



UPPSALA
UNIVERSITET

*Digital Comprehensive Summaries of Uppsala Dissertations
from the Faculty of Medicine 418*

Molecular Mechanisms of Frontotemporal Lobar Degeneration

LENA SKOGLUND



ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2009

ISSN 1651-6206
ISBN 978-91-554-7405-8
urn:nbn:se:uu:diva-9550

Dissertation presented at Uppsala University to be publicly examined in Rudbecksalen, Rudbecklaboratoriet, Dag Hammarskjölds väg 20, Uppsala, Friday, March 6, 2009 at 09:15 for the degree of Doctor of Philosophy. The examination will be conducted in English.

Abstract

Skoglund, L. 2009. Molecular Mechanisms of Frontotemporal Lobar Degeneration. Acta Universitatis Upsaliensis. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 418. 52 pp. Uppsala. ISBN 978-91-554-7405-8.

The aim of this thesis was to identify genetic factors involved in frontotemporal lobar degeneration (FTLD), a neurodegenerative disorder clinically characterised by a progressive change in personality, behaviour and language. FTLD is a genetically complex disorder and a positive family history is found in up to 40% of the cases.

In 10-20% of the familial cases the disease can be explained by mutations in the gene encoding the microtubule associated protein tau (MAPT). In the first study we describe the clinical and neuropathological features of a Finnish family with FTLD caused by a mutation in MAPT. We also provide evidence that the pathogenic mechanism of this mutation is through altered splicing of MAPT transcripts.

Recently, mutations in the gene encoding progranulin (PGRN) were identified as a major cause of FTLD. In the second study we describe a Swedish family with FTLD caused by a frameshift mutation in PGRN. We provide a clinical and neuropathological description of the family, as well as evidence that the pathogenicity of this mutation is through nonsense-mediated decay of the mutant mRNA transcripts and PGRN haploinsufficiency.

In the third study we describe a novel PGRN splice site mutation and a previously described PGRN frameshift mutation, found in a mutation screen of 51 FTLD patients. We describe the clinical and neuropathological characteristics of the mutation carriers and demonstrate that haploinsufficiency is the pathogenic mechanism of the two mutations.

In the fourth study we investigate the prevalence of PGRN and MAPT gene dosage alterations in 39 patients with FTLD. No gene dosage alterations were identified, indicating that variations in copy number of the PGRN and MAPT genes are not a common cause of disease, at least not in this FTLD patient collection.

Keywords: Frontotemporal lobar degeneration, Frontotemporal dementia, Tau, Progranulin, Alternative splicing, Nonsense-mediated decay, Haploinsufficiency

Lena Skoglund, Department of Public Health and Caring Sciences, Uppsala Science Park, Uppsala University, SE-75183 Uppsala, Sweden

© Lena Skoglund 2009

ISSN 1651-6206

ISBN 978-91-554-7405-8

urn:nbn:se:uu:diva-9550 (<http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-9550>)

Till min familj

Supervisors:

Anna Glaser, PhD
Department of Public Health and Caring Sciences
Uppsala University
Uppsala, Sweden

Martin Ingelsson, MD, PhD
Department of Public Health and Caring Sciences
Uppsala University
Uppsala, Sweden

Lars Lannfelt, MD, PhD
Department of Public Health and Caring Sciences
Uppsala University
Uppsala, Sweden

Faculty opponent:

Stuart Pickering-Brown, PhD
Department of Medicine
University of Manchester
Manchester, United Kingdom

Examining committee:

Irina Alafuzoff, MD, PhD
Department of Neurology
University of Kuopio
Kuopio, Finland

Catharina Lavebratt, PhD
Department of Molecular Medicine and Surgery
Karolinska Institutet
Stockholm, Sweden

Tobias Sjöblom, PhD
Department of Genetics and Pathology
Uppsala University
Uppsala, Sweden

Chairman:

Lars Nilsson, PhD
Department of Public Health and Caring Sciences
Uppsala University
Uppsala, Sweden

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

- I **Skoglund L**, Viitanen M, Kalimo H, Lannfelt L, Jönhagen ME, Ingelsson M, Glaser A, Herva R. The tau S305S mutation causes frontotemporal dementia with parkinsonism. *Eur J Neurol*. 2008 Feb;15(2):156-61
- II **Skoglund L**, Brundin R, Olofsson T, Kalimo H, Ingvast S, Blom ES, Giedraitis V, Ingelsson M, Lannfelt L, Basun H, Glaser A. Frontotemporal dementia in a large Swedish family is caused by a progranulin null mutation. *Neurogenetics*. 2009 Feb;10(1):27-34
- III **Skoglund L**, Matsui T, Freeman SH, Wallin A, Blom ES, Frosch MP, Growdon JH, Hyman BT, Lannfelt L, Ingelsson M, Glaser A. Identification of a novel deletion in the splice donor site of progranulin exon 6. *Manuscript*
- IV **Skoglund L**, Ingvast S, Matsui T, Freeman SH, Frosch MP, Brundin R, Giedraitis V, Growdon JH, Hyman BT, Lannfelt L, Ingelsson M, Glaser A. No evidence of PGRN or MAPT gene dosage alterations in a collection of patients with frontotemporal lobar degeneration. *Manuscript*

Reprints were made with permission from the respective publishers.

Contents

Introduction.....	9
Genetic diseases	9
The human genome.....	9
Disease causing mutations.....	11
Inheritance of disease	12
Frontotemporal lobar degeneration	14
Background.....	14
Clinical presentation, diagnosis and treatment	14
Neuropathological features	15
Genetics of FTLD	16
Present investigations.....	25
Aims of the thesis	25
Paper I	26
Background.....	26
Methods	26
Results and discussion	26
Paper II	28
Background.....	28
Methods	28
Results and discussion	29
Paper III.....	30
Background.....	30
Methods	30
Results and discussion	31
Paper IV	32
Background.....	32
Methods	33
Results and discussion	33
Concluding remarks and future perspectives	35
Populärvetenskaplig sammanfattning	37
Acknowledgements.....	40
References.....	43

Abbreviations

3R tau	Tau protein containing three microtubule binding repeats
4R tau	Tau protein containing four microtubule binding repeats
AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
CBD	Corticobasal degeneration
cDNA	Complementary deoxyribonucleic acid
CHMP2B	Chromatin-modifying protein 2B
CNS	Central nervous system
DNA	Deoxyribonucleic acid
ESCRT-III	Endosomal sorting complex required for transport-III
FTD	Frontotemporal dementia
FTDP-17	Frontotemporal dementia and parkinsonism linked to chromosome 17
FTLD	Frontotemporal lobar degeneration
IBMPFD	Inclusion body myopathy with Paget's disease of bone and frontotemporal dementia
MAPT	Microtubule associated protein tau
MND	Motor neuron disease
mRNA	Messenger ribonucleic acid
MT	Microtubule
NMD	Nonsense-mediated decay
PGRN	Progranulin
PNFA	Progressive non-fluent aphasia
PSP	Progressive supranuclear palsy
RNA	Ribonucleic acid
SD	Semantic dementia
TDP-43	Transactivation response DNA binding protein-43
VCP	Valosin-containing protein

Introduction

Genetic diseases

Genetic factors are believed to play a role in many human diseases, but their relative importance varies. Some diseases, for example Huntington's disease, are purely genetic conditions. In other diseases the genetic contribution is more modest and disease is caused by several genetic factors, often in combination with environmental factors. Identifying the genes involved in a disease can improve our understanding of the pathogenesis and possibly lead to development of novel treatment strategies.

The aim of this thesis was to identify genetic factors involved in the dementia disorder frontotemporal lobar degeneration (FTLD). A substantial number of FTLD patients have additional family members affected by the disease, indicating an important role for genetics. Here, patients with a familial history of FTLD were investigated for genetic defects in two genes that are associated with the disease.

The human genome

The deoxyribonucleic acid (DNA) molecule contains the genetic "blueprint" needed for the development, function and reproduction of an organism (Fig. 1). DNA resides predominantly in the nucleus of cells in the human body and is organised as 23 pairs of chromosomes, including 22 pairs of autosomes (non-sex determining chromosomes) and one pair of sex chromosomes (XX or XY). DNA consists of four nucleotide bases; adenine (A), thymine (T), guanine (G) and cytosine (C), which are covalently linked as a polynucleotide chain with a deoxyribose-phosphate backbone.

The DNA sequence harbours smaller units called genes; each encoding an active ribonucleic acid (RNA) molecule or a protein. Normally, an individual has two copies of each gene, one inherited from each parent. A gene consists of coding regions, called exons, and non-coding regions between the exons, called introns. Located prior to the first exon is a promoter sequence, which regulates gene activity. In the coding regions, a triplet of DNA bases (a codon) codes for one of 20 different amino acids, which are the building blocks of a protein.

The production of a protein from DNA begins with the synthesis (transcription) of a primary RNA transcript, using the gene's DNA sequence as

template. The RNA transcript is then modified through capping at the 5' end, polyadenylation at the 3' end, and splicing leading to the removal of non-coding introns and production of messenger RNA (mRNA). The mRNA is transported through nuclear pores into the cytoplasm where the ribosome translates the mRNA sequence into a chain of amino acids that forms a protein.

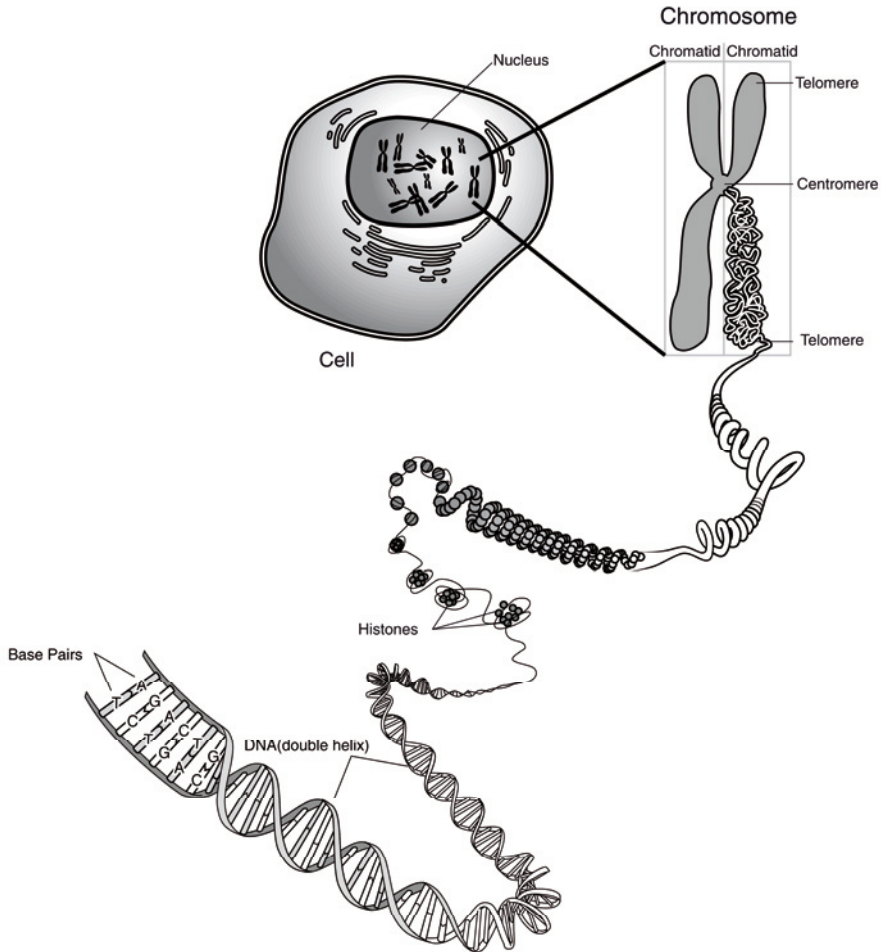


Figure 1. The DNA molecule carries the genetic information for the development, maintenance and reproduction of all cellular organisms. DNA is organised into 23 pairs of chromosomes located predominantly in the nucleus of cells in our body. *Adapted from National Human Genome Research Institute.*

The human genome is predicted to be comprised of 20-25 000 genes which may produce as many as a million different proteins¹. This protein diversity is the result of a process called alternative splicing (Fig. 2), which together with other posttranslational modifications of the protein, e.g. phosphoryla-

tion, methylation and glycosylation, generates multiple protein variants from a single gene

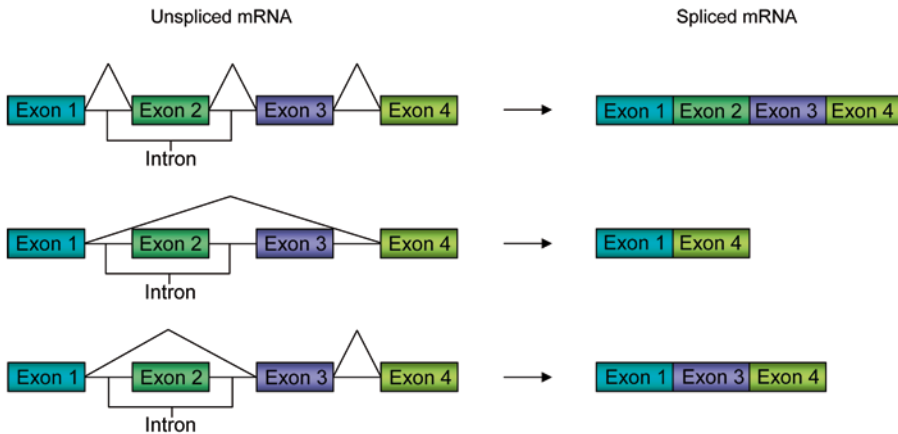


Figure 2. Alternative splicing allows several different variants of a protein to be produced from a single gene. In this process the primary transcript is spliced leading to the removal of introns, and occasionally exons, and production of transcripts containing different sets of exons which are then translated into different proteins.

Disease causing mutations

During cell division a new copy of the DNA sequence has to be produced. In this process of DNA replication, copying errors that alter the DNA sequence can occur and these changes in the DNA sequence are called mutations. Most mutations are benign and contribute to the genetic diversity, observed between and within all species. However, some mutations located within genes or in regulatory sequences of genes (e.g. the promoter) have a detrimental effect on the protein (or RNA molecule) that is encoded by the gene. Sometimes the effect is so severe that it results in disease. If such a mutation occurs in the germ cell, the disease is inherited by the offspring.

There are many types of mutations which could give rise to a genetic disorder. A single base pair substitution (also called point mutation) occurs when a single nucleotide base becomes replaced by another. Single base pair substitutions can be divided into missense, nonsense and silent mutations. In a missense mutation the base pair substitution results in a different amino acid being incorporated into the protein. A nonsense mutation leads to the introduction of a premature termination codon in the DNA sequence and a truncated protein. However, mutant mRNA transcripts containing a premature termination codon are often degraded through nonsense-mediated decay (NMD), a cellular mechanism which prevents the expression of truncated or erroneous proteins². The NMD mechanism is dependent on exon-junction complexes, deposited close to the exon-exon junctions following splicing of

pre-mRNA. These complexes are usually removed by the ribosome during protein translation. However, if a premature termination codon is located upstream of an exon-junction complex the complex will not be removed by the ribosome, triggering the degradation of the mutant mRNA transcript by NMD.

In a silent mutation the base pair substitution has no effect on the amino acid sequence as several codons may encode the same amino acid. However, if the base pair substitution is located at an exon/intron border it may alter a splice site and thus have an effect on the alternative splicing of the mRNA. This can result in a change in isoform production of the protein or the expression of an erroneous protein. Mutations leading to aberrant splicing are found in many genetic disorders and may constitute up to 50% of disease causing mutations³.

Frameshift mutations are another cause of disease which result from base pairs being added to (insertion) or removed from (deletion) the DNA sequence. Insertions and deletions result in a shift of the DNA reading frame, often introducing a premature termination codon. Disease can also be caused by the deletion of larger chromosomal regions, resulting in the loss of a number of genes; or by duplications of one or several genes. These rearrangements have an effect on the levels of protein produced. Other rearrangements in the genome (e.g. translocations and inversions) can also lead to disease.

Mutations can also be subdivided according to their effect on protein function. Loss-of-function mutations result in the mutant protein having little or no remaining normal function. Mutations causing complete loss of protein function are referred to as null mutations. Haploinsufficiency occurs when a mutation on one gene copy leads to a reduced expression of protein and the expression from the healthy gene copy is not sufficient for normal protein function. Mutations can also result in novel or enhanced protein function. These are referred to as gain-of-function mutations. A dominant negative mutation results in an altered protein with an opposite function to its normal, or to the suppression of the function of proteins that are derived from the healthy gene copy.

Inheritance of disease

Genetic disorders can be divided into monogenic or complex (multifactorial) disorders. While monogenic disorders are caused by a mutation in a single gene, complex disorders are caused by a combination of several genetic factors, often under the influence of environmental factors. Monogenic disorders are rare and most common diseases, e.g. cancer, diabetes and cardiovascular disease are complex genetic disorders.

Monogenic disorders can display a dominant or recessive mode of inheritance (Fig. 3). In a dominant disorder, bearing one mutant gene copy is suf-

ficient to develop the disease and there is a 50% risk that the mutant gene copy will be passed on to the children of an affected individual. In a recessive disorder, two defective gene copies are required to develop disease. However, a person carrying one mutant gene copy can pass the defective gene on to his/her children. If the parents are both carriers of a mutated gene, the offspring has a 25% risk of inheriting two defective gene copies and develop disease, and a 50% risk of inheriting one defective gene copy and becoming a carrier of the disease. Dominant and recessive inheritance is sometimes complicated by reduced or incomplete penetrance, where individuals fail to develop disease, even though they carry the mutant gene.

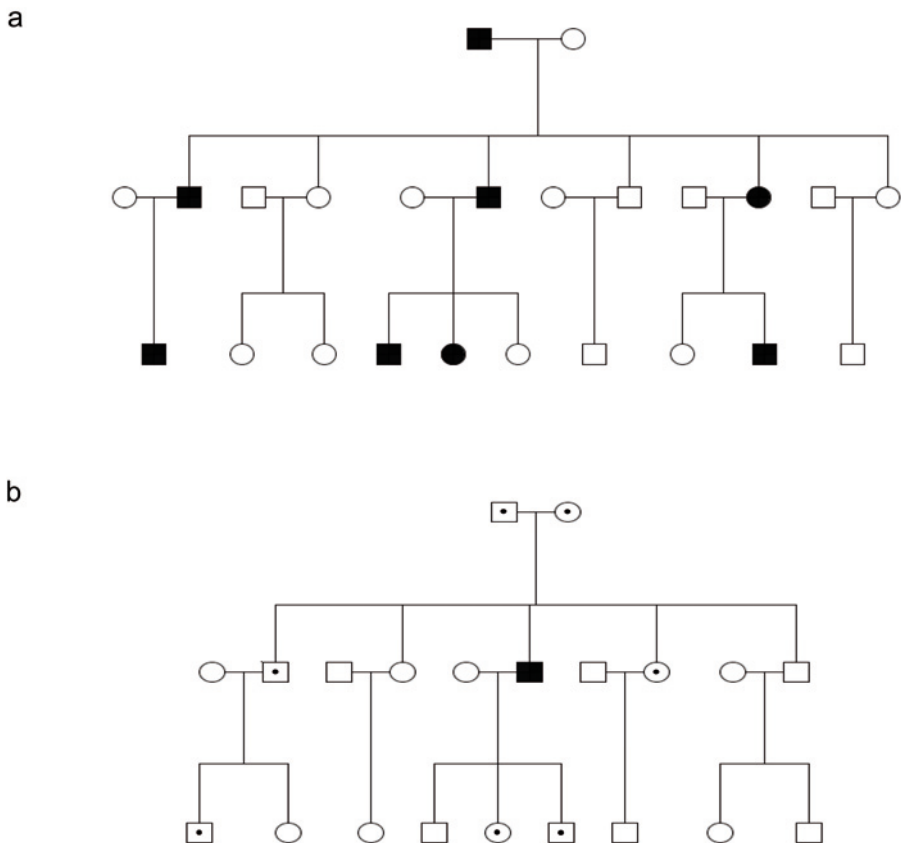


Figure 3. Monogenic disorders can display a dominant or recessive mode of inheritance. A dominant inheritance pattern is seen when one mutant gene copy is sufficient to cause the disease (a). A recessive mode of inheritance is seen when two mutant gene copies are required to develop the disease (b). Circles represent women and squares men. Affected individuals are indicated by black symbols and unaffected subjects by white symbols. Unaffected mutation carriers are indicated by white symbols with a black dot.

Frontotemporal lobar degeneration

Background

In 1892, the Czech-German neuropsychiatrist Arnold Pick described a patient presenting with a progressive loss of speech and dementia associated with severe atrophy of the frontal and temporal lobes of the brain⁴. This was the first description of what is today known as FTLD. The term FTLD refers to a heterogeneous group of neurodegenerative disorders clinically characterized by a progressive change in behaviour, personality and language, with disease onset usually in the fifth or sixth decade of life⁵. FTLD accounts for approximately 10% of all dementia cases and is, next to Alzheimer's disease (AD), the second most common cause of dementia in individuals under 65 years⁶⁻⁸.

Clinical presentation, diagnosis and treatment

FTLD can be divided into three major clinical subtypes⁵: Frontotemporal dementia (FTD), semantic dementia (SD) and progressive non-fluent aphasia (PNFA). FTD represents the most common clinical entity and is characterised by early changes in behaviour and personality including impulsive and inappropriate behaviour, emotional blunting, loss of insight, apathy, and stereotypic behaviours⁵. The change in behaviour and personality is often accompanied by language deficits in later stages of the disease. Patients with SD and PNFA present with language impairment as an initial symptom with behavioural changes appearing later in the course of the disease⁵. Patients with SD are characterised by a loss of ability to understand the meaning of words and to recognise faces, objects and other sensory stimuli, but otherwise their speech is usually fluent, effortless and grammatically correct. Patients with PNFA present with expressive language problems with effortful speech production, phonological and grammatical errors as well as severe problems with word retrieval. These patients may become mute as the disease progresses. The three FTLD subtypes may also be associated with motor dysfunction, as a proportion of the patients develop parkinsonism, including rigidity, bradykinesia and postural instability; or motor neuron disease (MND)⁵.

The clinical diagnosis of FTLD is made by a neuropsychological assessment and physical examination together with an evaluation of the patient's medical history. Structural imaging e.g. computed tomography, magnetic resonance imaging, positron emission tomography or single photon emission computerised tomography are supportive diagnostic tools and can exclude conditions that may cause the same signs and symptoms, e.g. cardiovascular conditions and tumours. Current treatment of FTLD is purely symptomatic and no medication has yet been developed that successfully slows or stops disease progression.

Neuropathological features

Brains of patients with FTLD are characterised by severe atrophy of the frontal and temporal lobes with neuronal loss, and varying degrees of microvacuolation (spongiform changes) and gliosis⁹. Atrophy of the basal ganglia is also seen in a proportion of cases. The majority of patients show bilateral symmetric involvement of frontal and temporal lobes, but patients with PNFA have a predominant left temporal lobe pathology. On the histological level FTLD can be divided into several subtypes according to the presence or absence of neuronal protein inclusions⁹. The most common underlying pathology in FTLD is characterised by intraneuronal inclusions of the transactivation response DNA binding protein-43 (TDP-43)¹⁰⁻¹². The pathological form of TDP-43 is abnormally phosphorylated and ubiquitinated¹². Cases present with variable densities of neuronal cytoplasmic inclusions (Fig. 4a) and dystrophic neurites (degenerating axonal or dendritic processes) in frontotemporal cortices, hippocampus and spinal cord. In some patients neuronal intranuclear inclusions of a “cat’s eye” or “lentiform” appearance are also detected (Fig. 4b)⁹.

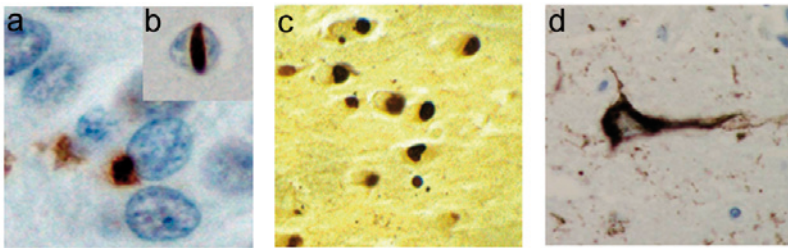


Figure 4. Histopathological subtypes of FTLD. The majority of FTLD patients are characterised by variable densities of TDP-43 positive intraneuronal cytoplasmic inclusions (a), dystrophic neurites and lentiform intraneuronal inclusions (b). The second major pathological subtype of FTLD is characterised by intraneuronal inclusions consisting of the hyperphosphorylated protein tau. Tau inclusions can either be spherical shaped Pick bodies (c) or flame-shaped neurofibrillary tangles (d). Pictures from Hannu Kalimo (a, b and d) and Martin Ingelsson (c).

The second pathological subtype of FTLD is characterised by intraneuronal protein inclusions consisting of abnormally phosphorylated forms of the microtubule associated protein tau⁹. The neuronal protein deposits are either spherical cytoplasmic inclusions (Pick bodies) or flame-shaped inclusions (neurofibrillary tangles) (Fig 4c and d). In addition, a subset of FTLD patients have a pathological picture characterised by ubiquitin-positive inclusions, negative for TDP-43 and tau⁹. Finally, a few percent of FTLD cases lack intraneuronal inclusions altogether, a picture referred to as dementia lacking distinctive histology⁹.

Genetics of FTLTLD

Approximately 30-40% of FTLTLD cases are familial and most display an autosomal dominant mode of inheritance^{6, 13, 14}. To date, four different genes have been found to be involved in the disease; the genes for the microtubule associated protein tau (*MAPT*)¹⁵⁻¹⁷, progranulin (*PGRN*)^{18, 19}, chromatin-modifying protein 2B (*CHMP2B*)²⁰ and valosin-containing protein (*VCP*)²¹. Mutations in *MAPT* and *PGRN* are the most common known causes of familial FTLTLD each representing 10-20% of the familial cases, while mutations in *CHMP2B* and *VCP* are only found in rare familial and sporadic FTLTLD cases^{19, 22-24}.

Tau

In 1994, a family with FTD was linked to a region on chromosome 17q21²⁵. Over the following years several families with linkage to chromosome 17 were identified. While the clinical presentation in these families was variable, the term frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) was introduced to best describe this subtype of FTLTLD²⁶. Neuropathologically, the FTDP-17 cases were characterised by the presence of tau inclusions in affected brain regions, making the *MAPT* gene located on chromosome 17 a candidate gene for the disease. In 1998, several mutations in the *MAPT* gene were identified in families with FTDP-17¹⁵⁻¹⁷.

The tau protein

Tau is a member of the microtubule associated protein family. It is an abundant protein in the central nervous system (CNS), where it is mainly located in axons of growing and mature neurons²⁷. The main function of tau is to promote microtubule (MT) assembly and maintain MT stability, which is essential for correct neuronal outgrowth, neuronal morphology and axonal transport of vesicles and proteins²⁸⁻³¹.

Tau protein expression is regulated by alternative mRNA splicing, generating six different isoforms of tau in the adult human brain (Fig. 5)³². These six isoforms differ in the presence or absence of two N-terminal regions encoded by exons 2 and 3, and a C-terminal region encoded by exon 10. The interaction of tau with the MTs is mediated through C-terminal repeat regions encoded by exons 9-12³³. Isoforms containing exon 10 have four MT binding repeat regions (4R tau), while isoforms lacking exon 10 contain three MT binding repeat regions (3R tau). The N-terminal part of the protein harbours the projection domain, which interacts with the plasma membrane and determines spacing between MTs in neurons^{34, 35}.

Phosphorylation is the major posttranslational modification of the tau protein. Tau phosphorylation is developmentally regulated with a higher degree of tau phosphorylation in foetal brain compared with adult brain³⁶. Increased phosphorylation reduces the binding of tau to the MTs and an ele-

vated level of phosphorylation in foetal tau is believed to correlate with the need of more dynamic MTs in the developing brain^{36,37}.

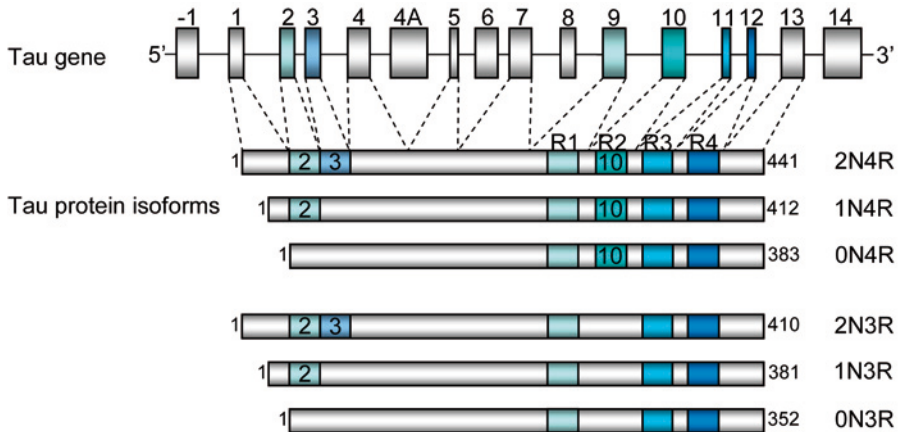


Figure 5. Schematic representation of the six tau isoforms expressed in the adult human brain. The six isoforms are produced by alternative splicing, leading to tau proteins with zero, one or two inserts in the N-terminal region and three or four microtubule binding repeats in the C-terminal region (R1-R2). N designates the number of N-terminal repeats and R the number of C-terminal microtubule binding repeats.

MAPT mutations

To date, over 40 *MAPT* mutations have been identified in more than 100 families worldwide (Fig. 6)³⁸. Most mutations are located in or near the MT binding repeat regions in exons 9-13, except for two mutations found in exon 1. Most mutations located in exons 1, 9, 11, 12 and 13 have been shown to decrease the ability of tau to bind to the MTs and promote MT assembly *in vitro*³⁸. This decreased binding of tau is believed to lead to a destabilisation of the MTs. With the dissociation of tau from the MTs it also becomes hyperphosphorylated, further reducing its ability to interact with the MTs³⁷. The decreased binding of tau to the MTs increases the pool of unbound protein, which may initiate aggregation of tau and the formation of intracellular deposits. Many of the mutations also increase the ability of tau proteins to self-aggregate³⁸.

Some mutations located in exon 10 and the mutations in the intron following exon 10 influence the alternative splicing of this exon. This shifts the ratio of 3R tau to 4R tau most often increasing the amount of 4R tau³⁸. In normal adult brain the amount of 3R tau and 4R tau is equal. However, in foetal brain only the shortest 3R tau isoform is expressed³⁹. It has been shown that 4R tau binds to and stabilises MTs more efficiently than 3R tau⁴⁰⁻⁴². The developmental regulation of 3R tau and 4R tau isoforms is therefore believed to correlate to the need for a more flexible MT network in the de-

veloping brain and a more stable network in adult brain. The effect of the splicing mutations causing overproduction of 4R tau could therefore result in an overly stabilised and rigid MT network, eventually leading to neuronal dysfunction.

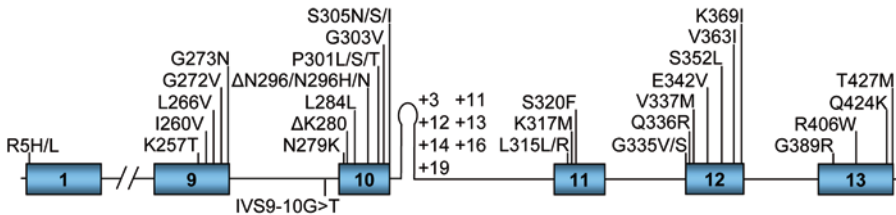


Figure 6. Mutations in the *MAPT* gene identified in patients with FTDP-17. Most mutations located in exons 1, 9, 11, 12 and 13 decrease the ability of tau to bind to the MTs and many also enhance the ability of tau to self-aggregate. Some mutations located in exon 10 and in the intron following exon 10 shift the ratio of 3R tau to 4R tau, most often resulting in increased production of 4R tau.

Clinical and neuropathological features

Significant variability in the clinical and pathological presentation has been described for *MAPT* mutation carriers, not only between different families with the same mutation, but also between patients within the same family. Several *MAPT* mutations result in clinical presentations from FTD to progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD), two other neurodegenerative disorders characterised by tau pathology⁴³⁻⁴⁹. As the substantia nigra is severely affected in PSP and CBD, motor deficits are a predominant clinical feature. However, degeneration of the frontotemporal cortices may also result in symptoms of FTLD. Consequently, a significant clinical and neuropathological overlap is seen for patients with FTDP-17, PSP and CBD⁵⁰. The high variability in clinical presentation emphasises the involvement of additional unidentified genetic or environmental factors in the disease.

Neuropathologically, FTDP-17 patients typically show frontotemporal atrophy with neuronal loss, gliosis and microvacuolation³⁸. Patients with associated parkinsonism also often show destruction of the basal ganglia and substantia nigra. All neuropathologically examined *MAPT* mutation cases display neuronal inclusions of hyperphosphorylated tau, and in some cases also glial tau inclusions. The morphology, isoform composition and distribution of tau deposits are highly variable³⁸. Mutations located outside exon 10 most often lead to a predominant neuronal tau pathology and inclusions are generally composed of equal amounts of 3R tau and 4R tau. Mutations affecting splicing of exon 10 lead to widespread neuronal and glial pathology. Similarly, mutations in exon 10 which do not affect splicing generally result in both neuronal and glial tau pathology. The tau inclusions contain pre-

dominantly or exclusively 4R tau isoforms, which is in agreement with mutations in exon 10 and the intron following exon 10 only affecting 4R tau.

Pathogenic mechanism of MAPT mutations

The pathogenic mechanism by which *MAPT* mutations lead to neuronal cell death is still unclear. It has been proposed that *MAPT* mutations lead to neuronal dysfunction due to an inability to properly regulate MT dynamics. According to this theory, correct regulation of MT dynamics and stability is essential for optimal function. Many *MAPT* mutations have been found to decrease the binding of tau to the MTs³⁸, most likely resulting in MT destabilisation. This is supported by studies on cultured cells where expression of different tau mutants lead to greater instability of MTs⁵¹⁻⁵⁴.

Changes in the balance of 3R tau and 4R tau may also affect MT dynamics and lead to neuronal dysfunction. The *MAPT* mutations resulting in overproduction of 4R tau are predicted to lead to an overly stabilised MT network, which is supported by cell culture studies^{41, 42}. A change in MT dynamics could have a detrimental effect on axonal transport. Over-expression of 4R tau in mice results in axonal damage and degeneration, and axonal transport has been found to be impaired in transgenic models over-expressing both wild-type or mutant tau⁵⁵⁻⁵⁹. However, conflicting reports from studies on cultured cells showed no effect on axonal transport^{60, 61}.

The second pathological possibility of *MAPT* mutations is a gain-of-toxic-function, caused by aggregation and deposition of tau protein. Recent findings confirm that tau aggregation is toxic to cells⁶²⁻⁶⁴. In cell models, inhibition of tau aggregation and elimination of tau deposits prevents tau toxicity⁶². In support of a dysfunction in MT dynamics as the cause of neurodegeneration, it has been shown in transgenic models expressing wild-type or mutant tau that tau-mediated neurodegeneration may precede or occur independently of tau deposition⁶⁵⁻⁶⁷. However, the two proposed mechanisms for the pathogenesis of *MAPT* mutations are not necessarily exclusive, and both likely contribute to neurodegeneration.

Progranulin

At the same time as mutations in the *MAPT* gene were found as a cause for FTDP-17, a number of families with conclusive linkage to the same region on chromosome 17q21 were identified⁶⁸⁻⁷⁵. However, despite extensive analysis of *MAPT*, no mutations could be identified^{74, 76, 77}. Neuropathological examination of these patients revealed intraneuronal inclusions which were negative for tau, but positive for another protein, ubiquitin⁷²⁻⁷⁵. These findings suggested another cause of disease not related to tau dysfunction. In 2006, mutations in *PGRN*, a gene located 1.7 Mb centromeric of *MAPT* on chromosome 17, were found in affected members from these families^{18, 19}. To date, over 60 *PGRN* mutations have been described in FTLD families worldwide⁷⁸.

PGRN protein

The *PGRN* gene encodes a 593 amino acid protein which is cleaved to form a family of cysteine-rich granulin peptides⁷⁹. The PGRN protein is expressed in a wide variety of tissues, particularly in epithelial and hematopoietic cells⁸⁰. In the CNS, progranulin is expressed in pyramidal neurons of the cerebral cortex and hippocampus, and in Purkinje cells of the cerebellum⁸⁰. PGRN is involved in multiple processes including development, wound healing and inflammation⁸¹. In addition, high expression of PGRN has been associated with increased tumour growth⁸¹. Little is known about the normal function of PGRN in the CNS. However, a recent study showed that PGRN promotes neuronal survival and stimulates axonal outgrowth in cultured neurons, indicating that PGRN may serve as a neurotrophic factor involved in the development of the nervous system⁸². Moreover, PGRN has been implicated in the sexual determination of the embryonic brain⁸³. PGRN has also been suggested to play a role in neuroinflammation and microglial activation. PGRN expression is up-regulated in a number of inflammatory neurodegenerative disorders associated with microglial activation, including amyotrophic lateral sclerosis (ALS), Creutzfeldt-Jakob disease and AD^{18, 84-86}. The brains of patients with *PGRN* mutations stain intensely for PGRN in activated microglia cells¹⁸.

The PGRN mutations

The majority of *PGRN* mutations identified in FTLD are frameshift, non-sense or splice site mutations that lead to the introduction of premature termination codons in the mRNA sequence (Fig. 7)⁷⁸. It has been shown that the mutant mRNA is degraded through NMD^{18, 19}. Loss-of-function leading to haploinsufficiency has therefore been suggested as the pathogenic mechanism of these mutations, which is further supported by the recent identification of *PGRN* locus deletions in patients with FTLD^{87, 88}. A few *PGRN* mutations have also been described that impair PGRN function due to mechanisms other than NMD. The *PGRN* IVS0+3A>T and IVS0+5G>C mutations are predicted to lead to retention of intron 0, leading to nuclear degradation of the unspliced transcripts^{19, 89}. In addition, several mutations affecting the Kozak sequence surrounding the translation initiation codon have been reported, which may result in loss of translation of the mutant transcripts^{18, 19, 22, 90}. Finally, a missense mutation, Ala9Asp, has been identified in the signal peptide-coding sequence of *PGRN*. This mutation has no effect on PGRN mRNA levels but leads to the entrapment of mutant PGRN protein within the Golgi apparatus, thereby reducing the level of secreted protein^{22, 86, 91}. Besides the Ala9Asp mutation, a number of additional missense mutations in *PGRN* have been identified. However, for the majority of these mutations the pathogenic nature remains unclear. It is possible that missense mutations affecting highly conserved regions of the protein could cause disease due to

partial loss of PGRN function without completely abolishing the mutant allele. Cellular studies of two of these missense mutations also indicated reduced secretion of mutant PGRN⁹².

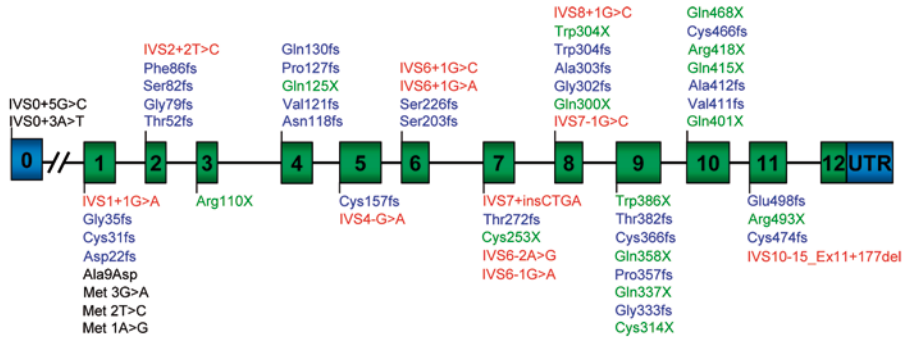


Figure 7. Mutations in the *PGRN* gene causing FTLD. Most *PGRN* mutations are frameshift, nonsense or splice site mutations that introduce premature termination codons in the mRNA sequence, resulting in degradation of mutant mRNA by nonsense-mediated decay.

Clinical and neuropathological features

Significant heterogeneity in the clinical presentation has been described for *PGRN* mutation carriers. However, the most common clinical diagnoses are FTD, PNFA or corticobasal syndrome, a clinical syndrome characterised by cognitive deficits and motor dysfunction^{22, 24, 89, 90}. A highly variable age-at-onset among *PGRN* mutation carriers has also been described and reduced penetrance is found in some *PGRN* mutation families, suggesting that the *PGRN* disease pathway is highly susceptible to other genetic or environmental factors^{19, 22}. In agreement with this, possession of the A allele of the common *PGRN* variant rs9897526 has been found to delay mean age at onset in an association study of 192 FTLD patients²⁴.

Neuropathologically, the *PGRN* mutation carriers are characterised by neuronal loss and gliosis in the frontal and temporal cortex, with variable involvement of the basal ganglia^{18, 19, 93}. The major protein constituent of the ubiquitin-positive inclusions found in the brains of *PGRN* mutation carriers was recently identified as TDP-43¹². The *PGRN* mutation carriers develop a characteristic TDP-43 pathology with an abundance of dystrophic neurites and neuronal cytoplasmic inclusions in affected brain regions (Fig. 4a). The presence of lentiform shaped neuronal intranuclear inclusions is another consistent feature of *PGRN* mutations carriers (Fig. 4b)^{12, 18, 19, 93}.

PGRN as a risk factor for FTLD

Recently a common genetic variant, rs5848 located in the 3'-untranslated region of *PGRN*, has been identified as a susceptibility factor for FTLD associated with TDP-43 pathology⁹⁴. Carriers homozygous for the T-allele of

rs5848 have a 3.2-fold higher risk of developing disease compared with homozygous C-allele carriers. The rs5848 variant is located in a binding-site for micro-RNA miR-659. Micro-RNAs play an important role in gene regulation by binding to partially complementary sequences in the 3'-untranslated region of mRNA transcripts and thereby inhibiting translation. Cell experiments suggest that micro-RNA miR-659 binds more efficiently to the risk T-allele resulting in translational inhibition of PGRN⁹⁴. Gene expression analysis of PGRN in brain extracts confirmed a 30% decrease in PGRN protein levels in the TT carriers when compared to CC carriers.

Pathogenic mechanism of PGRN mutations

The mechanism by which haploinsufficiency of PGRN leads to neurodegeneration in FTLTLD remains unclear. PGRN may function as a neurotrophic factor and is speculated to play a role in microglial activation and neuroinflammation^{18, 82, 84-86}. A loss of functional PGRN could therefore affect neuronal maintenance, neuronal survival and glial inflammatory processes. How mutations in *PGRN* lead to the accumulation of pathological TDP-43 also needs to be clarified. A recent study has shown that suppression of PGRN expression results in caspase-dependent cleavage of TDP-43 and accumulation of TDP-43 fragments in the cytoplasm of cultured cells⁹⁵. This agrees with the pathology found in *PGRN* mutation carriers, where TDP-43 is cleaved to form C-terminal fragments that accumulate in the cytoplasm¹².

Chromatin-modifying protein 2B

In 2005, a mutation in the *CHMP2B* gene was found in a large FTD family from Denmark²⁰. The *CHMP2B* gene is located on chromosome 3q13 and encodes a protein component of the endosomal sorting complex required for transport-III (ESCRT-III)⁹⁶. The ESCRT-III complex is important for sorting of endocytosed transmembrane proteins into multivesicular bodies, late endosomal structures destined for lysosomal degradation.

The Danish *CHMP2B* mutation is located at the splice acceptor site of exon 6 and results in the production of two aberrant transcripts, translated to C-truncated CHMP2B proteins. In addition to the Danish *CHMP2B* mutation, a nonsense mutation in exon 5, also resulting in a C-truncated protein, has been described in a familial FTLTLD patient from Belgium⁹⁷. Three additional *CHMP2B* missense mutations have been described, but the pathogenic nature of these mutations remains unclear^{20, 98}.

Over-expression of truncated *CHMP2B* mutants in cell culture leads to accumulation of mutant protein on abnormal endosomal structures^{20, 97}. However, it is still unclear how the *CHMP2B* mutations lead to neurodegeneration. Impairment in autophagy-mediated clearance of proteins has been suggested as a potential disease mechanism⁹⁹. A recent cell culture study showed that depletion of ESCRT subunits or expression of CHMP2B mutants inhibit autophagic protein degradation, leading to accumulation of

ubiquitin- and p62-positive protein aggregates⁹⁹. This agrees with the neuropathological picture in the *CHMP2B* mutation carriers from the Danish family. These brains display ubiquitin- and p62-positive, but TDP-43 negative, neuronal cytoplasmic inclusions in the hippocampus and frontotemporal cortices¹⁰⁰. Thus, impaired protein degradation resulting in accumulation of abnormal proteins could be the pathogenic mechanism of *CHMP2B* mutations, ultimately leading to neurodegeneration.

FTD-MND

There is increasing recognition of a clinical, neuropathological and genetic overlap between FTLD and ALS, a MND characterised by progressive muscle weakness, muscle wasting and spasticity. Approximately 15% of the FTLD cases display symptoms of MND, and this FTLD subtype is referred to as FTD-MND^{101, 102}. In addition to frontotemporal cortical atrophy, FTD-MND patients display degeneration of the brain stem and spinal motor neurons. Histologically, these patients are characterised by TDP-43 pathology, predominantly as neuronal cytoplasmic inclusions^{103, 104} (Fig. 4a). TDP-43 pathology has also been found to be a consistent feature in a significant number of ALS patients with no signs of dementia¹². Recently, several mutations in the *TDP-43* gene were described in patients with ALS^{105, 106}. So far, no *TDP-43* mutations have been identified in patients with FTLD. However, a common genetic mechanism of FTLD and ALS is suggested by the linkage of several families with familial FTD-MND to regions on chromosome 9p and 9q¹⁰⁷⁻¹¹¹. Once the responsible genes are identified, they will add yet another vital clue toward an understanding of the pathogenic mechanisms causing FTLD and ALS.

Valosin-containing protein

In 2004, mutations in the *VCP* gene located on chromosome 9p13 were identified in patients with the multisystem disorder, inclusion body myopathy with Paget's disease of bone and frontotemporal dementia (IBMPFD)²¹. As the name implies these patients develop a complex syndrome including FTD. Patients with *VCP* mutations display TDP-43 positive pathology characterised by numerous neuronal intranuclear inclusions (Fig. 4b) and dystrophic neurites in affected cortical regions¹¹². The VCP protein is involved in various cellular activities, including cell cycle control, membrane fusion and degradation of misfolded proteins by the endoplasmic reticulum-associated pathway¹¹³. Studies on cell culture and mouse models have suggested that *VCP* mutants lead to neurodegeneration through impaired clearance and degradation of protein aggregates¹¹⁴⁻¹¹⁶.

TDP-43 in FTLD

The discovery of pathological TDP-43 in a majority of patients with FTLD and ALS suggests a central role for this protein in neurodegeneration. Very little is known about the biological function of TDP-43. However, it has been implicated in regulation of alternative splicing and transcription^{117, 118}. TDP-43 is widely expressed in a number of tissues including the brain, where it is concentrated in the nuclei of neurons¹². In TDP-43 positive FTLD cases, pathological forms of TDP-43 are ubiquitinated, phosphorylated and cleaved to produce C-terminal fragments¹². The C-terminal fragments form cytoplasmic aggregates in the affected neurons resulting in reduced nuclear levels of TDP-43. The abnormal metabolism of TDP-43 is most likely central to the pathogenesis. This is supported by findings in a yeast model over-expressing TDP-43¹¹⁹. This model was able to recapitulate key features of TDP-43 pathology, including mislocalisation of TDP-43 and the formation of cytoplasmic aggregates and provides evidence of cellular toxicity caused by TDP-43 aggregation.

The mechanism by which TDP-43 pathology results in cellular toxicity remains unclear. Neurodegeneration may result from a loss of nuclear TDP-43 function, due to the mislocalisation of TDP-43 to the cytoplasm. This is supported by a recent publication where depletion of TDP-43 in human cells resulted in abnormal nuclear morphology, misregulation of the cell cycle and increased apoptosis¹²⁰. Neurodegeneration could also be caused by the accumulation of toxic TDP-43 in the cytoplasm, similar to the formation of tau aggregates in FTDP-17.

Investigation of the *TDP-43* mutations found in ALS patients may shed some light upon the pathogenic mechanism of TDP-43. Many of the identified *TDP-43* mutations are clustered in the highly conserved C-terminal glycine-rich domain of the protein, known to be important in regulation of alternative splicing and transcriptional repression^{105, 106}. The *TDP-43* mutations are therefore suggested to have detrimental effects on the normal function of TDP-43 in regulation of gene expression.

Present investigations

Aims of the thesis

The overall aim of this thesis was to investigate the genetic cause of disease in familial cases with FTL D. The specific aim for each paper was:

Paper I

To investigate if the disease in a Finnish family with FTD and parkinsonism is caused by a mutation in the *MAPT* gene.

Paper II

To determine if the disease in a Swedish FTD family is caused by a mutation in the *PGRN* gene.

Paper III

To examine the genetic contribution of *PGRN* mutations in patients with FTL D.

Paper IV

To estimate the prevalence of *PGRN* and *MAPT* gene dosage alterations in patients with FTL D.

Paper I

Genetic study of the MAPT gene in a Finnish family with FTD

Background

In paper I we wanted to investigate the genetic cause of disease in a Finnish family with FTD. The family consisted of eleven siblings of whom three brothers were affected by the disease. Both parents died in their fifties before onset of disease. The most prominent clinical features in the family were early signs of personality change, speech disturbance and cognitive decline followed by parkinsonism. Neuropathological examination of one affected family member revealed cortical atrophy with neuronal loss especially in the frontal and temporal lobes. Tau positive inclusions were observed in neurons and glial cells in the frontal and temporal cortices as well as in the brain stem. The clinical picture and the neuropathological findings of tau inclusions suggested a mutation in the *MAPT* gene as the cause of disease in this family, accordingly we decided to perform a *MAPT* sequence analysis.

Methods

Three affected brothers and one unaffected sister were investigated for mutations in the *MAPT* gene by DNA sequencing. To biochemically assess soluble and insoluble (deposited) tau isoforms in one affected family member, frontal lobe brain tissue was homogenised in a low-salt buffer and centrifuged to remove cell debris. The remaining supernatant was incubated with sarkosyl-detergent and ultracentrifuged, resulting in detergent-soluble tau in the supernatant and detergent-insoluble tau in the pellet¹²¹. The soluble and insoluble tau fractions were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and detected by western blot using a tau antibody which recognises all six isoforms of tau.

Results and discussion

Sequencing of *MAPT* revealed a silent mutation, S305S, located in the last codon of exon 10 for all three affected brothers (Fig. 8). Since this mutation is located close to the splice donor site of exon 10 it is likely to affect the splicing of this exon by destabilisation of a stem loop structure and thereby changing the ratio of 3R tau to 4R tau¹²². An exon-trapping experiment of this mutation has previously shown increased production of 4R tau compared with wild-type constructs^{46, 122}. We biochemically analysed tau from frontal cortex tissue of one of the affected cases of the Finnish FTD family to investigate the ratio of 3R tau to 4R tau. As predicted, we found an increase of 4R tau in the detergent-insoluble fraction, supporting the theory that this mutation increases production and deposition of 4R tau.

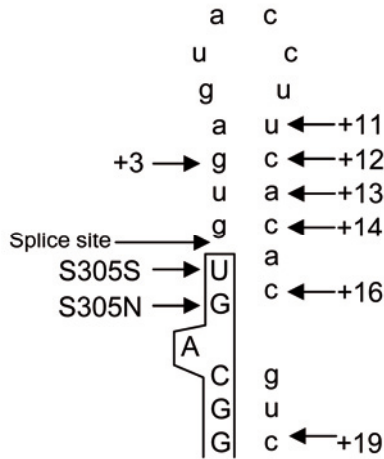


Figure 8. Schematic representation of the stem loop structure located at the junction of exon 10 and its flanking intron. The *MAPT* S305S mutation is located in the last codon of exon 10.

The *MAPT* S305S mutation has previously been described in an Australian family with a predominant PSP phenotype⁴⁶. As mentioned, a clinical and neuropathological overlap is seen for patients with PSP and FTDP-17⁵⁰. However, patients with PSP have some clinical features rarely seen in FTDP-17, e.g. supranuclear vertical gaze palsy, dysarthria and frequent falls¹²³. These clinical features were all described in the Australian S305S-family⁴⁶. Neuropathologically, the frontal and temporal lobes of PSP patients are usually mildly affected, but there is a marked atrophy of the basal ganglia and brain stem¹²³. Another common PSP feature, which is rare in patients with FTDP-17, is the glial tau pathology of tufted astrocytes, observed in the Australian S305S-family^{46, 124}.

A third family with the *MAPT* S305S mutation has also been described¹²⁵. This family presented with behavioural changes without parkinsonism. Variability in the clinical and pathological presentation has been described previously for a number of *MAPT* mutations, with variation not only between different families with the same mutation, but also between patients within the same family^{43-45, 47-49}. The different phenotypes described in all three families with the *MAPT* S305S mutation indicate that other genetic factors or environmental factors could influence the disease phenotype in FTDP-17.

The identification of *MAPT* mutations affecting splicing of exon 10 shows that an alteration in the ratio of 3R tau to 4R tau is sufficient to cause neurodegeneration. However, the exact mechanism for this is at present unclear. A disturbance in the regulation of MT dynamics has emerged as a possible explanation for the pathogenicity of *MAPT* mutations. Most mutations located in the coding regions of the *MAPT* gene lead to a reduced bind-

ing of tau to the MTs resulting in a destabilised MT network³⁸. The *MAPT* mutations affecting the splicing of exon 10 may also affect MT dynamics leading to overly stabilised MTs³⁸. More studies are needed to elucidate the pathogenic mechanism of *MAPT* splicing mutations. Understanding the molecular mechanisms by which an altered ratio of 3R tau to 4R tau leads to disease will provide new insights into the mechanisms of tau-related FTL and may help to identify therapeutic targets for treatment.

Paper II

Genetic study of the PGRN gene in a Swedish family with FTD

Background

In 1997, we described a Swedish family with a rapidly progressive FTD with linkage to chromosome 17q21^{69, 126}. In 1998, mutations in the *MAPT* gene, located on chromosome 17q21, were described in families with FTDP-17¹⁵⁻¹⁷. However, despite a thorough investigation no *MAPT* mutation could be identified in the Swedish family⁷⁶. Several additional families with FTL were around the same time found to be linked to the chromosome 17q21-region and also lacked pathogenic mutations in the *MAPT* gene^{68, 70-75}. In 2006, mutations in the *PGRN* gene were found in affected individuals in some of these families^{18, 19}, therefore we wanted to investigate if the disease in the Swedish FTD-family was caused by a mutation in the same gene.

Methods

Twenty-one members of the Swedish FTD family, including seven affected individuals were screened for *PGRN* mutations by DNA sequencing. To investigate the effect of the identified *PGRN* mutation on *PGRN* mRNA levels, RNA was extracted from lymphoblast cell lines established from two mutation carriers and two unaffected family members, and complementary DNA (cDNA) was synthesised. The cDNA was amplified using *PGRN* specific primers in a quantitative PCR based on SYBR green chemistry¹²⁷. The SYBR green dye binds non-specifically to double-stranded DNA, resulting in a fluorescent signal. Therefore, an increase in PCR product, during amplification, is detected as an increase in fluorescence, allowing DNA levels to be quantified.

Sequence and PCR fragment size analyses were also performed on lymphocyte cDNA to determine the relative levels of wild-type and mutant mRNA alleles. To confirm that degradation of mutant *PGRN* mRNA was due to NMD, lymphocyte cell lines were treated with cycloheximide, an inhibitor of translation. Since NMD depends upon translation, cyclo-

heximide can be used as an NMD-inhibitor. Subsequently, the mRNA was extracted and compared with mRNA from untreated cells, using PCR fragment size analysis.

Results and discussion

Sequencing of the *PGRN* gene in affected members from the Swedish FTD family revealed a 1 bp deletion in exon 1, causing a frameshift of the coding sequence and introducing a premature termination codon in exon 2 (Gly35GlufsX19) (Fig. 9). Previous studies of *PGRN* mutations have revealed that the mutant *PGRN* mRNA transcripts are degraded by NMD, leading to a loss of functional *PGRN* protein^{18, 19}. In our family, lymphoblast *PGRN* mRNA levels of two mutation carriers were considerably decreased when compared with healthy relatives, and fragment size separation and sequence analyses confirmed that the mutant mRNA allele had been degraded. NMD was confirmed as the mechanism of mutant mRNA allele degradation by its inhibition with cycloheximide, resulting in increased levels of the mutant mRNA allele, consistent with previous studies^{18, 19}.

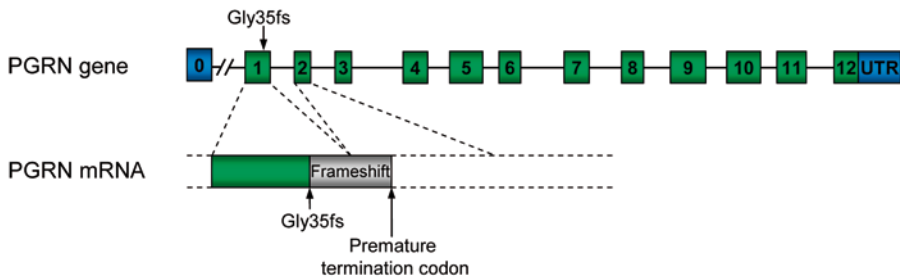


Figure 9. Schematic representation of the location of the Gly35fs mutation in *PGRN* exon 1. The Gly35fs mutation causes a frameshift of the coding sequence introducing a premature termination codon in exon 2.

Patients with mutations in *PGRN* display significant heterogeneity in their clinical presentation, however common diagnoses are FTD, PNFA or corticobasal syndrome^{22, 24, 89, 90}. There was a large variation of the initial presenting symptoms in the Swedish FTD family, but all affected family members shared a rapid disease progression with non-fluent aphasia, personality and behavioural changes. Neuropathologically, affected members of the family displayed severe atrophy, especially of the frontal lobes, microvacuolation, and gliosis. Tissue from one affected individual was available for additional immunohistochemical staining and displayed intraneuronal cytoplasmic and intranuclear inclusions positive for ubiquitin and TDP-43 in superficial cortical layers, consistent with the pathology reported for other patients carrying *PGRN* mutations^{12, 18, 19, 93}.

The mean age at onset in the Swedish FTD family is 54 years, varying from 46 to 59 years. The Gly35fs mutation was identified in seven affected family members as well as in three relatives unaffected by disease at ages 60, 62 and 65 years. The Gly35fs mutation has been previously described in two unrelated patients with FTLD²². One of these Gly35fs mutation carriers had an onset age of 83 years, high above the mean age at onset in our family. Previous studies have shown evidence of highly variable age at onset among *PGRN* mutation carriers and reduced penetrance in some *PGRN* mutation families, suggesting that the *PGRN* disease pathway is susceptible to modifying factors^{19, 22}.

The identification of mutations in the *PGRN* gene is a great breakthrough in FTLD research. However, the mechanisms by which a loss of functional *PGRN* lead to neurodegeneration are still unclear. How *PGRN* mutations lead to accumulation of pathological TDP-43 also needs to be clarified. The development of cell and animal models with reduced *PGRN* expression will be important for this work, and most likely contribute to the development of therapeutic intervention strategies, current suggestions include replacement of *PGRN*. However, this might be complicated by the fact that over-expression of *PGRN* has been associated with tumorigenesis⁸¹. The identification of modifying factors, resulting in reduced penetrance of the disease in some *PGRN* mutation families, may represent another potential target for therapeutic approaches to treat FTLD.

Paper III

Mutation screen of the PGRN gene in patients with FTLD

Background

The discovery of mutations in the *PGRN* gene as a major cause for familial FTLD^{18, 19} prompted us to screen for *PGRN* mutations in a series of FTLD patients. The mutation analysis included 51 FTLD patients, 32 clinical cases from Sweden and 19 neuropathologically confirmed FTLD cases from the United States, all previously found negative for mutations in the *MAPT* gene.

Methods

The 51 FTLD patients were investigated for mutations in *PGRN* by DNA sequencing. To investigate the effect of identified *PGRN* mutations on *PGRN* mRNA levels, total RNA was extracted from frontal lobe tissue and cDNA was synthesised. The cDNA was amplified using *PGRN* specific primers in a quantitative SYBR green PCR analysis. Sequence and PCR fragment size analyses were performed on brain cDNA to determine the

relative levels of wild-type and mutant mRNA alleles. PCR analysis was performed on brain cDNA to determine splicing effects of one of the identified *PGRN* mutations.

Results and discussion

Mutation analysis of the *PGRN* gene in our series of FTLD patients revealed two frameshift mutations. A novel deletion of four nucleotides, IVS6+5_8delGTGA, was identified in the splice donor site of *PGRN* exon 6 in two patients and a previously described deletion of four nucleotides, Gln130fs, was identified in *PGRN* exon 4 in another patient (Fig. 10a)^{18, 22}. Both *PGRN* mutations are predicted to result in premature termination codons, NMD and haploinsufficiency.

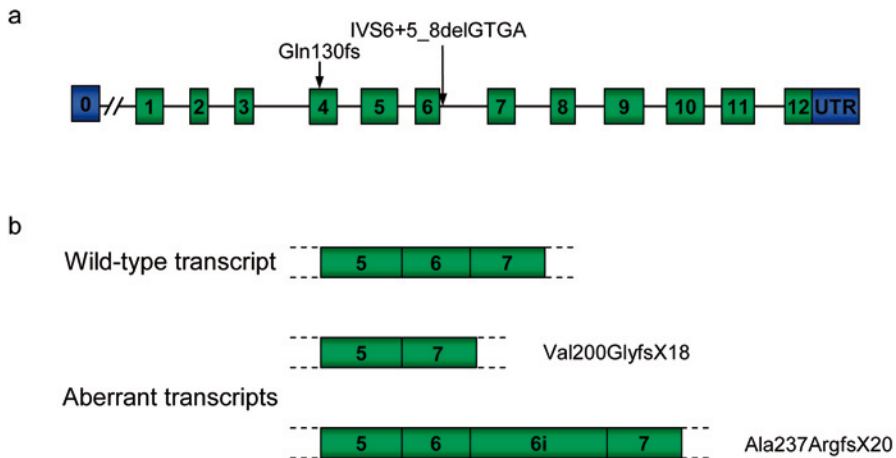


Figure 10. (a) Schematic representation of the location of the Gln130fs and IVS6+5_8delGTGA mutations. (b) Schematic representation of the two aberrant splice forms produced as a result of the IVS6+5_8delGTGA mutation. Both aberrant transcripts are predicted to result in a frameshift of the coding sequence and the introduction of premature termination codons.

The IVS6+5_8delGTGA deletion is located in the splice donor site of *PGRN* exon 6. Altering this splice site could result in the exclusion of exon 6 in the *PGRN* transcript and a premature termination codon in exon 7 when spliced onto exon 5 (Val200GlyfsX18) (Fig. 10b). Another possible outcome is retention of the intron following exon 6, which would also create a premature termination codon in exon 7 (Ala237ArgfsX20) (Fig. 10b). Both these transcripts were detected in a brain cDNA sample from a mutation carrier, although only in minor amounts as the majority of the mutated transcripts are most likely degraded through NMD. The reduction of mutant *PGRN* mRNA was supported by qPCR analysis although the difference in total *PGRN* levels between the IVS6+5_8delGTGA deletion carrier and a healthy control

was quite modest. However, cDNA sequence analysis of a synonymous polymorphism within the *PGRN* exon 4 in the mutation carrier confirmed that the wild type allele represented the major transcript and that the mutated transcript levels were very low.

The Gln130fs mutation leads to a frameshift of the sequence and a premature termination codon in exon 7 (Gln130SerfsX125). By qPCR analysis, we were able to demonstrate a 40% reduction of PGRN mRNA levels in brain tissue from the case carrying the Gln130fs mutation when compared with a healthy control. Additional sequence and PCR fragment size separation analyses of brain cDNA confirmed that the mutant mRNA allele was almost absent in the Gln130fs mutation carrier.

As small amounts of aberrant transcripts are seen as a result of the IVS6+5_8delGTGA deletion, it is possible that resulting truncated forms of PGRN could exert a pathogenic effect through a gain-of-function mechanism. Due to the lack of additional tissue samples or cell lines from the IVS6+5_8delGTGA mutation cases we were unable to investigate the stability of these aberrant transcripts or the potential presence of low levels of truncated protein. Therefore it is currently difficult to determine the significance, if any, of these splice variant transcripts. However, previous studies have provided convincing evidence that the pathogenic mechanism of *PGRN* mutations results from a loss of functional PGRN protein and this is supported by the recent discovery of deletions of one *PGRN* gene copy in patients with FTLD^{87, 88}. Furthermore, some *PGRN* mutations resulting in reduced PGRN function through non-NMD mediated mechanisms have been described. For example, the *PGRN* IVS0+3A>T and IVS0+5G>C mutations are predicted to lead to the retention of intron 0, causing nuclear degradation^{19, 89}. In addition, several mutations affect the Kozak sequence, resulting in a loss of translation of the mutant transcripts^{18, 19, 22, 90}. Finally, the Ala9Asp mutation, located in the signal peptide of *PGRN*, is believed to cause mislocalisation of mutant PGRN protein to the Golgi apparatus and reduced synthesis of mutant PGRN protein^{22, 86, 91}. These findings strongly suggest that haploinsufficiency is the pathogenic mechanism of *PGRN* mutations in FTLD.

Paper IV

Analysis of PGRN and MAPT gene dosage alterations in patients with FTLD

Background

Recently, gene dosage alterations have been implicated in several neurodegenerative disorders. In AD, duplications of the gene encoding the amyloid

precursor protein have been described and α -synuclein gene duplications or triplications have been identified in patients with Parkinson's disease¹²⁸⁻¹³⁰. In addition, hemizygous deletions of the *PGRN* locus were recently described in patients with TDP-43 positive FTLD^{87, 88}. In study IV, we therefore wanted to investigate the prevalence of *PGRN* gene deletions in FTLD. Thirty-nine patients negative for *MAPT* and *PGRN* mutations were analysed. We hypothesised that *MAPT* gene duplications could cause tau-positive FTLD through increased production of tau protein. Therefore, *MAPT* gene dosage alterations were investigated in the same set of patients. Due to the clinico-pathological overlap between FTLD, PSP and CBD we also included 20 patients with PSP and five patients with CBD in the *MAPT* gene dosage analysis.

Methods

The *PGRN* and *MAPT* gene dosage analysis was performed using multiplex ligation dependent probe amplification (MLPA)¹³¹. In an MLPA analysis multiple probes targeting different exons, within a specific gene (and a number of reference genes), are added in the same reaction. Each MLPA probe consists of two oligonucleotides that hybridise to adjacent sequences of the target gene. One oligonucleotide contains a sequence recognised by a forward primer, and the other oligonucleotide contains a sequence recognised by a reverse primer and an additional sequence of variable length, the "stuffer" sequence. After hybridising to the adjacent target sites, the two oligonucleotides are ligated. The resulting ligation product is subsequently exponentially amplified in a PCR reaction. Oligonucleotides that are not hybridised to adjacent sites will not be ligated or PCR amplified. The amplification product of each probe has a unique length due to the stuffer sequence allowing PCR products from different probes to be separated by electrophoresis. Since the forward primer used for probe amplification is fluorescently labelled, the signal strength of the PCR amplification product can be detected and compared to a control sample, giving the relative quantity of the target sequence.

Results and discussion

Our analysis was unable to demonstrate deletions of the *PGRN* locus as a cause of disease in the 39 FTLD patients. However, the study was limited by a relatively small number of patients, where most patients only had a clinical diagnosis and just seven patients had known TDP-43 pathology. This might explain the lack of *PGRN* gene deletions in our sample.

We were also unable to find evidence of *MAPT* gene duplications in our set of FTLD patients, which included seven patients with known tau-positive pathology. Previous studies have also been unable to find *MAPT* gene dupli-

cations in patients with FTLD^{132, 133}. If duplications of *MAPT* exist they are most likely not a common cause of FTLD. However, given that gene multiplications have been identified as a cause for other neurodegenerative disorders, characterised by protein aggregation, it is not unlikely that tau-positive FTLD could be caused by a similar mechanism, through increased production and deposition of tau. This is supported by transgenic mouse models, where over-expression of tau recapitulates key features of FTLD, including the formation of tau deposits^{57, 134, 135}. We also found no evidence for *MAPT* duplications in the PSP and CBD cases investigated. However, studies involving larger patient cohorts will be necessary to rule out the possibility of *MAPT* gene duplications as a cause of PSP and CBD.

In conclusion, we were unable to demonstrate *PGRN* or *MAPT* gene dosage alterations in our series of patients with FTLD. However, additional studies involving larger patient series are needed to fully elucidate the importance of gene dosage alterations of *PGRN* and *MAPT* in FTLD.

Concluding remarks and future perspectives

Recent genetic and neuropathological discoveries have brought the FTLN field closer to an understanding of the molecular pathogenesis and suggest new strategies for the development of disease-modifying treatments. It is now clear that FTLN is a genetically, clinically and neuropathologically complex disorder. The identification of mutations within the genes for MAPT and PGRN and their correlation to two distinct types of neuropathology, tau-positive pathology and TDP-43 positive pathology, provides the basis for the characterisation of two FTLN subtypes. Therefore it is possible to correlate molecular causes to the neuropathological outcome, even though the genetics behind many cases of tau and TDP-43 positive pathology is currently unknown. The different pathological subtypes of FTLN most likely represent separate mechanisms of disease presenting with similar symptomatology. Strategies for therapeutic prevention and intervention of FTLN will likely target these disease pathways and it seems reasonable to assume that the different subtypes of FTLN will require different treatments. Development of diagnostic methods to determine subtype of FTLN when no genetic indication can be identified will be essential and characterisation of relevant biomarkers is very important.

For tau-related FTLN, a number of treatment strategies have been suggested. For example, MT-stabilising drugs may have a therapeutic benefit in counteracting the negative effect of tau dissociation and MT destabilisation. In a recent study, treatment with the MT-stabilising drug paclitaxel (Taxol[®]), increased the number of MTs and restored axonal transport in tau transgenic mice¹³⁶. Also, by inhibiting phosphorylation of tau, which reduces the binding of tau to the MTs, the formation of tau aggregates may be prevented and MT dynamics maintained. Indeed, kinase inhibition in tau transgenic mice prevents tau hyperphosphorylation and aggregation¹³⁷⁻¹³⁹. Agents inhibiting tau aggregation are another possible treatment strategy for tau-related FTLN and have already proven successful in cell culture studies^{62, 140, 141}. Immunisation, aiming to reduce the level of toxic tau species in the brain has also been suggested as treatment of tau-positive FTLN. Immunisation has previously proven promising in AD, where disease is presumed to be caused by aggregation of toxic forms of the amyloid- β protein¹⁴². In a tau transgenic mouse model, immunisation with a phosphorylated tau-peptide led to reduced tau aggregates and a slower progression of behavioural deficits¹⁴³.

In the absence of a clear understanding of the mechanism of TDP-43 related neurodegeneration, it is currently difficult to formulate strategies for treatment of this FTLD subtype. If neurodegeneration is caused by toxicity due to aggregation of TDP-43 in the cytoplasm, therapies aiming at removing these toxic species could prove successful. However, if disease is caused by the loss of normal nuclear TDP-43 function, such treatment could instead be detrimental. Since reduced levels of PGRN lead to neurodegeneration, PGRN replacement has been proposed as a potential treatment strategy for patients with *PGRN* mutations and TDP-43 pathology. However, this approach could result in severe side effects since an increase in PGRN expression has been associated with tumour growth⁸¹. Studies have suggested that the PGRN disease pathway is highly susceptible to modifying factors, resulting in highly variable age at onset among *PGRN* mutation carriers and reduced penetrance in some *PGRN* mutation families. The identification of such modifying factors might represent another potential target for therapeutic approaches to treat FTLD, if replacement therapy of PGRN proves unsuccessful.

Current treatment strategies for FTLD are purely symptomatic and have no effect on disease progression. However, with recent advances in the understanding of FTLD pathogenesis, specific disease-modifying therapies for FTLD appear realistic within the near future. Tau-based treatment strategies may also be helpful for other neurodegenerative diseases characterised by tau-pathology e.g. PSP, CBD and AD. Furthermore, therapies targeting the pathogenic pathways involving TDP-43 could prove beneficial in other neurodegenerative diseases, since pathological TDP-43 has also been found in patients with ALS.

There is a desperate need for improved prevention, diagnosis and treatment of neurodegenerative diseases. The past years' rapid progress in the identification of genetic factors and molecular mechanisms involved in the pathogenesis of FTLD and other neurodegenerative conditions provides a framework for efforts towards slowing down or halting the progression of these detrimental disorders.

Populärvetenskaplig sammanfattning

Frontotemporal demens (FTD) är en neurodegenerativ sjukdom som, näst efter Alzheimers sjukdom och vaskulär demens, är den tredje vanligaste formen av demens. FTD orsakas av att nervceller i hjärnans frontal- och temporallobber förtvinar och dör. Hjärnans frontallobber styr mycket av vår personlighet och vårt beteende. Denna hjärnregion är viktig för vårt omdöme, för social interaktion och vår förmåga att känna empati och läsa andras känslor. En förtvinning av frontallobberna hos FTD-patienter leder därför till en smygande beteendeförändring som kan yttra sig i sämre omdöme, olämpligt beteende och känslomässig avtrubbnig. Temporallobberna innehåller områden som är viktiga för vårt språk och en förtvinning av dessa regioner leder till att FTD-patienter även drabbas av en gradvis försämring av språket, med problem att finna ord eller ett mödosamt tal som resultat. FTD skiljer sig från andra demenssjukdomar som exempelvis Alzheimers sjukdom, genom att det inte finns någon, eller endast en mycket liten försämring av minnet i början av sjukdomen. Minnesproblem kan dock uppkomma i senare skeden av sjukdomen.

Varför nervcellerna i hjärnan förtvinar hos patienter med FTD har länge varit okänt. Tittar man närmare på de drabbade hjärnregionerna i mikroskop kan man se sjukliga inlagringar av protein i nervcellerna. Majoriteten av patienterna har inlagringar som består av ett protein kallat TAR DNA-binding protein-43 (TDP-43), medan andra har inlagringar bestående av proteinet tau. Under de senaste åren har vi börjat förstå varför dessa proteininlagringar uppstår och hur de skadar och dödar nervcellerna i hjärnan. Genetiska studier av familjer med ärftlig FTD har varit mycket viktiga för förståelsen av de mekanismer som leder till sjukdom.

Syftet med denna avhandling var att identifiera genetiska defekter som leder till sjukdom i patienter med ärftlig FTD. Upp emot 40% av alla FTD-fall är av ärftlig form där sjukdomen orsakas av en genetisk defekt som kan gå i arv inom en familj. För tio år sedan upptäcktes sjukdomsframkallande mutationer i genen för proteinet tau i familjer med ärftlig FTD.

Den första studien i denna avhandling beskriver en FTD-familj från Finland där sjukdomen orsakas av en tau-mutation. Tau-proteinets normala funktion är att stabilisera mikrotubuli vilka är viktiga för att bibehålla nervcellens struktur och för transport av näringsämnen inom nervcellen. Vissa mutationer i tau-genen förändrar tau-proteinets egenskaper så att det lossnar från mikrotubuli och bildar proteininlagringar inuti nervcellerna i hjärnan,

vilket resulterar i att mikrotubuli försvagas. Andra tau-mutationer leder istället till att tau binder hårdare till mikrotubuli, vilket gör cellskelettet i nervcellen för stelt. Möjligen orsakas förtviningen av nervceller av förändrad stabilitet av mikrotubuli med påverkan på nervcellens struktur och den intracellulära transporten av näringsämnen. Alternativt kan de proteininlagringar som uppstår stora viktiga processer i nervcellen och leda till att nervcellen förtvinar.

Nyligen identifierades sjukdomsframkallande mutationer i genen som kodar för proteinet progranulin i familjer med FTD. Progranulin är en tillväxtfaktor som spelar en viktig roll vid sårhäkning och inflammation. Förhöjd produktion av progranulin kan ge upphov till tumörer. Mutationerna i progranulin-genen hos FTD-patienter medför att endast hälften av den normala mängden av progranulin produceras, men hur detta leder till sjukdom är inte klarlagt.

I den andra studien beskrivs en svensk FTD-familj med en mutation i genen för progranulin. Vi kunde i denna studie, i likhet med tidigare studier, visa att celler från sjuka individer i familjen bildar hälften så mycket progranulin som friska individer. Undersökning av hjärnan från en avlidet familjemedlem som ärvt den sjukdomsframkallande mutationen påvisade inlagringar av TDP-43 proteinet. Hur en minskad mängd progranulin kan orsaka att detta protein inlagras i nervcellerna är idag okänt.

I den tredje studien har vi studerat förekomsten av mutationer i progranulin i 51 patienter med FTD. Vi hittade två olika mutationer i tre obesläktade patienter. Detta ger en förekomst av mutationer i progranulin hos 6% av FTD-fallen, vilket stämmer överens med andra studier. I likhet med mutationen i progranulin i den svenska familjen fann vi att även dessa mutationer leder till minskad mängd protein. Inlagringar av TDP-43 i hjärnans nervceller återfanns även hos dessa patienter.

Förutom mutationer kan sjukdomar uppkomma på grund av förändrat antal kopior av en gen ("gen-dos") då detta förändrar mängden protein i kroppen. Vanligtvis bär vi människor på två kopior av en gen, där en ärvt från vardera föräldern. Ibland kan en gen av misstag kopieras upp (dupliceras) i fler än två upplagor, eller alternativt så försvinner (deleteras) en av våra två genkopior. Vid Alzheimers sjukdom har man nyligen visat att en duplikation av genen för amyloid-prekursor proteinet leder till sjukdom på grund av ökad produktion av proteinet. Vid FTD har man kunnat visa att en deletion av en av genkopiorna för progranulin leder till sjukdom på grund av minskad mängd av progranulin, i likhet med de tidigare beskrivna mutationerna i progranulin-genen. I den fjärde studien ville vi undersöka förekomsten av deletioner av progranulin-genen hos 39 patienter med FTD. Vi undersökte även om duplikationer av tau-genen kunde orsaka sjukdom i samma patientgrupp. Vi kunde inte identifiera några förändringar i antalet genkopior av progranulin och tau i dessa patienter vilket antyder att förändringar i gen-

dos av progranulin och tau inte är en vanligt förekommande orsak till FTD, åtminstone inte i vår patientgrupp.

Studier för att utröna de mekanismer som orsakar FTD är viktiga för utvecklingen av nya behandlingsmetoder. I dagsläget kan patienter med FTD endast erbjudas symptomatisk behandling som inte påverkar de bakomliggande orsakerna till sjukdomen. Genetiska studier av familjer med ärftlig FTD har lett till en ökad kunskap om sjukdomens orsaker. Strategier för att behandla patienter med tau-relaterad sjukdom är redan under utveckling och har visat lovande resultat i cell- och djurmodeller. De mekanismer som leder till bildning av TDP-43 inlagringar är ännu oklara och behöver undersökas närmare, bland annat genom att klargöra kopplingen mellan progranulin och TDP-43. Tack vare den forskning som bedrivits ser framtiden ljus ut och en behandling som riktar sig mot de bakomliggande orsakerna till FTD är fullt möjlig.

Acknowledgements

I would like to express my sincere gratitude to everyone who has contributed to this thesis and helped me along the way. I especially would like to thank the following persons:

My supervisor, Anna Glaser, for all your encouragement, support and good advice over the years. For all our interesting discussions and for sharing your scientific knowledge with me.

My co-supervisor, Martin Ingelsson, for all your encouragement, support and good ideas, and for introducing me to another world “outside” genetics.

My co-supervisor, Lars Lannfelt, for generously offering me a place in your research group, for introducing me into the field of dementia research and for all your support with this thesis.

All my co-authors who made this thesis possible, especially; Matti Viitanen and Hans Basun for clinical descriptions and helpful advice during preparation of paper I and II, Hannu Kalimo for all your help with the neuropathology for paper I and II and for helpful advice during manuscript preparation, and the people from Bradley Hyman’s research group for our nice collaboration regarding paper III and IV.

All the wonderful co-workers at Molecular Geriatrics:

RoseMarie Brundin, whose laughter always lifts my spirit. Thanks for all your hard work with “our” FTLD families and for being a perfect travel partner in San Francisco and Rotterdam.

Sofie Ingvast, for all the help you have given me these last years in the lab and for always being such a good friend.

Mimmi Hedlund, for making me feel very welcome in the group when I first arrived as a very young student.

Lars Nilsson, our lab expert. What you don’t know isn’t worth knowing. Thanks for helpful advice regarding my thesis.

The “genetics group”; Elin Blom, for keeping me company in the field of genetics and for all your help with my thesis. Vilmantas Giedraitis, for your genetic knowledge and for your help with computer and statistical problems.

The “immunology group”; Frida Ekholm Petterson, for all our nice talks over the years. Hillevi Englund, my desk neighbour, for always offering a helpful hand, especially lately with my thesis. Dag Sehlin, for nice company during lunch and coffee breaks. Barbro Simu, for keeping me company in the “genetics” lab this autumn. Sofia Söllvander, for being a nice addition to our research group.

The “transgenics group”; Anna Lord, for your nice laughter and funny pranks. Ola Philipson, for your positive attitude. Astrid Gumucio, for being so kind and nice.

The “ α -synuclein group”; Joakim Bergström, for nice talks and excellent company during the medical student-labs. Charlotte Sahlin, for always being so nice and helpful. Tomas Näsström, for being a very nice desk neighbour. Therese Wahlberg, for keeping me company in the tau field (at least for a while).

The “heparan-sulfate group”; Xiao Zhang, for your uplifting laughter. Paul O’Callaghan, for always being so helpful. Thanks for proofreading my thesis.

My former students; Johan Fredriksson, Yi-jing Yang, and especially my former master thesis students; Moa Fransson, Anna Börjesson and Therese Wahlberg.

Former members of the group; Pär Gellerfors, Jovanka Ostojic, Sara Häggblad, Fredrik Noborn and Ann-Sofi Johansson.

Former and present co-workers at the Clinic of geriatrics, especially; Lena Kilander for teaching me a lot about the clinical features of FTLD, Maria Lindau for bringing back my interest for ballet, Alexander Frizell Santillo for “eye-of-the-tiger” and nice times in SanFran and Rotterdam, Cicki for being a nice travel partner in Rotterdam and all the ladies from Maria’s ballet group.

The administrative staff at the Department of Public Health and Caring Sciences, especially Karin Torbratt and the head of the department Marianne Carlsson.

The staff at the Uppsala Genome Centre, especially Inger Jonasson, for all your help and advice regarding my sequencing and genotyping projects.

The following foundations are acknowledged for financial support: Alzheimerfonden, Demensfonden, Lions forskningsfond, Gun och Bertil Stohnes stiftelse, Gamla tjänarinnors stiftelse and Emma Petterssons testamente.

Jag vill också rikta ett stort tack till några andra viktiga personer i mitt liv:

Tack till min underbara familj. Mamma och pappa som alltid har stöttat och trott på mig. Världens bästa systrar, Monica och Yvonne, och deras familjemedlemmar Tommy, Josefine, Amanda, Andreas och Per. Ig älsker ir!

Tack även till alla andra medlemmar av min stora tjocka släkt och till min ”nya” familj i fina Krokek.

Mina Uppsala-vänner; Sofia, Marika, Lina, Therese och Anna. Tack för alla underbara resor, fester och annat kul genom åren. Tack även till mina barn-domsvänner; Linda, Anna och Jenny, som alltid finns där trots alla år som har gått.

Mitt sista tack går till Rickard, för utan dig hade jag inte klarat det här ♥

References

1. Finishing the euchromatic sequence of the human genome. *Nature* 431, 931-945 (2004).
2. Chang, Y.F., Imam, J.S. & Wilkinson, M.F. The nonsense-mediated decay RNA surveillance pathway. *Annual review of biochemistry* 76, 51-74 (2007).
3. Wang, G.S. & Cooper, T.A. Splicing in disease: disruption of the splicing code and the decoding machinery. *Nature reviews* 8, 749-761 (2007).
4. Pick, A. Uber die Beziehungen der senilen Hirnatrophie zur Aphasie. *Prager Med Wochenschr* 17, 165-167 (1892).
5. Neary, D. *et al.* Frontotemporal lobar degeneration: a consensus on clinical diagnostic criteria. *Neurology* 51, 1546-1554 (1998).
6. Ratnavalli, E., Brayne, C., Dawson, K. & Hodges, J.R. The prevalence of frontotemporal dementia. *Neurology* 58, 1615-1621 (2002).
7. Harvey, R.J., Skelton-Robinson, M. & Rossor, M.N. The prevalence and causes of dementia in people under the age of 65 years. *J Neurol Neurosurg Psychiatry* 74, 1206-1209 (2003).
8. Ikeda, M., Ishikawa, T. & Tanabe, H. Epidemiology of frontotemporal lobar degeneration. *Dement Geriatr Cogn Disord* 17, 265-268 (2004).
9. Cairns, N.J. *et al.* Neuropathologic diagnostic and nosologic criteria for frontotemporal lobar degeneration: consensus of the Consortium for Frontotemporal Lobar Degeneration. *Acta neuropathologica* 114, 5-22 (2007).
10. Taniguchi, S. *et al.* The neuropathology of frontotemporal lobar degeneration with respect to the cytological and biochemical characteristics of tau protein. *Neuropathol Appl Neurobiol* 30, 1-18 (2004).
11. Lipton, A.M., White, C.L., 3rd & Bigio, E.H. Frontotemporal lobar degeneration with motor neuron disease-type inclusions predominates in 76 cases of frontotemporal degeneration. *Acta Neuropathol (Berl)* 108, 379-385 (2004).
12. Neumann, M. *et al.* Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* 314, 130-133 (2006).
13. Stevens, M. *et al.* Familial aggregation in frontotemporal dementia. *Neurology* 50, 1541-1545 (1998).
14. Rosso, S.M. *et al.* Frontotemporal dementia in The Netherlands: patient characteristics and prevalence estimates from a population-based study. *Brain* 126, 2016-2022 (2003).

15. Hutton, M. *et al.* Association of missense and 5'-splice-site mutations in *tau* with the inherited dementia FTDP-17. *Nature* 393, 702-705 (1998).
16. Poorkaj, P. *et al.* Tau is a candidate gene for chromosome 17 frontotemporal dementia. *Ann Neurol* 43, 815-825 (1998).
17. Spillantini, M.G. *et al.* Mutation in the tau gene in familial multiple system tauopathy with presenile dementia. *Proc Natl Acad Sci U S A* 95, 7737-7741 (1998).
18. Baker, M. *et al.* Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. *Nature* 442, 916-919 (2006).
19. Cruts, M. *et al.* Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. *Nature* 442, 920-924 (2006).
20. Skibinski, G. *et al.* Mutations in the endosomal ESCRTIII-complex subunit CHMP2B in frontotemporal dementia. *Nat Genet* 37, 806-808 (2005).
21. Watts, G.D. *et al.* Inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia is caused by mutant valosin-containing protein. *Nat Genet* 36, 377-381 (2004).
22. Gass, J. *et al.* Mutations in progranulin are a major cause of ubiquitin-positive frontotemporal lobar degeneration. *Hum Mol Genet* 15, 2988-3001 (2006).
23. Bronner, I.F. *et al.* Progranulin mutations in Dutch familial frontotemporal lobar degeneration. *Eur J Hum Genet* 15, 369-374 (2007).
24. Pickering-Brown, S.M. *et al.* Frequency and clinical characteristics of progranulin mutation carriers in the Manchester frontotemporal lobar degeneration cohort: comparison with patients with MAPT and no known mutations. *Brain* 131, 721-731 (2008).
25. Wilhelmsen, K.C., Lynch, T., Pavlou, E., Higgins, M. & Nygaard, T.G. Localization of disinhibition-dementia-parkinsonism-amyotrophy complex to 17q21-22. *Am J Hum Genet* 55, 1159-1165 (1994).
26. Foster, N.L. *et al.* Frontotemporal dementia and parkinsonism linked to chromosome 17: a consensus conference. Conference Participants. *Ann Neurol* 41, 706-715 (1997).
27. Binder, L.I., Frankfurter, A. & Rebhun, L.I. The distribution of tau in the mammalian central nervous system. *J Cell Biol* 101, 1371-1378 (1985).
28. Weingarten, M.D., Lockwood, A.H., Hwo, S.Y. & Kirschner, M.W. A protein factor essential for microtubule assembly. *Proc Natl Acad Sci U S A* 72, 1858-1862 (1975).
29. Caceres, A. & Kosik, K.S. Inhibition of neurite polarity by tau antisense oligonucleotides in primary cerebellar neurons. *Nature* 343, 461-463 (1990).
30. Esmaeli-Azad, B., McCarty, J.H. & Feinstein, S.C. Sense and antisense transfection analysis of tau function: tau influences net

- microtubule assembly, neurite outgrowth and neuritic stability. *Journal of cell science* 107 (Pt 4), 869-879 (1994).
31. Sato-Harada, R., Okabe, S., Umeyama, T., Kanai, Y. & Hirokawa, N. Microtubule-associated proteins regulate microtubule function as the track for intracellular membrane organelle transports. *Cell structure and function* 21, 283-295 (1996).
 32. Goedert, M., Spillantini, M.G., Jakes, R., Rutherford, D. & Crowther, R.A. Multiple isoforms of human microtubule-associated protein tau: sequences and localization in neurofibrillary tangles of Alzheimer's disease. *Neuron* 3, 519-526 (1989).
 33. Lee, G., Neve, R.L. & Kosik, K.S. The microtubule binding domain of tau protein. *Neuron* 2, 1615-1624 (1989).
 34. Chen, J., Kanai, Y., Cowan, N.J. & Hirokawa, N. Projection domains of MAP2 and tau determine spacings between microtubules in dendrites and axons. *Nature* 360, 674-677 (1992).
 35. Brandt, R., Leger, J. & Lee, G. Interaction of tau with the neural plasma membrane mediated by tau's amino-terminal projection domain. *J Cell Biol* 131, 1327-1340 (1995).
 36. Rosner, H., Rebhan, M., Vacun, G. & Vanmechelen, E. Developmental expression of tau proteins in the chicken and rat brain: rapid down-regulation of a paired helical filament epitope in the rat cerebral cortex coincides with the transition from immature to adult tau isoforms. *Int J Dev Neurosci* 13, 607-617 (1995).
 37. Lindwall, G. & Cole, R.D. Phosphorylation affects the ability of tau protein to promote microtubule assembly. *J Biol Chem* 259, 5301-5305 (1984).
 38. Gasparini, L., Terni, B. & Spillantini, M.G. Frontotemporal dementia with tau pathology. *Neuro-degenerative diseases* 4, 236-253 (2007).
 39. Goedert, M., Spillantini, M.G., Potier, M.C., Ulrich, J. & Crowther, R.A. Cloning and sequencing of the cDNA encoding an isoform of microtubule-associated protein tau containing four tandem repeats: differential expression of tau protein mRNAs in human brain. *Embo J* 8, 393-399 (1989).
 40. Goode, B.L., Chau, M., Denis, P.E. & Feinstein, S.C. Structural and functional differences between 3-repeat and 4-repeat tau isoforms. Implications for normal tau function and the onset of neurodegenerative disease. *J Biol Chem* 275, 38182-38189 (2000).
 41. Panda, D., Samuel, J.C., Massie, M., Feinstein, S.C. & Wilson, L. Differential regulation of microtubule dynamics by three- and four-repeat tau: implications for the onset of neurodegenerative disease. *Proc Natl Acad Sci U S A* 100, 9548-9553 (2003).
 42. Bunker, J.M., Wilson, L., Jordan, M.A. & Feinstein, S.C. Modulation of microtubule dynamics by tau in living cells: implications for development and neurodegeneration. *Mol Biol Cell* 15, 2720-2728 (2004).

43. Bugiani, O. *et al.* Frontotemporal dementia and corticobasal degeneration in a family with a P301S mutation in tau. *J Neuropathol Exp Neurol* 58, 667-677 (1999).
44. Spillantini, M.G. *et al.* A novel tau mutation (N296N) in familial dementia with swollen achromatic neurons and corticobasal inclusion bodies. *Ann Neurol* 48, 939-943 (2000).
45. Delisle, M.B. *et al.* A mutation at codon 279 (N279K) in exon 10 of the Tau gene causes a tauopathy with dementia and supranuclear palsy. *Acta Neuropathol (Berl)* 98, 62-77 (1999).
46. Stanford, P.M. *et al.* Progressive supranuclear palsy pathology caused by a novel silent mutation in exon 10 of the tau gene: expansion of the disease phenotype caused by tau gene mutations. *Brain* 123 (Pt 5), 880-893 (2000).
47. Pastor, P. *et al.* Familial atypical progressive supranuclear palsy associated with homozygosity for the delN296 mutation in the tau gene. *Ann Neurol* 49, 263-267 (2001).
48. Poorkaj, P. *et al.* An R5L tau mutation in a subject with a progressive supranuclear palsy phenotype. *Ann Neurol* 52, 511-516 (2002).
49. Ros, R. *et al.* A new mutation of the tau gene, G303V, in early-onset familial progressive supranuclear palsy. *Arch Neurol* 62, 1444-1450 (2005).
50. Murray, B., Lynch, T. & Farrell, M. Clinicopathological features of the tauopathies. *Biochemical Society transactions* 33, 595-599 (2005).
51. Dayanandan, R. *et al.* Mutations in tau reduce its microtubule binding properties in intact cells and affect its phosphorylation. *FEBS Lett* 446, 228-232 (1999).
52. Matsumura, N., Yamazaki, T. & Ihara, Y. Stable expression in Chinese hamster ovary cells of mutated tau genes causing frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17). *Am J Pathol* 154, 1649-1656 (1999).
53. Sahara, N., Tomiyama, T. & Mori, H. Missense point mutations of tau to segregate with FTDP-17 exhibit site-specific effects on microtubule structure in COS cells: a novel action of R406W mutation. *Journal of neuroscience research* 60, 380-387 (2000).
54. Bunker, J.M., Kamath, K., Wilson, L., Jordan, M.A. & Feinstein, S.C. FTDP-17 mutations compromise the ability of tau to regulate microtubule dynamics in cells. *J Biol Chem* 281, 11856-11863 (2006).
55. Ebner, A. *et al.* Overexpression of tau protein inhibits kinesin-dependent trafficking of vesicles, mitochondria, and endoplasmic reticulum: implications for Alzheimer's disease. *J Cell Biol* 143, 777-794 (1998).
56. Spittaels, K. *et al.* Prominent axonopathy in the brain and spinal cord of transgenic mice overexpressing four-repeat human tau protein. *Am J Pathol* 155, 2153-2165 (1999).

57. Ishihara, T. *et al.* Age-dependent emergence and progression of a tauopathy in transgenic mice overexpressing the shortest human tau isoform. *Neuron* 24, 751-762 (1999).
58. Probst, A. *et al.* Axonopathy and amyotrophy in mice transgenic for human four-repeat tau protein. *Acta neuropathologica* 99, 469-481 (2000).
59. Zhang, B. *et al.* Retarded axonal transport of R406W mutant tau in transgenic mice with a neurodegenerative tauopathy. *J Neurosci* 24, 4657-4667 (2004).
60. Utton, M.A. *et al.* The slow axonal transport of the microtubule-associated protein tau and the transport rates of different isoforms and mutants in cultured neurons. *J Neurosci* 22, 6394-6400 (2002).
61. Yuan, A., Kumar, A., Peterhoff, C., Duff, K. & Nixon, R.A. Axonal transport rates in vivo are unaffected by tau deletion or overexpression in mice. *J Neurosci* 28, 1682-1687 (2008).
62. Khlistunova, I. *et al.* Inducible expression of Tau repeat domain in cell models of tauopathy: aggregation is toxic to cells but can be reversed by inhibitor drugs. *J Biol Chem* 281, 1205-1214 (2006).
63. Bandyopadhyay, B., Li, G., Yin, H. & Kuret, J. Tau aggregation and toxicity in a cell culture model of tauopathy. *J Biol Chem* 282, 16454-16464 (2007).
64. Mocanu, M.M. *et al.* The potential for beta-structure in the repeat domain of tau protein determines aggregation, synaptic decay, neuronal loss, and coassembly with endogenous Tau in inducible mouse models of tauopathy. *J Neurosci* 28, 737-748 (2008).
65. Wittmann, C.W. *et al.* Tauopathy in *Drosophila*: neurodegeneration without neurofibrillary tangles. *Science* 293, 711-714 (2001).
66. Kraemer, B.C. *et al.* Neurodegeneration and defective neurotransmission in a *Caenorhabditis elegans* model of tauopathy. *Proc Natl Acad Sci U S A* 100, 9980-9985 (2003).
67. Santacruz, K. *et al.* Tau suppression in a neurodegenerative mouse model improves memory function. *Science* 309, 476-481 (2005).
68. Bird, T.D. *et al.* Chromosome 17 and hereditary dementia: linkage studies in three non-Alzheimer families and kindreds with late-onset FAD. *Neurology* 48, 949-954 (1997).
69. Froelich, S. *et al.* Mapping of a disease locus for familial rapidly progressive frontotemporal dementia to chromosome 17q12-21. *Am J Med Genet (Neuropsych Genet)* 74, 380-385 (1997).
70. Lendon, C.L. *et al.* Hereditary dysphasic disinhibition dementia: a frontotemporal dementia linked to 17q21-22. *Neurology* 50, 1546-1555 (1998).
71. Kertesz, A. *et al.* Familial frontotemporal dementia with ubiquitin-positive, tau-negative inclusions. *Neurology* 54, 818-827 (2000).
72. Rosso, S.M. *et al.* Familial frontotemporal dementia with ubiquitin-positive inclusions is linked to chromosome 17q21-22. *Brain* 124, 1948-1957 (2001).

73. Rademakers, R. *et al.* Tau negative frontal lobe dementia at 17q21: significant finemapping of the candidate region to a 4.8 cM interval. *Mol Psychiatry* 7, 1064-1074 (2002).
74. Mackenzie, I.R. *et al.* A family with tau-negative frontotemporal dementia and neuronal intranuclear inclusions linked to chromosome 17. *Brain* 129, 853-867 (2006).
75. van der Zee, J. *et al.* A Belgian ancestral haplotype harbours a highly prevalent mutation for 17q21-linked tau-negative FTLD. *Brain* 129, 841-852 (2006).
76. Froelich Fabre, S., Axelman, P., Almkvist, A., Basun, H. & Lannfelt, L. Extended investigation of tau and mutation screening of other candidate genes on chromosome 17q21 in a Swedish FTDP-17 family. *Am J Med Genet (Neuropsych Genet)* 121B, 112-118 (2003).
77. Cruts, M. *et al.* Genomic architecture of human 17q21 linked to frontotemporal dementia uncovers a highly homologous family of low-copy repeats in the tau region. *Hum Mol Genet* 14, 1753-1762 (2005).
78. Gijssels, I., Van Broeckhoven, C. & Cruts, M. Granulin mutations associated with frontotemporal lobar degeneration and related disorders: an update. *Hum Mutat* 29, 1373-1386 (2008).
79. Bhandari, V., Palfrey, R.G. & Bateman, A. Isolation and sequence of the granulin precursor cDNA from human bone marrow reveals tandem cysteine-rich granulin domains. *Proc Natl Acad Sci U S A* 89, 1715-1719 (1992).
80. Daniel, R., He, Z., Carmichael, K.P., Halper, J. & Bateman, A. Cellular localization of gene expression for progranulin. *J Histochem Cytochem* 48, 999-1009 (2000).
81. He, Z. & Bateman, A. Progranulin (granulin-epithelin precursor, PC-cell-derived growth factor, acrogranin) mediates tissue repair and tumorigenesis. *Journal of molecular medicine (Berlin, Germany)* 81, 600-612 (2003).
82. Van Damme, P. *et al.* Progranulin functions as a neurotrophic factor to regulate neurite outgrowth and enhance neuronal survival. *J Cell Biol* 181, 37-41 (2008).
83. Suzuki, M., Yoshida, S., Nishihara, M. & Takahashi, M. Identification of a sex steroid-inducible gene in the neonatal rat hypothalamus. *Neurosci Lett* 242, 127-130 (1998).
84. Malaspina, A., Kaushik, N. & de Belleruche, J. Differential expression of 14 genes in amyotrophic lateral sclerosis spinal cord detected using gridded cDNA arrays. *J Neurochem* 77, 132-145 (2001).
85. Baker, C.A. & Manuelidis, L. Unique inflammatory RNA profiles of microglia in Creutzfeldt-Jakob disease. *Proc Natl Acad Sci U S A* 100, 675-679 (2003).
86. Mukherjee, O. *et al.* HDDD2 is a familial frontotemporal lobar degeneration with ubiquitin-positive, tau-negative inclusions caused by a missense mutation in the signal peptide of progranulin. *Ann Neurol* 60, 314-322 (2006).

87. Gijssels, I. *et al.* Progranulin locus deletion in frontotemporal dementia. *Hum Mutat* 29, 53-58 (2008).
88. Rovelet-Lecrux, A. *et al.* Deletion of the progranulin gene in patients with frontotemporal lobar degeneration or Parkinson disease. *Neurobiology of disease* 31, 41-45 (2008).
89. Le Ber, I. *et al.* Progranulin null mutations in both sporadic and familial frontotemporal dementia. *Hum Mutat* 28, 846-855 (2007).
90. Le Ber, I. *et al.* Phenotype variability in progranulin mutation carriers: a clinical, neuropsychological, imaging and genetic study. *Brain* 131, 732-746 (2008).
91. Mukherjee, O. *et al.* Molecular characterization of novel progranulin (GRN) mutations in frontotemporal dementia. *Hum Mutat* (2008).
92. Shankaran, S.S. *et al.* FTLD-U linked missense mutations in the progranulin gene reduce progranulin production and secretion. *J Biol Chem* (2007).
93. Mackenzie, I.R. *et al.* The neuropathology of frontotemporal lobar degeneration caused by mutations in the progranulin gene. *Brain* 129, 3081-3090 (2006).
94. Rademakers, R. *et al.* Common variation in the miR-659 binding-site of GRN is a major risk factor for TDP43-positive frontotemporal dementia. *Hum Mol Genet* 17, 3631-3642 (2008).
95. Zhang, Y.J. *et al.* Progranulin mediates caspase-dependent cleavage of TAR DNA binding protein-43. *J Neurosci* 27, 10530-10534 (2007).
96. Babst, M., Katzmann, D.J., Estepa-Sabal, E.J., Meerloo, T. & Emr, S.D. Escrt-III: an endosome-associated heterooligomeric protein complex required for mvb sorting. *Developmental cell* 3, 271-282 (2002).
97. van der Zee, J. *et al.* CHMP2B C-truncating mutations in frontotemporal lobar degeneration are associated with an aberrant endosomal phenotype in vitro. *Hum Mol Genet* 17, 313-322 (2008).
98. Parkinson, N. *et al.* ALS phenotypes with mutations in CHMP2B (charged multivesicular body protein 2B). *Neurology* (2006).
99. Filimonenko, M. *et al.* Functional multivesicular bodies are required for autophagic clearance of protein aggregates associated with neurodegenerative disease. *J Cell Biol* 179, 485-500 (2007).
100. Holm, I.E., Englund, E., Mackenzie, I.R., Johannsen, P. & Isaacs, A.M. A reassessment of the neuropathology of frontotemporal dementia linked to chromosome 3. *J Neuropathol Exp Neurol* 66, 884-891 (2007).
101. Hodges, J.R., Davies, R., Xuereb, J., Kril, J. & Halliday, G. Survival in frontotemporal dementia. *Neurology* 61, 349-354 (2003).
102. Lomen-Hoerth, C. Characterization of amyotrophic lateral sclerosis and frontotemporal dementia. *Dement Geriatr Cogn Disord* 17, 337-341 (2004).

103. Mackenzie, I.R. *et al.* Heterogeneity of ubiquitin pathology in frontotemporal lobar degeneration: classification and relation to clinical phenotype. *Acta neuropathologica* 112, 539-549 (2006).
104. Cairns, N.J. *et al.* TDP-43 in familial and sporadic frontotemporal lobar degeneration with ubiquitin inclusions. *Am J Pathol* 171, 227-240 (2007).
105. Kabashi, E. *et al.* TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. *Nat Genet* 40, 572-574 (2008).
106. Sreedharan, J. *et al.* TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science* 319, 1668-1672 (2008).
107. Hosler, B.A. *et al.* Linkage of familial amyotrophic lateral sclerosis with frontotemporal dementia to chromosome 9q21-q22. *JAMA* 284, 1664-1669 (2000).
108. Vance, C. *et al.* Familial amyotrophic lateral sclerosis with frontotemporal dementia is linked to a locus on chromosome 9p13.2-21.3. *Brain* 129, 868-876 (2006).
109. Morita, M. *et al.* A locus on chromosome 9p confers susceptibility to ALS and frontotemporal dementia. *Neurology* 66, 839-844 (2006).
110. Valdmanis, P.N. *et al.* Three families with amyotrophic lateral sclerosis and frontotemporal dementia with evidence of linkage to chromosome 9p. *Arch Neurol* 64, 240-245 (2007).
111. Luty, A.A. *et al.* Pedigree with frontotemporal lobar degeneration--motor neuron disease and Tar DNA binding protein-43 positive neuropathology: genetic linkage to chromosome 9. *BMC neurology* 8, 32 (2008).
112. Forman, M.S. *et al.* Novel ubiquitin neuropathology in frontotemporal dementia with valosin-containing protein gene mutations. *J Neuropathol Exp Neurol* 65, 571-581 (2006).
113. Wang, Q., Song, C. & Li, C.C. Molecular perspectives on p97-VCP: progress in understanding its structure and diverse biological functions. *Journal of structural biology* 146, 44-57 (2004).
114. Weihl, C.C., Dalal, S., Pestronk, A. & Hanson, P.I. Inclusion body myopathy-associated mutations in p97/VCP impair endoplasmic reticulum-associated degradation. *Hum Mol Genet* 15, 189-199 (2006).
115. Weihl, C.C., Miller, S.E., Hanson, P.I. & Pestronk, A. Transgenic expression of inclusion body myopathy associated mutant p97/VCP causes weakness and ubiquitinated protein inclusions in mice. *Hum Mol Genet* 16, 919-928 (2007).
116. Ju, J.S., Miller, S.E., Hanson, P.I. & Weihl, C.C. Impaired protein aggregate handling and clearance underlie the pathogenesis of p97/VCP-associated disease. *J Biol Chem* 283, 30289-30299 (2008).
117. Ou, S.H., Wu, F., Harrich, D., Garcia-Martinez, L.F. & Gaynor, R.B. Cloning and characterization of a novel cellular protein, TDP-

- 43, that binds to human immunodeficiency virus type 1 TAR DNA sequence motifs. *Journal of virology* 69, 3584-3596 (1995).
118. Buratti, E. *et al.* Nuclear factor TDP-43 and SR proteins promote in vitro and in vivo CFTR exon 9 skipping. *Embo J* 20, 1774-1784 (2001).
 119. Johnson, B.S., McCaffery, J.M., Lindquist, S. & Gitler, A.D. A yeast TDP-43 proteinopathy model: Exploring the molecular determinants of TDP-43 aggregation and cellular toxicity. *Proc Natl Acad Sci U S A* 105, 6439-6444 (2008).
 120. Ayala, Y.M., Misteli, T. & Baralle, F.E. TDP-43 regulates retinoblastoma protein phosphorylation through the repression of cyclin-dependent kinase 6 expression. *Proc Natl Acad Sci U S A* 105, 3785-3789 (2008).
 121. Goedert, M., Spillantini, M.G., Cairns, N.J. & Crowther, R.A. Tau proteins of Alzheimer paired helical filaments: abnormal phosphorylation of all six brain isoforms. *Neuron* 8, 159-168 (1992).
 122. Grover, A. *et al.* 5' splice site mutations in tau associated with the inherited dementia FTDP-17 affect a stem-loop structure that regulates alternative splicing of exon 10. *J Biol Chem* 274, 15134-15143 (1999).
 123. Dickson, D.W., Rademakers, R. & Hutton, M.L. Progressive supranuclear palsy: pathology and genetics. *Brain pathology (Zurich, Switzerland)* 17, 74-82 (2007).
 124. Halliday, G.M. *et al.* Neuropathology in the S305S tau gene mutation. *Brain* 129, E40 (2006).
 125. Dickson, D.W. *et al.* Familial frontotemporal dementia with a novel tau exon-10 splice site mutation: nature confirms a theoretical construct. *Neurobiology of Aging* 21, S65 (2000).
 126. Basun, H. *et al.* Clinical characteristics of a chromosome 17-linked rapidly progressive familial frontotemporal dementia. *Arch Neurol* 54, 539-544 (1997).
 127. Zipper, H., Brunner, H., Bernhagen, J. & Vitzthum, F. Investigations on DNA intercalation and surface binding by SYBR Green I, its structure determination and methodological implications. *Nucleic acids research* 32, e103 (2004).
 128. Rovelet-Lecrux, A. *et al.* APP locus duplication causes autosomal dominant early-onset Alzheimer disease with cerebral amyloid angiopathy. *Nat Genet* 38, 24-26 (2006).
 129. Singleton, A.B. *et al.* alpha-Synuclein locus triplication causes Parkinson's disease. *Science* 302, 841 (2003).
 130. Chartier-Harlin, M.C. *et al.* Alpha-synuclein locus duplication as a cause of familial Parkinson's disease. *Lancet* 364, 1167-1169 (2004).
 131. Schouten, J.P. *et al.* Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. *Nucleic acids research* 30, e57 (2002).
 132. Johnson, J. *et al.* No evidence for tau duplications in frontal temporal dementia families showing genetic linkage to the tau locus in

- which tau mutations have not been found. *Neurosci Lett* 363, 99-101 (2004).
133. Llado, A. *et al.* MAPT gene duplications are not a cause of fronto-temporal lobar degeneration. *Neurosci Lett* 424, 61-65 (2007).
 134. Ishihara, T. *et al.* Age-dependent induction of congophilic neurofibrillary tau inclusions in tau transgenic mice. *Am J Pathol* 158, 555-562 (2001).
 135. Andorfer, C. *et al.* Hyperphosphorylation and aggregation of tau in mice expressing normal human tau isoforms. *J Neurochem* 86, 582-590 (2003).
 136. Zhang, B. *et al.* Microtubule-binding drugs offset tau sequestration by stabilizing microtubules and reversing fast axonal transport deficits in a tauopathy model. *Proc Natl Acad Sci U S A* 102, 227-231 (2005).
 137. Noble, W. *et al.* Inhibition of glycogen synthase kinase-3 by lithium correlates with reduced tauopathy and degeneration in vivo. *Proc Natl Acad Sci U S A* 102, 6990-6995 (2005).
 138. Engel, T., Goni-Oliver, P., Lucas, J.J., Avila, J. & Hernandez, F. Chronic lithium administration to FTDP-17 tau and GSK-3beta overexpressing mice prevents tau hyperphosphorylation and neurofibrillary tangle formation, but pre-formed neurofibrillary tangles do not revert. *J Neurochem* 99, 1445-1455 (2006).
 139. Le Corre, S. *et al.* An inhibitor of tau hyperphosphorylation prevents severe motor impairments in tau transgenic mice. *Proc Natl Acad Sci U S A* 103, 9673-9678 (2006).
 140. Pickhardt, M. *et al.* Anthraquinones inhibit tau aggregation and dissolve Alzheimer's paired helical filaments in vitro and in cells. *J Biol Chem* 280, 3628-3635 (2005).
 141. Pickhardt, M. *et al.* Phenylthiazolyl-hydrazide and its derivatives are potent inhibitors of tau aggregation and toxicity in vitro and in cells. *Biochemistry* 46, 10016-10023 (2007).
 142. Hardy, J. & Selkoe, D.J. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* 297, 353-356 (2002).
 143. Asuni, A.A., Boutajangout, A., Quartermain, D. & Sigurdsson, E.M. Immunotherapy targeting pathological tau conformers in a tangle mouse model reduces brain pathology with associated functional improvements. *J Neurosci* 27, 9115-9129 (2007).

Acta Universitatis Upsaliensis

*Digital Comprehensive Summaries of Uppsala Dissertations
from the Faculty of Medicine 418*

Editor: The Dean of the Faculty of Medicine

A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)

Distribution: publications.uu.se
urn:nbn:se:uu:diva-9550



ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2009