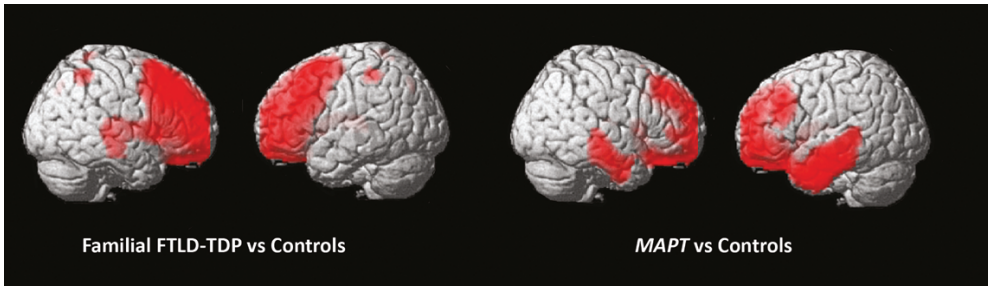
(A) Frontal atrophy on axial fluid attenuated inversion recovery MRI of a patient with behavioural variant of FTD (bvFTD). (B) Axial T1-weighted image with left temporal lobe atrophy in a patient with semantic dementia (SD). (C) Coronal T1-weighted MR image of a patient with progressive non-fluent aphasia (PNFA) and left inferior frontal and superior temporal atrophy. (D) Axial T1-weighted MR image in a patient with predominant right temporal lobe atrophy.

Figure 2 Clinical, genetic and pathological spectrum of FTLD



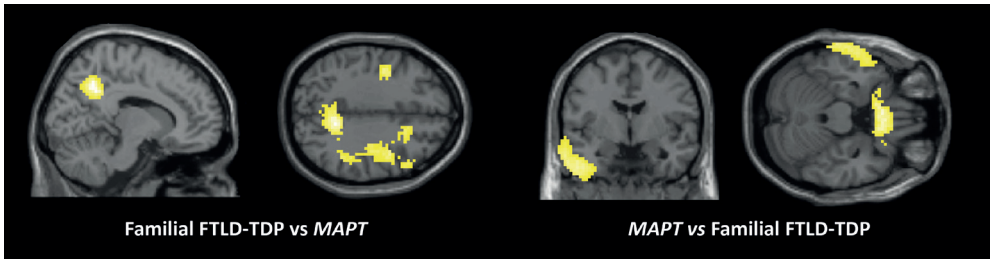
Chapter 2.1

Figure 1 Perfusion pattern of familial FTLD-TDP and MAPT compared to controls



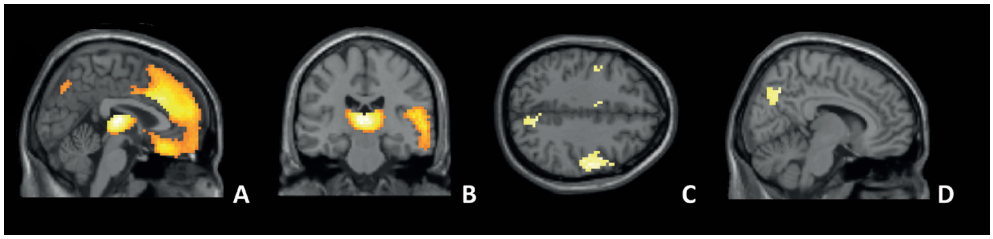
Significant relative hypoperfusion superimposed on a SPM8 canonical single subject template, with a threshold p<.001.

Figure 2 Perfusion pattern of familial FTLD-TDP compared to *MAPT*



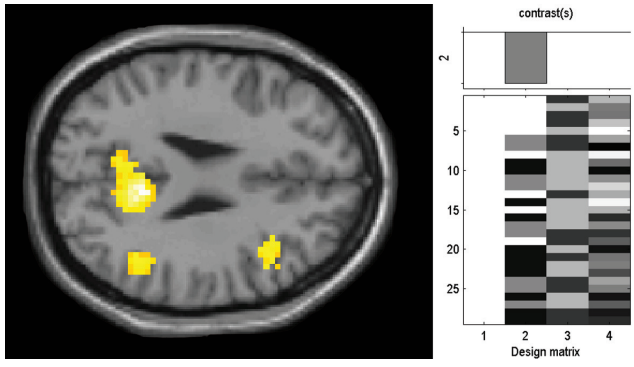
Significant relative hypoperfusion superimposed on a SPM8 canonical single subject template, with a threshold p<.001.

Figure 3 Familial FTLD-TDP UGD compared to controls and *MAPT*



Significant relative hypoperfusion superimposed on a SPM8 canonical single subject template, with a threshold p<.001. Familial FTLD-TDP UGD versus controls **(A and B)**. Familial FTLD-TDP UGD compared to *MAPT*, masked by familial FTLD-TDP UGD compared to controls **(C and D)**.

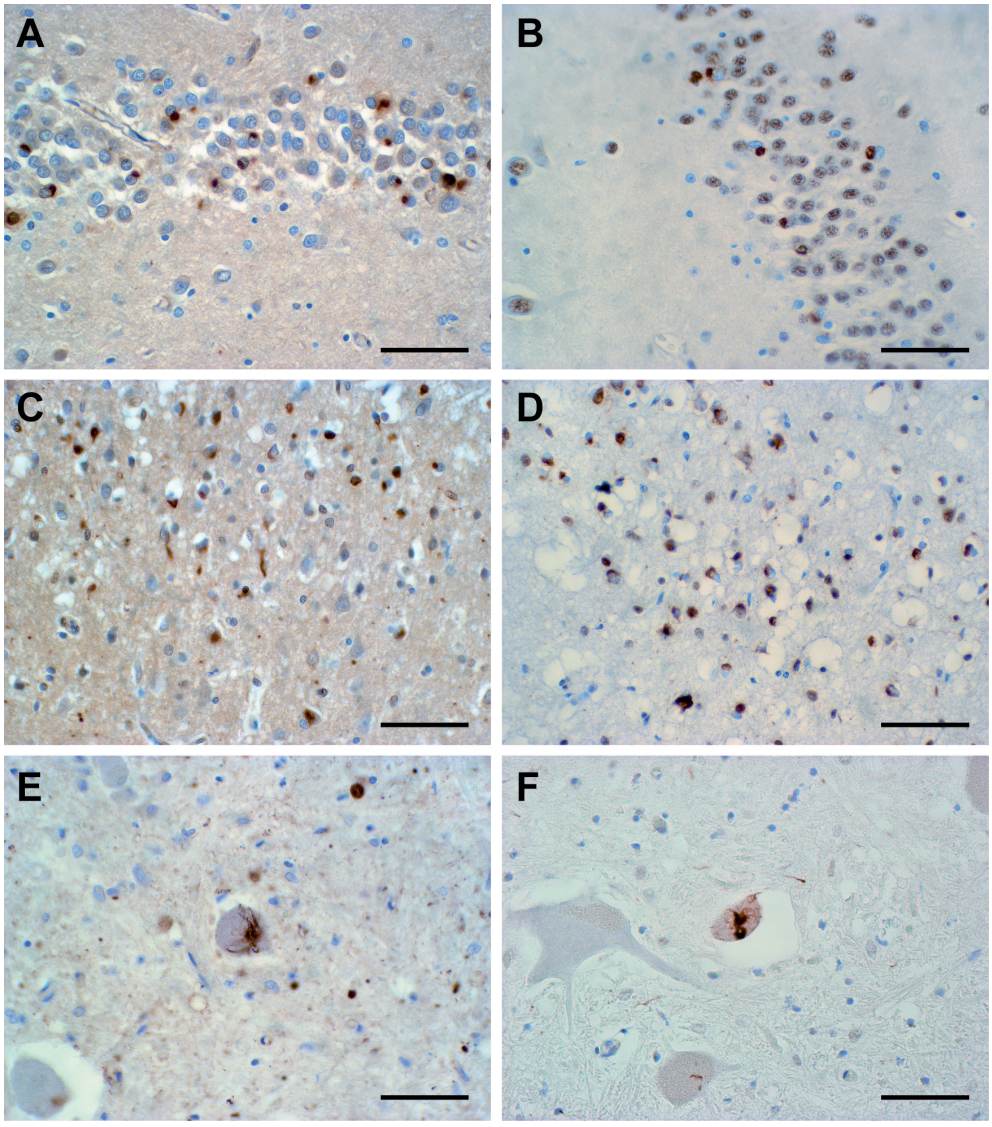
Figure 4 Correlation with memory performance in 29 FTLD patients



Correlation of memory deficits (p<.001) with perfusion levels in posterior cingulate, precuneus, right inferior parietal lobule and right middle frontal gyrus projected on a SPM8 canonical single subject template.

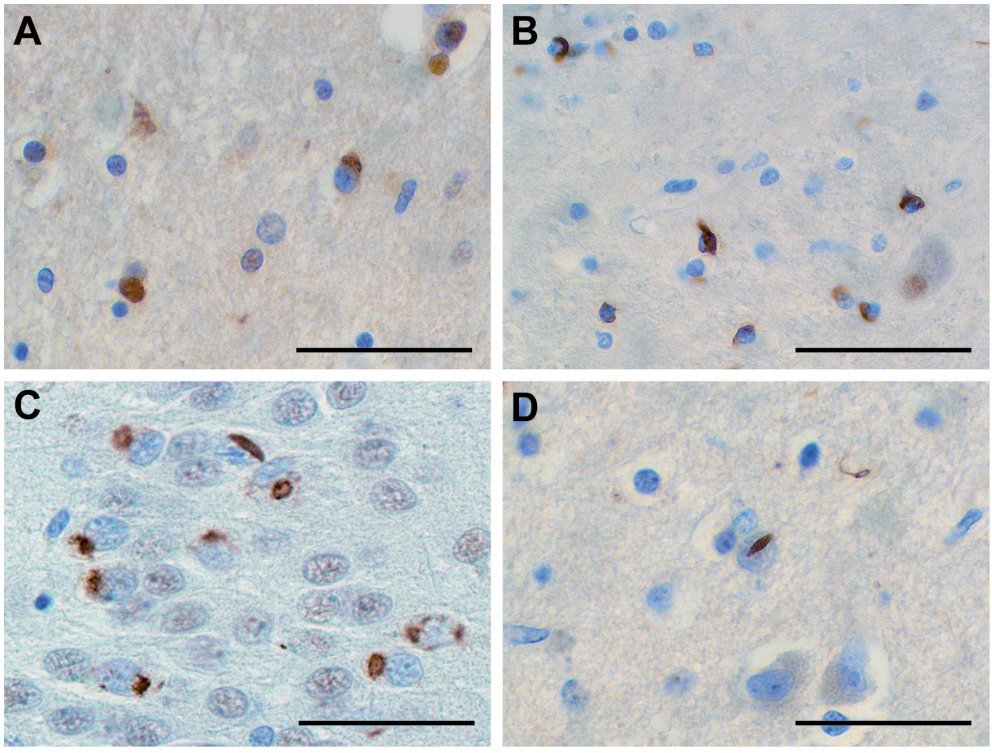
Chapter 4.1

Figure 2



Positive staining with anti-ubiquitin and TDP-43 antibody neuronal cytoplasmatic inclusions in the granular cells of the dentate gyrus were seen in the hippocampus in all cases with ubiquitin (A) and TDP-43 (B). NCI in the neocortex with ubiquitin (C) and TDP43 (D) and (skein-like) inclusions were seen in all spinal cords with both ubiquitin (E) and TDP-43 (F). Scale bar = 100 mm.

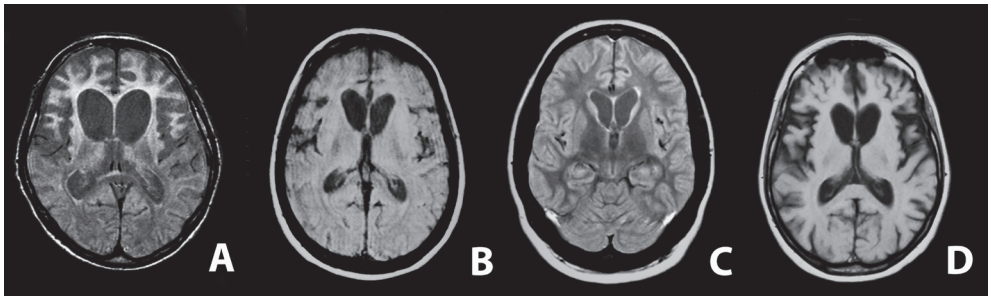
Figure 3



Ub-positive neuronal cytoplasmatic inclusions were found in the caudatus **(A)** and the glial cells stained positive with TDP-43 **(B)**. Lentiform or cat-eye shaped NII in the dentate gyrus **(C)** and neocortex (**D)** also stained positive with TDP-43. Scale bar = 100 μm.

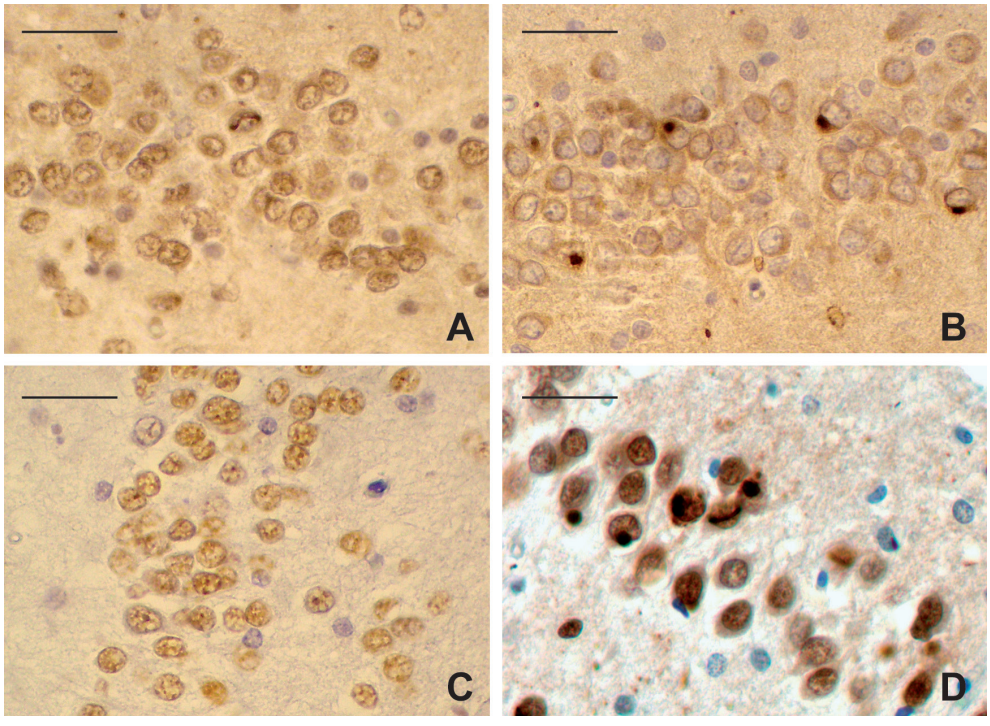
Chapter 4.2

Figure 1 MRI scans of FTLD-FUS cases



(A) Patient 1 with severe atrophy (PD-weighted MR image). **(B)** Patient 2 with severe atrophy (PD-weighted MR image). **(C)** Patient 3 with mild-moderate atrophy (FLAIR MR image). **(D)** Patient 4 with severe atrophy (T1-weighted MR image).

Figure 2 Immunohistochemistry of the FTLD-FUS cases.



Ubiquitin-positive neuronal intranuclear inclusions (NII) in the granular cells of the dentate gyrus, which are worm-like **(A)**. Only the ubiquitin-positive neuronal cytoplasmatic inclusions (NCI) stained positive with p62 **(B)**, and NII not. TDP-43-immunohistochemistry only stained normal nuclei, and did neither stain ubiquitin-positive NCI nor NII **(C)**. The NCI as well as NII stained positive with FUS antibody **(D)**. Scale bar = 50 µm