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# Biomarkers in genetic frontotemporal dementia – findings from the GENFI study

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Stockholm 2021

Published by Karolinska Institutet.
Printed by Universitetsservice US-AB, 2021

ISBN 978-91-8016-330-9
Cover illustration: Sara Spånghagen

# Biomarkers in frontotemporal dementia – findings from the GENFI study

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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The thesis will be defended in public at Karolinska University Hospital, Lars Klareskog NBU1:02, Gävlegatan 55. January 20, 2022, at 9 am.

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*“It is our choices that show what we truly are,*
*far more than our abilities.”*

*Albus Dumbledore*

# POPULAR SCIENCE SUMMARY OF THE THESIS

Frontotemporal dementia (FTD) is a collective term for a group of complex brain disorders. In FTD, the neurons in the frontal and temporal lobes of the brain die, leading to loss of brain volume (atrophy), but the mechanisms of how this is happening are not known. The frontal and temporal lobes are responsible for cognitive functions such as attention, decision-making, judgement, and language. The brain atrophy seen in FTD consequently results in patients having symptoms related to these functions. Typical symptoms of FTD are changes in personality, loss of empathy, and language difficulties. However, the symptoms are not the same in all individuals and it is sometimes hard to distinguish FTD from other types of dementia and from psychiatric disorders. There is no investigation nor test that can be used to confirm FTD and the diagnosis is based on the clinician's judgement. To make it even more complicated, the disease is ongoing inside the body and brain before any symptoms are noticeable. One example of this is the detection of brain atrophy in individuals already ten to fifteen years before clinical symptoms of FTD appear. Detectable traits of a disease, measured for example by imaging, as protein levels in spinal fluid, on cognitive tests etc, are called biomarkers. The purpose of the with this thesis was to investigate early changes and biomarkers in FTD. This could hopefully lead to improved diagnostic accuracy, and the development of effective treatments for FTD which unfortunately are lacking.

We know that FTD can be inherited and is sometimes caused by alterations in the genetic code (so-called mutations). Any person with a parent with FTD due to one of these mutations have 50% risk of developing FTD themselves. In the GENFI study, the participants belong to families with heritable FTD, and come for annual research visits. Each visit includes a thorough medical examination, imaging of the brain, cognitive testing, and collection of blood and spinal fluid. When the data is analysed, we can compare the results from participants with a mutation (but with no symptoms of FTD yet) to the results from participants without a mutation. The differences in for example protein level or cognitive performance might be signs of early FTD. We can also compare participants with FTD to the ones without symptoms, to investigate biomarkers that hopefully can verify that a person has developed FTD and not another disease.

This thesis presents results from the GENFI study. First, we confirmed that a certain mutation in chromosome 9 (*C9orf72*) is particularly common in patients with FTD in Sweden. We were more likely to find a mutation in a patient if other members of their family also had dementia. Second, we found that most individuals in the GENFI study improved their performance on cognitive tests at the follow-up visits. This is not surprising because the same tests were included on each occasion and the participants got familiar with them. Interestingly, individuals with a mutation did not have the same improvement over time as the other participants. We found the smallest improvement on cognitive tests in those individuals that were expected to soon show symptoms of FTD. This may be helpful for identifying individuals close to symptom onset so they can be offered support at an early stage in the disease. Finally, we discovered that the levels of several proteins in the spinal fluid and a few proteins in blood were different between individuals with and without symptoms of FTD. We do not yet know if the identified proteins can be used in the clinic as a biomarker of FTD. More studies are needed before we can evaluate the benefit of these measurements.

# POPULÄRVETENSKAPLIG SAMMANFATTNING

Frontallobsdemenssjukdom (FTD) är ett samlingsnamn för olika typer av mycket svåra demenssjukdomar. Vid FTD förtvinar nervcellerna i pann- och tinningloberna vilket leder till att hjärnans volym minskar (så kallad atrofi) men sjukdomsmekanismerna för hur och varför detta inträffar är inte klarlagda. De delar av hjärnan som drabbas av atrofi vid FTD reglerar funktioner såsom uppmärksamhet, beslutsfattande, bedömningsförmåga och språk. Därför är symptomen vid FTD kopplade till dessa funktioner och de vanligaste är personlighetsförändringar, minskad empati och språksvårigheter. Symptomen kan dock variera mycket mellan olika individer och likna andra sjukdomar vilket gör att det ibland kan vara svårt att skilja FTD från andra typer av demenssjukdomar eller från psykiatriska sjukdomar. Det finns ingen undersökning eller något test som kan bekräfta FTD och diagnosen baseras på en samlad medicinsk bedömning. Sannolikt börjar sjukdomsprocessen vid FTD innan några symptom är märkbara. Anledningen till att man tror sig veta det är bland annat att det är möjligt att påvisa atrofi i hjärnan upp till 15 år innan förväntat insjuknande. Mätbara förändringar som sker i kroppen och som är kopplade till en viss sjukdom kallas för biomarkörer. Målet med studierna i denna avhandling var att undersöka de tidiga stadierna av FTD och försöka hitta biomarkörer för sjukdomen. Detta för att på sikt förbättra diagnostiken och förhoppningsvis kunna utveckla en botande behandling mot FTD, något som tyvärr saknas idag.

FTD kan vara ärftlig och orsakas i dessa fall av en förändring i arvsmassan (så kallad mutation). I en familj med ärftlig FTD kommer ungefär hälften att insjukna och varje individ med en drabbad förälder har 50% risk att ärva den sjukdomsorsakande mutationen. I GENFI-studien inkluderas individer som tillhör dessa familjer och forskningsbesöken planeras en gång per år. Vid varje forskningsbesök får deltagaren genomföra flera olika moment (läkarbesök, magnetkameraundersökning av hjärnan, kognitiv testning, provtagning av blod och ryggvätska m.m.). När det insamlade materialet sedan analyseras jämförs resultaten mellan de personer som är bärare av mutationen (och sedermera kommer att insjukna i FTD) och de som saknar mutationen för att se skillnader på undersökningarna. På detta sätt kan vi studera de tidiga stadierna av FTD. Vi kan också jämföra individer med och utan symptom för att hitta biomarkörer som kan bekräfta att en individ drabbats av just FTD och inte någon annan sjukdom.

I denna avhandling presenteras resultat från GENFI-studien. Bland annat har vi bekräftat att mutation i genen *C9orf72* är särskilt vanligt förekommande hos personer med FTD i Sverige och att FTD generellt sett ofta är en ärftlig sjukdom. Vidare upptäckte vi att de flesta deltagare i GENFI förbättrar sina resultat på de kognitiva testerna vid återbesöken. Det är förvisso inte förvånande eftersom samma tester genomförs vid varje besök och deltagarna blir välbekanta med dem under studiens gång. Det intressanta är dock att individer som bär på en mutation inte visar samma mått av inlärning som övriga deltagare. En inlärning lägre än förväntat observerades framförallt hos individer som förväntas insjukna inom en snar framtid och detta skulle möjligtvis kunna användas för att tidigt upptäcka dem som snart kommer att bli sjuka. Slutligen identifierade vi skillnader i proteinnivåer i ryggvätska och blod mellan individer med och utan symptom på FTD. Det är för tidigt att veta huruvida det kommer vara användbart att mäta dessa proteiner inom sjukvården, till exempel som en del av diagnostiken vid FTD. Fler studier behövs för att utvärdera värdet av resultaten som presenteras här.

# ABSTRACT

Frontotemporal dementia (FTD) is a group of neurodegenerative diseases including a wide range of clinical phenotypes, neuropathological hallmarks, and genetic causes. People with FTD typically present with deficits in behaviour and/or language which largely overlap with symptoms of other types of dementia and primary psychiatric disorders. FTD is associated with considerable suffering for both patients and their next of kin, and there is unfortunately no effective treatment for FTD yet. In genetic FTD, the disease is inherited in an autosomal dominant pattern where several causative mutations have been identified. The Genetic frontotemporal Initiative study (GENFI) is a prospective study enrolling individuals with a 50% risk of genetic FTD. Research visits are performed annually including medical and neuropsychological assessments, magnetic resonance imaging of the brain, and collection of biofluids. The purpose of this thesis was to investigate FTD at different stages with the aim to find biomarkers for FTD. Currently, no biomarkers specific for FTD are being used in clinical practice, and finding reliable biomarkers is essential for diagnostic and prognostic purposes as well as for the development of therapeutic interventions.

In study I, we performed a genetic screen in an FTD cohort from Sweden and found that mutations were particularly frequent in the *C9orf72* gene. Interestingly, mutations were found in patients with apparent sporadic FTD suggesting that there are additional factors contributing to the development of disease.

In study II, practice effects of repeated neuropsychological testing were investigated in the GENFI cohort. Presymptomatic individuals carrying either a *C9orf72* or a *GRN* mutation had lower practice effects than controls. This study warrants for caution when interpreting potential treatment effects unless practice effects have been considered.

In studies III-V, different biomarkers in cerebrospinal fluid (CSF) and plasma were explored using a multiplexed suspension bead array technique. Several proteins in CSF, and some in plasma, were found at altered levels in patients with FTD compared to unaffected individuals. In addition, we present indications that a couple of CSF proteins may be altered already in a presymptomatic stage.

In summary, genetic and potential cognitive and fluid biomarkers were identified in this thesis. Additional studies are required to determine each biomarker's respective relevance in FTD, including their future value in a clinical setting.

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V. **Öijerstedt L***, Ullgren A*, Olofsson J, Bergström S, Remnestål J, Månberg A, van Swieten J, Jiskoot L, Seelaar H, Borroni B, Sanchez-Valle R, Moreno F, Laforce R, Synofzik M, Galimberti D, Rowe J, Masellis M, Tartaglia MC, Finger E, Vandenberghe R, de Mendonça A, Tagliavini F, Santana I, Ducharme S, Butler CR, Gerhard A, Levin J, Danek A, Otto M, Frisoni G, Ghidoni R, Sorbi S, Rohrer JD, Nilsson P, Graff C, on behalf of the Genetic Frontotemporal Dementia Initiative (GENFI). **A large-scale proteomic profiling of plasma in genetic FTD: a GENFI study**. *Manuscript.*

* shared first authors

# CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| AD | Alzheimer disease |
| ALS | Amyotrophic lateral sclerosis |
| AMC | Affected mutation carriers |
| AQP4 | Aquaporin 4 |
| ASO | Antisense oligonucleotide |
| AU | Arbitrary unit |
| AUC | Area under curve |
| AZGP1 | Zinc-alpha-2-glycoprotein |
| Aβ | Amyloid beta |
| bvFTD | Behavioural variant FTD |
| C9orf72 | Chromosome 9 open reading frame 72 |
| CBD | Corticobasal degeneration |
| CDR | Clinical dementia rating |
| CHGA | Chromogranin A |
| CHIT-1 | Chitotriosidase |
| CHMP2B | Charged multivesicular body protein 2b |
| CSF | Cerebrospinal fluid |
| CV | Coefficient of variance |
| DNA | Deoxyribonucleic acid |
| DPP6 | Dipeptidyl peptidase like 6 |
| DPR | Dipeptide repeat |
| EDTA | Ethylenediaminetetraacetic acid |
| EYO | Expected years to symptom onset |
| FCRST | The free and cued selective reminding test |
| FDG | $^{18}$F-fluoro-2-deoxyglucose |
| FDR | False discovery rate |
| FPI | Frontotemporal prevention initiative |
| FTD | Frontotemporal dementia |
| FTLD | Frontotemporal lobar degeneration |
| FUS | Fused in sarcoma |
| GENFI | Genetic Frontotemporal dementia Initiative |
| GFAP | Glial fibrillary acidic protein |
| GRN | Progranulin |
| HLA-DQA2 | Major histocompatibility complex-DQA2 |
| ICD-10 | International Statistical Classification of Diseases and Related Health Problems – Tenth Revision |
| LASSO | Least absolute shrinkage and selection operator |
| LRP1 | Low density lipoprotein receptor-related protein 1 |
| lvPPA | Logopenic variant PPA |

| | |
|---|---|
| M6PR | Mannose 6-phosphate receptor |
| MAPT | Microtubule associated protein tau |
| MFI | Median fluorescence intensity |
| Mini-SEA | Mini social cognition and emotional assessment |
| MRI | Magnetic resonance imaging |
| NACC | National Alzheimer's Coordinating Center |
| NC | Non-carriers |
| NEFL/NfL | Neurofilament light polypeptide |
| NEFM/NF-M | Neurofilament medium polypeptide |
| nfvPPA (PNFA) | Non-fluent variant PPA |
| NPTXs | Neuronal pentraxins |
| PCA | Principle component analysis |
| PDYN | Prodynorphin |
| PET | Possitron emission tomography |
| PMC | Presymptomatic mutation carriers |
| PPA | Primary progressive aphasia |
| PSAP | Prosaposin |
| PSP | Progressive supranuclear palsy |
| p-tau | Phosphorylated tau |
| RBP | RNA binding protein |
| RNA | Ribonucleic acid |
| S100A12 | S100 calcium binding protein A12 |
| SNAP-25 | Synaptosome associated protein 25 |
| SNV | Single nucleotide variant |
| SORT1 | Sortilin 1 |
| SweFTDi | Swedish FTD Initiative |
| svPPA (SD) | Semantic variant PPA |
| TBK1 | TANK-binding kinase 1 |
| TDP-43/TARDBP | TAR DNA-binding protein 43 |
| TMEM | Transmembrane protein |
| TN-R | Tenacin R |
| TREM2 | Triggering receptor expressed on myeloid cells 2 |
| t-tau | Total tau |
| UNC13A | Unc-13 homologue A |
| VCP | Valosin containing protein |
| VGF | Neurosecretory protein VGF |
| VUS | Variant of uncertain significance |
| XPO5 | Exportin 5 |
| YKL-40 | Chitinase 3 like 1 (also otherwise abbriviated CHI3L1) |

# 1 INTRODUCTION

## 1.1 FRONTOTEMPORAL DEMENTIA – AN OVERVIEW

Frontotemporal dementia (FTD) is a collective term for a group of neurodegenerative diseases with heterogeneous phenotypes, genetic causes, and underlying neuropathology. FTD is characterised by progressive neurodegeneration in the frontal and temporal lobes of the brain (1,2). The underlying mechanisms leading to FTD are still unknown and the clinical diagnoses are based on consensus criteria (3,4). The first historical note of FTD was made by Dr Arnold Pick in 1892 who described a patient with progressive aphasia, behavioural disturbances, and focal left temporal lobe atrophy (5). A few years later, similar cases were found to have a common, distinct histopathology, and the disease was named "Pick's disease". Almost a hundred years after this discovery, a large series of patients were described as having frontal lobe degeneration. However, only few of the cases had histopathological findings corresponding to the ones in Pick's disease and the clinical terms "frontal lobe degeneration of non-Alzheimer type" or "dementia of frontal type" were instead established. The first diagnostic criteria for FTD were introduced in 1994 (Lund and Manchester) and have since then been modified and the term FTD now includes several different clinical phenotypes, covered in section 1.2 Clinical symptoms and overlapping disorders (3,4,6).

FTD accounts for approximately 10% of all dementia cases in individuals under the age of 65 years, but different prevalence and incidence rates have been reported globally (7,8). According to the National Board of Health and Welfare, the incidence rate of dementia in Sweden is 20 000 to 25 000 cases per year (9). The classification code used for clinical FTD in Sweden (ICD-10 F02.0 Picks sjukdom) is still linked to Pick's disease, which today is restricted to a neuropathological diagnosis, complicating accurate statistical analyses of incidence rates. Nevertheless, based on international reports, we would expect to find approximately 500 to 1000 new FTD cases per year in Sweden. The disease duration is highly variable, but the mean survival is around 6 to 12 years (10).

Up to half of the people with FTD have another family member with dementia (11). In some of these families, a disease-causing genetic variant (mutation) is identified which can explain the hereditary nature of the disease in the family. The mutation can be inherited from parent to offspring and the disease is passed on for generations. The three most common genes, where mutations causing genetic FTD are found, are chromosome 9 open reading frame 72 (*C9orf72*), progranulin (*GRN*), and microtubule-associated protein tau (*MAPT*) (12–16).

## 1.2 CLINICAL SYMPTOMS AND OVERLAPPING DISORDERS

The clinical presentation of FTD is diverse and includes several phenotypes and neurological syndromes (Figure 1) (17).

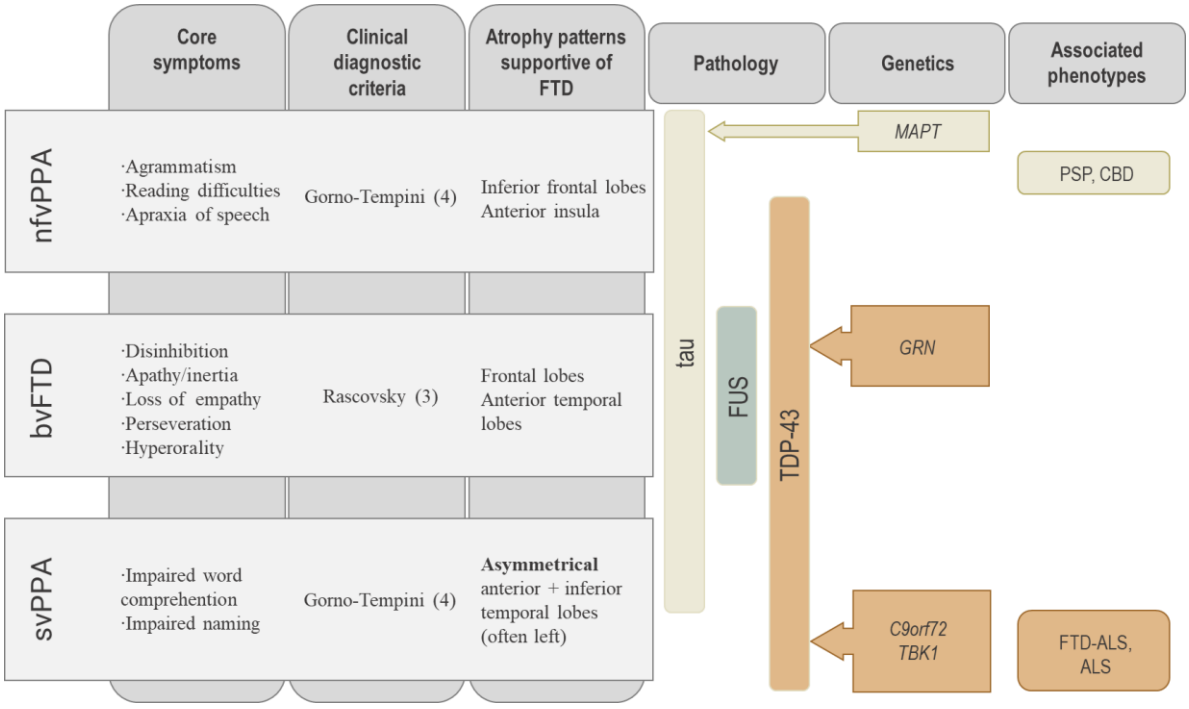


Figure 1. *Overview of characteristic features of the three main phenotypes in FTD. nfvPPA, non-fluent variant primary progressive aphasia; bvFTD, behavioural variant frontotemporal dementia; svPPA, semantic variant primary progressive aphasia; FUS, fused in sarcoma; TDP-43, 43 kDa transactive response DNA-binding; MAPT, microtubule associated protein tau; GRN, progranulin; C9orf72, chromosome 9 open reading frame 72; TBK1, TANK binding kinase 1; PSP, progressive supranuclear palsy; CBD, corticobasal degeneration; ALS, amyotrophic lateral sclerosis.*

A common feature in all clinical presentations is the progressive nature, meaning that a deterioration of cognitive functions and behaviour must be seen over time. Patients typically have little or no insight into their impairments. The phenotypes described below are more distinct from one another at an early stage and as the disease progresses the symptoms become more similar, i.e. behavioural symptoms can occur in language variants and vice versa.

### 1.2.1 Behavioural variant FTD

The behavioural variant (bvFTD) is the most common clinical phenotype, seen in around 60% of all FTD cases. Patients present predominantly with behavioural and personality changes. As described above, the current consensus criteria were developed in 2011 (3). There are three levels of diagnostic certainty: possible, probable, and definitive bvFTD. To fulfil the criteria for possible bvFTD, three of the following symptoms must be present and deteriorate over time: disinhibition, apathy/inertia, loss of sympathy/empathy, perseverative/stereotyped behaviour, dietary changes and/or typical neuropsychological profile (described as executive dysfunction but with preserved memory and visuospatial abilities) (3). However, recent literature regarding cognitive impairments in FTD shows ambiguous results and this "typical bvFTD neuropsychological profile" has been questioned (18,19) (see section 3.2 Clinical biomarkers). To meet the criteria for probable bvFTD, the patient should have functional decline, meaning that the behavioural and cognitive deficits interfere with independence in everyday activities (for example not being able to manage finances, prepare meals or care for other people etc.). Moreover, probable bvFTD requires imaging findings supportive of the disease and bvFTD is characterised by grey matter atrophy of the frontal lobes (prefrontal cortex, frontal insula) and/or anterior temporal lobes. The atrophy is primarily right-sided or including both hemispheres (20,21). The highest level of diagnostic certainty (definitive bvFTD) is limited to the individuals who fulfil criteria for probable bvFTD and in addition have histopathological evidence of frontotemporal lobar degeneration (see section 1.3 Neuropathology) or a known pathogenic mutation (see section 2.2 Genetics of FTD).

There is a considerable overlap in symptoms between different neurodegenerative diseases and the clinicopathological correlation is not complete, leading to misdiagnoses in clinical practice (22). Even so, longitudinal studies of the natural progression of FTD have shown that the majority of patients with probable bvFTD will deteriorate over time and eventually fulfil the criteria for definitive bvFTD (19,23). In contrast, the diagnostic reliability in possible bvFTD is not as high. Some of patients with possible bvFTD will in time fulfil criteria for probable bvFTD but a significant group has no, or very slow, progression. This latter group, labelled FTD phenocopy syndrome and consisting predominantly of men, will never have a frontotemporal atrophy or symptoms that impair their activities of daily living (24).

### 1.2.2 Primary progressive aphasia

Other major FTD phenotypes, besides bvFTD, are the primary progressive aphasias (PPA) (Figure 1). There are three PPA phenotypes; progressive non-fluent variant PPA (nfvPPA or PNFA), semantic variant PPA (svPPA or SD), and logopenic variant PPA (lvPPA) (4). LvPPA most often have underlying Alzheimer pathology which will not be covered in this thesis (25). In nfvPPA, patients have impaired speech production with hesitant speech and a lot of phonetic errors. Additionally, nfvPPA presents with agrammatism and problems reading, particularly unfamiliar words. Imaging findings supporting a diagnosis of nfvPPA are grey matter atrophy

of the inferior frontal lobe and anterior insula (4,26). Patients with svPPA present with impaired semantic memory, i.e. the meaning of words and knowledge that you have gained throughout your life. Thus, patients have difficulties comprehending words but the speech itself is effortless. The performance on verbal tasks is poor but they have preserved visuospatial and problem-solving skills when tested on non-verbal tasks (27). Grey matter atrophy localised predominantly to the left anterior and inferior temporal lobe is supportive of a svPPA diagnosis. However, cases with right temporal atrophy have been described and these patients present with more behavioural symptoms than what is typically seen in left-sided svPPA (28).

### 1.2.3 Overlapping syndromes

The challenges in diagnosing FTD are on the one hand contributed to the phenotypic diversity, making it hard to distinguish FTD from for example Alzheimer disease (AD) or primary psychiatric disorders, but also to the fact that there are many overlapping syndromes included in the term (29). About 15% of FTD cases are diagnosed with motor neuron disease and vice versa (30,31). The most common type of motor neuron disease is amyotrophic lateral sclerosis (ALS) and it is characterised by the progressive degeneration of both upper and lower motor neurons resulting in muscle atrophy and eventually loss of control of all voluntary movements (32). During the last ten years, the relationship between FTD and ALS has become more and more clear as they share both genetic and pathological features (Figure 1) (12,33). Even if there is a relatively small proportion of patients that fulfil the criteria for both diseases, some reports suggest that about 40% of FTD cases have symptoms of motor neuron disease and as many as 80% of ALS cases have behavioural impairment to some extent, and that these symptoms may predate motor symptoms (30,34).

Progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD), sometimes called atypical parkinsonism, are tauopathies (section 1.3 Neuropathology) and thus considered to be part of the umbrella term of FTD (Figure 1) (35). Besides cognitive decline, PSP is characterised by postural instability and supranuclear gaze palsy while CBD appears with limb apraxia, asymmetrical rigidity, and language impairment (36,37). In addition, about 20% of FTD cases have signs of parkinsonism and these are mainly associated with the bvFTD and nfvPPA phenotypes (1).

Furthermore, it is not uncommon that symptoms of FTD are misinterpreted as originating from a primary psychiatric disorder (29). Interestingly, patients with FTD, especially *C9orf72* mutation carriers, can also present with psychiatric symptoms such as delusions, hallucinations and mania which might precede other symptoms (38,39). In addition, there is an overrepresentation of neuropsychiatric disorders (autism spectrum disorders, attention deficit hyperactivity disorder etc.) as well as psychiatric diseases in families with genetic FTD, that might be underestimated (40).

## 1.3 NEUROPATHOLOGY

Patients with FTD show selective neurodegeneration in the frontal and temporal lobes which can be observed macroscopically in post-mortem brain and is termed frontotemporal lobar degeneration (FTLD). The neuronal loss is usually accompanied by microvacuolisation, astrocytic gliosis and myelin loss (41). Immunohistochemical staining of post-mortem brain tissue can identify the type, shape, and location of the protein aggregates/inclusions associated with FTLD. The first aggregated protein to be identified in FTLD was tau (primary tauopathies, FTLD-tau). In the tau-negative FTLD cases, a majority were initially found to have ubiquitin-positive inclusions. In 2006, the most common component of these inclusions were recognised as 43 kDa transactive response DNA-binding (FTLD-TDP) (Figure 1) (42,43). FTLD-TDP is further subclassified into four categories (A-D) based on the different inclusions. A revision of the current criteria has been proposed since some cases do not fit the current classification, for example due to features of multiple subtypes or completely novel inclusion patterns (44,45). Patients with mutations in the *C9orf72* or *GRN* genes have FTLD-TDP, whereas patients with mutations in *MAPT* have FTLD-tau (46). It is not possible to predict the underlying neuropathological subtypes based on clinical phenotype, even if svPPA is mostly associated with TDP-43 type C and nfvPPA with type A and B (47). A small proportion of FTLD cases are tau-negative and TDP43-negative but ubiquitin positive. Inclusions containing the protein fused in sarcoma (FUS) have been found in many of these cases (FTLD-FUS) (48). In a few ubiquitin-positive cases, the misfolded protein has not yet been identified (FTLD-UPS). Finally, in very rare cases, no inclusions have been found and they are categorised as FTLD-ni (no inclusions) (46).

# 2 RISK FACTORS ASSOCIATED WITH FTD

## 2.1 ENVIRONMENTAL RISK FACTORS

There are few studies on environmental and lifestyle risk factors in FTD (49). Among the factors investigated in several studies is cognitive reserve. Cognitive reserve is the individual differences in how the brain copes with damage making some people more resilient to pathological changes (50). Measures of the cognitive reserve are education, occupation, social and leisure activities among others, which have been associated with FTD (51,52). For example, low education correlates to decreased grey matter volume in genetic FTD (53). In addition, known risk factors of other types of dementia, for example traumatic brain injury, cardiovascular diseases and diabetes mellitus have been proposed in FTD (49). Retrospective studies indicate that having a traumatic brain injury increases the risk of developing FTD later in life (54,55). However, cardiovascular diseases (such as hypertension and hyperlipidaemia) and diabetes have not been associated with FTD (56).

## 2.2 GENETICS OF FTD

The most important and studied risk factors for FTD, in addition to aging, are genetic. FTD can be divided into genetic and sporadic cases. In families with genetic FTD, the inheritance pattern is autosomal dominant meaning that one gene copy carrying a mutation (inherited from either parent) is enough to develop the disease (11). There is a 50% risk for each child to inherit the mutation from an affected parent. To date, mutations in several genes are identified as the cause of genetic FTD but in some families with apparent inherited disease, the genetic cause remains undiscovered (57). Cases in families with a single affected individual, and where no causative mutation can be identified, are called sporadic FTD. In these cases, multiple genetic variations can increase the risk of FTD but the relationship between each variant and developing the disease is far weaker than in genetic FTD (58).

### 2.2.1 Causative mutations in FTD

#### *2.2.1.1 C9orf72 repeat expansion*

A non-coding repeat region of six base pairs, GGGGCC ($G_4C_2$), is located in intron 1 in the *C9orf72* gene in chromosome 9. When this $G_4C_2$ region is expanded, up to several hundred or thousand repeats, it causes FTD and/or ALS (12,13). Overall, a *C9orf72* expansion is identified in 6-8% of sporadic FTD and 25-42% of genetic FTD, making it the most common genetic cause of the disease (59,60). However, there is a large variation in mutation frequencies in FTD across different populations (61). The highest frequency is found in eastern North America and northern European countries, especially Sweden and Finland, while a much lower prevalence is found in Asian countries (62). Reports of *C9orf72* expansion frequency in African populations are still missing (63).

Exactly how the *C9orf72* repeat expansion is causing neurodegeneration is still largely unknown (64). Multiple disease mechanisms have been proposed (Figure 2).

Loss of function of the C9orf72 protein due to reduced transcription of the gene (haploinsufficiency, i.e. the protein production from the non-mutated allele is not sufficient to maintain normal function) has been suggested (65). The *C9orf72* gene is expressed in most human tissues and three different transcripts exist (variants 1, 2, and 3) (Figure 2**)**. Supporting the hypothesis of haploinsufficiency, lower levels of all three mRNA variants are found in blood cells and brain tissue from *C9orf72* mutation carriers (12,66). In addition, protein levels of C9orf72 are lower in the frontal cortex of mutation carriers with FTD compared to controls (67). However, as the normal functions of the C9orf72 protein are not fully understood, the downstream effects of reduced *C9orf72* translation have not been described in detail. C9orf72 has been suggested to be involved in membrane trafficking, synaptic activity and autophagy (64,65), which are all implicated as possible disease mechanisms in FTD and ALS. In *C. elegans* and zebrafish animal models, knockdown of *C9orf72* causes behavioural changes and motility/motor neuron defects (68,69). In contrast, Koppers et al. subsequently showed that *C9orf72* knockout mice do not develop signs of motor neuron degeneration, gliosis or TDP-43 pathology (70). Taken together, even if some *in vivo* experiments have shown that a loss of C9orf72 is associated with neurodegeneration and dementia-like phenotypes, there is still no knockdown/knockout model that directly links loss-of-function to the development of FTD related pathology, i.e. the formation of TDP-43 aggregates.

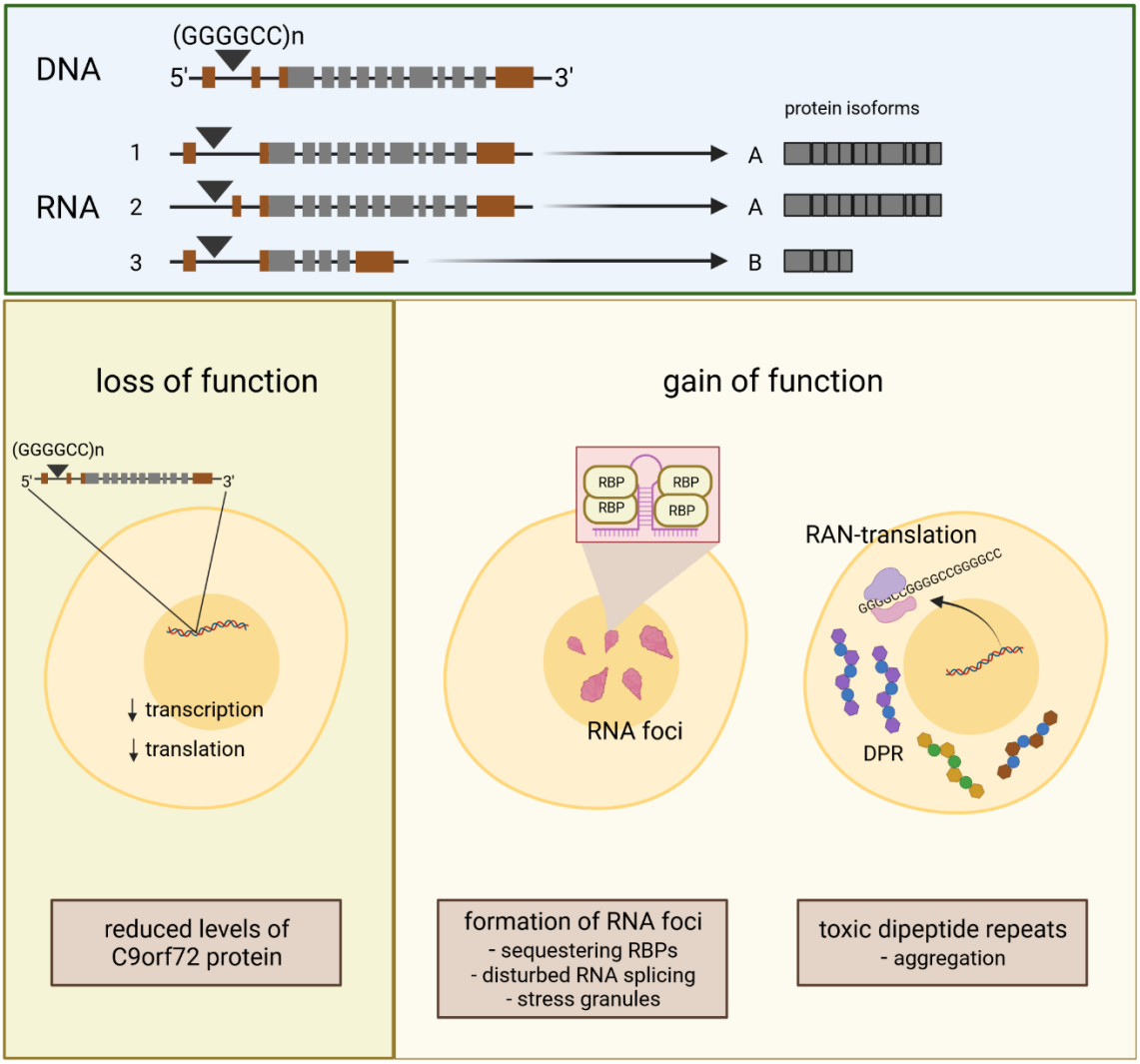


Figure 2. *Disease mechanisms in FTD due to the C9orf72 repeat expansion mutation. The GGGGCC repeat is located in a non-coding region and three different transcripts exist. Translation results in either a long (A) or short (B) protein isoform. Loss of function leading to reduced transcription and translation of C9orf72 protein. Gain of toxic function related to the formation of RNA foci and/or dipeptide repeats. RBP, RNA binding protein; DPR, dipeptide repeat. Figure created with BioRender.com*

Other proposed mechanisms of how the *C9orf72* expansion causes neuronal death are gain-of-function through the formation of RNA foci and insoluble dipeptides (Figure 2**)**. After the *C9orf72* gene is transcribed, the mRNA including the expanded region is forming RNA foci localised in the nucleus (12). Essential RNA binding proteins (RBPs) then bind to these structurally complex constructs, possibly prohibiting them to function properly. The sequestering of RBPs is proposed to lead to dysregulation of mRNA splicing and disruption of translation also of other proteins (65). Not long after the discovery of the *C9orf72* expansion, the research group of Leonard Petrucelli found that the expanded mRNA was translated in an unusual way independently of a start codon, so-called repeat-associated non ATG-initiated (RAN) translation (71,72). RAN translation has been observed in other repeat expansion diseases including Huntington disease and spinocerebellar ataxia and can produce proteins in all (three) different reading frames in both directions (73). Since the *C9orf72* expansion

constitutes six base pairs (GGGGCC), the repeat is translated into three dipeptides (DPRs) from the sense strand: poly(GA), poly(GP) and poly(GR), and three dipeptides from the antisense strand: poly(PR), poly(GP) and poly(PA) (Table 1). Note that poly(GP) is translated from both the sense and antisense strands. These dipeptides accumulate in the neurons and have been identified in different brain tissue samples from patients with FTD/ALS carrying the *C9orf72* repeat expansion mutation (72,74).

Table 1. *Possible products of the C9orf72 repeat expansion mutation. Gly, glycin; Ala, alanine; Pro, proline; Arg, arginine.*

| Repeat | RNA strand | Reading frame | RAN protein | Dipeptide name |
|---|---|---|---|---|
| $(GGGGCC)_n$ | Sense | GGG GCC GGG GCC GGG GCC etc. | $(Gly\ Ala)_n$ | Poly(GA) |
| | | GGG CCG GGG CCG GGG CCG etc. | $(Gly\ Pro)_n$ | Poly(GP) |
| | | GGC CGG GGC CGG GGC CGG etc. | $(Gly\ Arg)_n$ | Poly(GR) |
| $(CCCCGG)_n$ | Antisense | CCC CGG CCC CGG CCC CGG etc. | $(Pro\ Arg)_n$ | Poly(PR) |
| | | CCC GGC CCC GGC CCC GGC etc. | $(Pro\ Gly)_n$ | Poly(GP) |
| | | CCG GCC CCG GCC CCG GCC etc. | $(Pro\ Ala)_n$ | Poly(PA) |

Numerous studies have tried to elucidate the cytotoxicity of RNA foci and dipeptides, and their respective contributions to disease pathogenesis (75). Animal models using *D. melanogaster* have shown that both the formation of RNA foci and translation of DPRs causes neurodegeneration. When comparing fruit flies developed to express only RNA foci to those with only DPRs, the dipeptide aggregates were found to be the most cytotoxic (76). In mice expressing the *C9orf72* repeat expansion, both RNA foci and DPRs are present in the brain tissue. However, even if some studies have shown that mice with the *C9orf72* repeat expansion develop neurodegeneration and a phenotype including behavioural changes, others have failed to establish a connection to clinical presentation (77–79). A link between DPRs and the formation of TDP-43 aggregates was not uncovered until Cook et al. demonstrated that poly(GR) induces and accelerates TDP-43 aggregation in mice (80). Furthermore, they showed that poly(GR) alone is sufficient to cause neurodegeneration and TDP-43 pathology.

In summary, the pathological processes by which the *C9orf72* repeat expansion cause FTD and ALS include different aspects and are probably a combination of loss-of-function and toxic gain-of-function processes. Although it is clear that both RNA foci and DPRs are major components in these processes, the relative contribution of each part is yet to be explained.

#### 2.2.1.2 Progranulin

More than 130 unique mutations in *GRN* have been found to cause FTD (61,81). Different types of mutations have been discovered (substitutions, insertions and deletions). The most common are p.Thr272fs and p.Arg493X with 95 and 22 families identified worldwide, respectively. All causative mutations in *GRN* are null mutations meaning that no functional protein is being produced by the mutated allele and this leads to haploinsufficiency. *GRN* mutations account for approximately 15-25% of genetic FTD cases (82).

The *GRN* gene is located in chromosome 17 and translates into the progranulin precursor protein. Progranulin is expressed in neurons and microglia as well as in several peripheral tissues (83). Progranulin is either secreted intact or cleaved into different smaller granulins inside the lysosomes, and are involved in various processes such as cell migration, survival, repair, and inflammation, all of which will not be covered in this thesis (84). Homozygous carriers of a *GRN* mutation develop neuronal ceroid lipofuscinosis, a lysosomal disease presenting in childhood with symptoms of neurodegeneration including retinopathy, seizures and cerebellar ataxia (85). Heterozygous *GRN* carriers, on the other hand, develop FTD and the association between the diseases has generated interest in looking into the role of progranulin in lysosomes and its potential contribution to the pathogenesis of FTD (discussed below) (86). Since all FTD-related *GRN* mutations are causing mRNA degradation and haploinsufficiency, the consequence is an approximate 50% reduction in functional protein level. Indeed, progranulin protein levels are reduced in lymphoblasts and brain tissue from patients with FTD with a *GRN* mutation, and plasma progranulin levels can predict mutation status (14,15,87). Results from numerous animal model studies also confirm that *GRN* haploinsufficiency causes behavioural disturbances, neurodegeneration and neuroinflammation (88–92). During the past years, the role of progranulin in lysosomes has been discovered and the implication of lysosomal dysfunction as a protagonist in FTD pathology is particularly intriguing (86).

The lysosomal trafficking of progranulin is mediated by two independent pathways, either by binding to the receptor sortilin (SORT1) or indirectly via binding to the protein prosaposin (PSAP). When progranulin binds to SORT1, it is transported across the cell surface and further into the lysosomes (93). Blocking SORT1 results in an increase of extracellular progranulin. The other pathway of progranulin transportation is through the PSAP receptors (low density lipoprotein receptor-related protein 1, LRP1, or mannose 6-phosphate receptor, M6PR) while bound to PSAP. Likewise, PSAP can be transported via SORT1 when bound to progranulin. Progranulin and PSAP thus facilitate each other's lysosomal trafficking. Once inside the lysosomes, progranulin is cleaved into granulins but the lysosomal functions of both progranulin and granulins remain unknown (94,95). Taken together, it is well established that *GRN* haploinsufficiency is a major cause of neurodegeneration. However, the exact function of progranulin and how the reduction of functional protein contributes to the formation of TDP-43 aggregates are still actively investigated.

#### *2.2.1.3 Microtubule associated protein tau*

Another gene in chromosome 17 is *MAPT*, which encodes the protein tau. The presence of mutations in *MAPT* was the first recognised monogenic explanation for FTD (16,61). Mutations in *MAPT* can act on either the RNA or protein level. There are six different tau protein isoforms in the human brain, depending on the splicing of exons 2, 3, and 10 (96). Alternative splicing of exon 10 results in either three repeat tau (3R) or four repeat tau (4R) and the ratio between these two, in the normal brain, is 1. Splice-site mutations in exon 10 or intron 10, resulting in an altered ratio between 3R and 4R, have been shown to cause primary tauopathies (96). On the protein level, *MAPT* missense mutations mostly act by disrupting the ability of tau to bind to the microtubule, leading to unbound hyperphosphorylated tau which is prone to aggregate (97). How tau aggregation leads to neurodegeneration has not been entirely clarified. More recent findings indicate that tau has prion-like properties meaning that tau can be released from neurons and spread through the nervous system (98).

#### *2.2.1.4 Rare genetic causes*

There are also other rarer genetic causes of FTD (11). Mutations causing autosomal dominant FTD have for example been found in the genes charged multivesicular body protein 2b (*CHMP2B*), valosin-containing protein (*VCP*), and TANK binding kinase 1 (*TBK1*) (99–101). The latter has similarities with the *C9orf72* repeat expansion mutation as mutations in *TBK1* can cause both ALS and FTD. In addition, TDP-43 positive inclusions are found in post-mortem brain tissue from patients with FTD and *TBK1* mutations (102).

### 2.2.2 Genotype-phenotype correlations

Several studies have investigated the clinical characteristics associated with the different types of genetic FTD (103–105). The mean age at onset is younger in *MAPT* mutation carriers compared to *C9orf72* and *GRN* (61) (Table 2). However, the age range is particularly wide in *GRN* mutation carriers. Within the same family, the age at onset can vary with more than 30 years and *GRN* carriers might develop symptoms as late as in their eighties or not at all (61,106,107). All clinical phenotypes are represented in genetic FTD, where FTD-ALS have the strongest and svPPA the weakest associations to a genetic cause (11). Mutation carriers with the *C9orf72* repeat expansion mutation most often present with bvFTD and/or ALS. *C9orf72* cases are also more frequently associated with psychiatric symptoms, compared to cases without the expansion (108). PPA is not very common in *C9orf72* mutation carriers, and seldom a primary symptom, nor is parkinsonism. In *GRN* and *MAPT* mutation carriers, bvFTD is the most common phenotype. Furthermore, the proportion of nfvPPA is higher in *GRN* cases than in other mutation carriers (11). Parkinsonism is mostly associated with *GRN* and *MAPT* whereas FTD-ALS is rarely seen in these carriers (109).

Table 2. *Comparison of clinical phenotypes and symptoms of FTD between C9orf72, GRN and MAPT mutation carriers.*

| | ***C9orf72*** | ***GRN*** | ***MAPT*** |
|---|---|---|---|
| Mean age at onset | 58 years | 61 years | 50 years |
| bvFTD | ++++ | ++++ | ++++ |
| nfvPPA | + | +++ | ++ |
| svPPA | + | (+) | (+) |
| FTD-ALS | +++ | 0 | 0 |
| PSP | (+) | 0 | + |
| CBD | (+) | ++ | + |
| Parkinsonism | + | +++ | ++ |
| Psychiatric symptoms | ++ | 0 | 0 |

### 2.2.3 Genetic modifiers and reduced penetrance

Even if there are correlations between genotype and phenotype, these are not generalisable for the whole FTD population. Within the same family, some individuals with a *C9orf72* repeat expansion mutation may develop FTD, some ALS and others a combination of the two (12). In addition, the penetrance of *C9orf72* and *GRN* mutations is reduced, i.e. some individuals carry a pathogenic mutation without ever developing symptoms of cognitive impairment (110,111). Both the diverse phenotypes, variable ages at onset (especially in *GRN* cases) and reduced penetrance suggest the involvement of other (genetic) modifiers. Several studies have investigated whether the number of *C9orf72* repeats is correlated to disease severity or age at onset, as it is in the case of the CAG repeat expansion in Huntington disease (112,113). The cut-off where the *C9orf72* repeat is pathogenic is not established but more than 30 repeats is usually considered causative. In the general population, around 90% have less than 10 repeats, and more than 24 repeats are very uncommon (114). There is no convincing evidence that the number of repeats are associated with clinical phenotype, disease duration or age at onset in FTD or ALS (113). Moreover, it is not yet established what the effect is of an intermediate repeat length (20-30 repeats). It has been suggested that intermediate alleles are a risk factor of ALS but not FTD (108). Additionally, a variability in the number of repeats have been observed between tissues, meaning that even if an intermediate repeat length is measured in peripheral blood, longer repeats may be detected in neurons (115).

Apart from the rare causative mutations (pathogenic genetic variations), other genetic factors might modulate disease characteristics. To date, 19 genome-wide association studies (GWAS) have been reported, aiming to find genetic risk loci associated with FTD (116). The first variants were discovered in TDP-43-confirmed cases and located in the gene transmembrane protein 106 b (*TMEM106B*). Certain common single nucleotide variants (SNVs), all spanning

the locus of *TMEM106B*, were associated with FTD-TDP (117). The findings have later been replicated and *TMEM106B* SNVs are considered to be modifiers of penetrance and age at onset in both *GRN* and *C9orf72* mutation carriers (118,119). Interestingly, *TMEM106B* SNVs are not associated with ALS, despite that this phenotype has underlying TDP-43 pathology. On the other hand, having a *TMEM106B* SNV is correlated to lower cognitive function in patients with ALS (119,120). Besides *TMEM106B*, additional risk loci have been identified in a large cohort of FTLD-TDP without mutations in known FTD genes: rs5848, located in *GRN*, and variants in *DPP6*, *UNC13A* and *HLA-DQA2*, as well as genes known to be involved in the innate immune pathway (121). Variants in *UNC13A* were not associated with ALS but with increased risk of cognitive impairment and FTD in these patients.

# 3 BIOMARKERS IN FTD

## 3.1 BIOMARKER HYPOTHESIS

Once cognitive or behavioural symptoms become noticeable, and activities of daily life start to deteriorate, the neurodegeneration is already prominent (122). Finding biomarkers that reflect the early changes could elucidate the processes involved in FTD and potentially explain the underlying pathology. Even though there are many contributing factors influencing the clinical presentation of FTD, the genetic penetrance is generally high and mutation carriers are considered to be in a presymptomatic phase of the disease. To explore these presymptomatic phases of dementia, studies on the genetic forms have been particularly established in AD. Jack et al. have proposed a widely accepted hypothetical model of AD biomarkers where the relationship between different biomarkers and the change over time is described (123). According to the model, pathological processes precede apparent clinical symptoms were amyloid deposits (indirectly measured as reduced amyloid beta levels in CSF and by positron emission tomography) are the first to appear, followed by increased levels of tau in CSF, structural changes in the brain, and lastly cognitive decline. Several studies have presented evidence that supports this model (124–126).

Also in FTD, it has been suggested that biochemical and structural changes within the brain occur before any clinical symptoms become evident (122,127). No specific FTD biomarkers are currently being used in clinical practice and investigations such as magnetic resonance imaging (MRI), positron emission tomography (PET), CSF and blood sample analysis are primarily conducted to eliminate other underlying causes of cognitive and/or behavioural decline. To improve clinical diagnostics, biomarkers specific for FTD are essential. Reliable biomarkers can have different purposes, for example to differentiate FTD from other dementias and psychiatric disorders, identify different subtypes of FTD (clinical phenotypes, underlying pathology etc.), or discover pathophysiological mechanisms in FTD. Furthermore, robust

outcome measures in clinical trials are necessary to be able to evaluate a potential treatment response.

### 3.2 CLINICAL BIOMARKERS

The current clinical criteria for bvFTD have high sensitivity and specificity (3). However, there is no specification of what rating scales or neuropsychological tests to use in the evaluation of the different symptoms. The methods of obtaining information on which the assessment is based (e.g. clinical judgement, caregiver interview, questionnaires etc.) probably vary across clinics and the lack of standardisation will impact the accuracy of the diagnostic criteria. The core cognitive features stated in the bvFTD clinical criteria are *"executive/generation deficits with relative sparing of memory and visuospatial functions"*. Even if executive dysfunction is observed in a majority of patients with bvFTD, this does not seem to be a distinctive feature in all patients, and executive function tests are not sensitive for detecting early bvFTD (18,19). Moreover, recent literature shows that episodic memory is indeed impaired in bvFTD and that the performance lies at an intermediate level between controls and patients with AD (128–130). The focus has thus moved to investigating other cognitive domains, and deficits in for example social cognition have been proposed as a more sensitive marker for bvFTD than memory impairment and executive dysfunction (18). Social cognition is important for people's understanding of and interaction with other people, and it involves processing and applying information gained through contact with others. Patients with impaired social cognition may for instance have trouble recognising facial emotions and/or understanding other people's mental states (so-called theory of mind) including not being able to detect sarcasm (131). In bvFTD, the ability to recognise emotions is impaired, especially for negative emotions such as anger and fear (132). However, there is currently no single cognitive test nor test battery that can distinguish FTD from other dementias with high accuracy.

The widely used Clinical Dementia Rating (CDR®) was developed in 1982 as a disease staging scale in AD (133). CDR® is based on interviews with the patient or next of kin including six domains namely memory, orientation, judgement/problem solving, community affairs, home and hobbies, and personal care (Figure 3). It can be calculated either as a sum of the individual score from each domain (sum of boxes), or as a global score where 0 is no cognitive impairment, 0.5 uncertain cognitive impairment, 1 mild, 2 moderate, and 3 severe cognitive impairment. The memory domain is contributing more to the calculation of the global score for CDR®, relative to the other domains. CDR® is not a sensitive instrument for detecting early FTD (134). To increase the sensitivity, two modules have been added to the CDR®: behaviour and language (CDR NACC, previously FTLD-CDR or CDR-FTLD) (Figure 3) (135). CDR NACC was able to detect early FTD and distinguish between bvFTD and aphasia and is proposed as a screening tool in clinical trials (134). Within the GENFI consortium (section 5 GENFI – project description) another two modules have been proposed in addition to the existing CDR NACC (neuropsychiatric and motoric symptoms) but whether this improves the accuracy remains to be investigated (unpublished data).

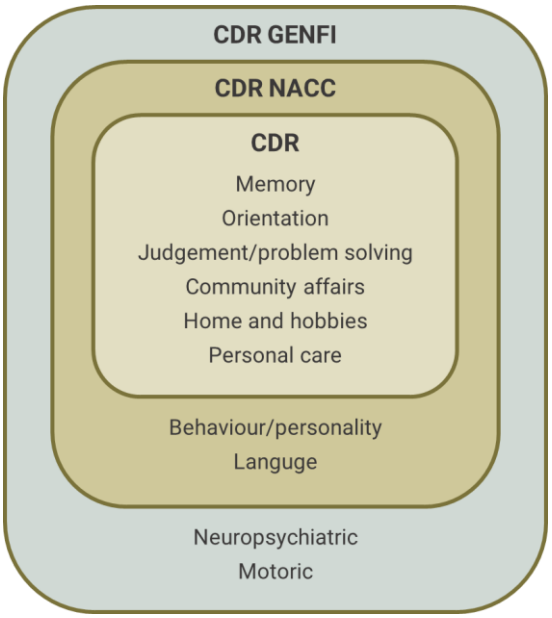


Figure 3. *Domains included in the different clinical instruments. CDR, clinical dementia rating; NACC, National Alzheimer's Coordinating Center.*

### 3.3 IMAGING BIOMARKERS

As mentioned in section 1.2, imaging findings of frontal and/or temporal lobe atrophy can support a diagnosis of FTD (measured by MRI or computerised tomography). In addition, grey matter atrophy, especially in the temporal lobes and insula, can be detected on MRI several years before clinical symptom onset (127). Distinct patterns of grey matter atrophy have been identified in the most common genetic forms of FTD (17). FTD with *GRN* mutations commonly have asymmetrical atrophy in the frontal, temporal, and parietal lobes. *MAPT* often presents with symmetrical atrophy of the anteromedial temporal and orbitofrontal lobes while *C9orf72* is associated with a more general atrophy including many regions.

Positron emission tomography (PET) is a nuclear imaging technique that can be used to measure for example the degree of neuronal metabolic activity using the tracer $^{18}$F-fluoro-2-deoxyglucose (FDG) (136). In regions of the brain with lower metabolism, the FDG uptake is decreased. Therefore, FDG hypometabolism is observed in the same regions as the grey matter atrophy in FTD. However, FDG-PET does not provide information about the aetiology of the changes. There are PET-tracers developed to bind to the pathological proteins accumulated in the brain of patients with neurodegenerative diseases, for example amyloid beta ($A\beta_{42}$) and tau (137). This has proven very successful in AD but so far, no tracer targeting the tau form seen in FTD has been developed. Unfortunately, tracers targeting TDP-43 are also still unavailable.

## 3.4 FLUID BIOMARKERS

In biomarker research, the typical element where proteins are measured is in CSF, the fluid surrounding the brain and spinal cord (138). The close proximity to the central nervous system makes CSF an attractive choice when studying brain disorders. In addition, plasma and serum are also potential sources for biomarker discovery. Advantages of blood-based biomarkers are that they would be easily accessible, minimally invasive, and low-cost. Other, less studied, body fluids such as saliva and urine might be of interest in the future. A schematic summary of suggested fluid biomarkers in FTD is shown in Figure 4.

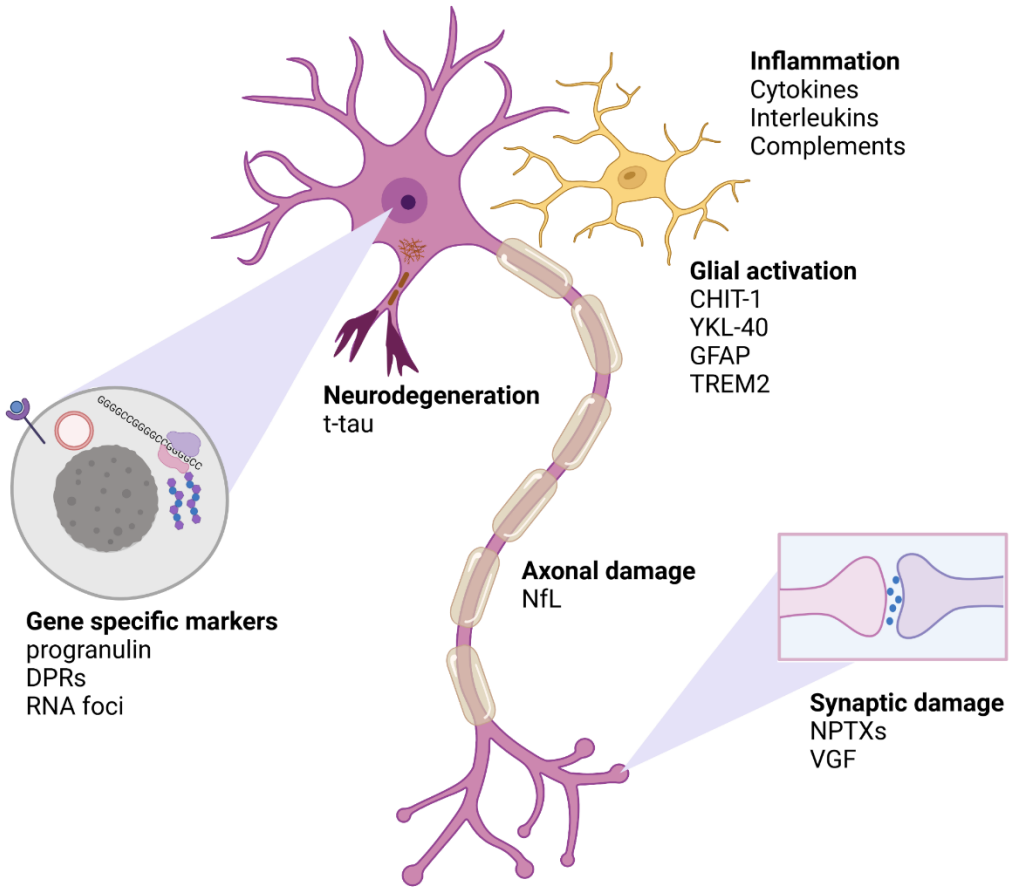


Figure 4. *Schematic illustration of different fluid biomarkers of potential relevance in FTD. Figure created with BioRender.com*

### 3.4.1 Amyloid beta and tau

Studies on autosomal dominant AD have shown altered levels of CSF $A\beta_{42}$, total-tau (t-tau) and phospho-tau ($p\text{-}tau_{181}$) in presymptomatic AD mutation carriers up to twenty years prior to the onset of clinical symptoms (125,126). These core AD biomarkers have also been investigated in FTD (139–142). Rivero-Santana et al. presented a systematic review on the diagnostic performance of CSF AD core biomarkers in distinguishing between FTD and AD (139). An elevated ratio of CSF $p\text{-}tau_{181}$ to $A\beta_{42}$ could separate FTD from AD with the highest

accuracy. However, neither $A\beta_{42}$, tau, nor p-$tau_{181}$:$A\beta_{42}$ ratio, were different between FTD and controls, and would thus only be useful in the differential diagnosis of FTD and AD (143). Recently, Palmqvist et al. showed that p-$tau_{217}$ measured in CSF or plasma performs better than the core biomarkers in recognising AD compared to other neurodegenerative diseases including FTD (144). Elevated levels of plasma p-$tau_{217}$ were specific for AD whereas patients with bvFTD, PPA or atypical parkinsonism had normal p-$tau_{217}$ levels. The reported diagnostic accuracy was more than 0.92. Different from what one might expect, CSF or plasma tau is not increased in primary tauopathies (with the exception of some specific *MAPT* mutation carriers with slightly raised levels of p-$tau_{181}$, presenting also with AD-like tau aggregates) (145). One could speculate that the elevated levels of tau measured in AD are specific for the co-occurrence of 3r and 4r tau pathology and thus not found in FTLD which present with either 3r or 4r tau.

### 3.4.2 Neurofilaments

Neurofilaments are a group of axonal proteins with three subunits: light chain (NfL or NEFL), medium chain (NF-M or NEFM) and heavy chain (146). Neurofilaments are part of the neuroaxonal cytoskeleton, and the extracellular levels increase upon axonal damage. NfL is the most studied fluid biomarker candidate in FTD (142,147,148). NfL levels in CSF are elevated in several conditions, such as neurodegenerative and neuroinflammatory diseases but also in the acute phase of traumatic brain injury (146,148). FTD and ALS are among the diseases with highest levels of CSF NfL. There is growing evidence that NfL is elevated also in plasma in FTD, and the levels correlate to NfL in CSF (149,150). In proximity, or just prior to symptom onset, there is a considerable increase of NfL, but the levels seem to reach a plateau and are stabilised at high levels in symptomatic FTD (147). Early in FTD (before the levels have reach the plateau), NfL has been suggested to be a prognostic or staging marker as it correlates to disease severity. However, data from longitudinal studies of NfL in FTD are limited and the temporal changes are largely unknown.

Since NfL raises around symptom onset, it could be used for selecting individuals that would benefit from disease modifying interventions, when those become available. In a recent study, plasma NfL levels predicted the conversion from a presymptomatic to a symptomatic stage in genetic FTD (151). Moreover, elevated plasma NfL is being used as an inclusion criterion in an ongoing phase 3 clinical trial (Alector, AL100) (Figure 5) (152). Furthermore, NfL has been suggested as a surrogate endpoint in clinical trials, meaning that reduced levels of NfL would indicate a successful treatment. Interestingly, in multiple sclerosis, NfL has been shown to decrease after disease-modifying treatment which would imply that it could be used as a biomarker of treatment response also in FTD (153). However, individual NfL levels may fluctuate, especially in *GRN* mutation carriers, complicating the evaluation of a potential treatment effect (150).

### 3.4.3 Synaptic markers

Synaptic dysfunction has been proposed as an early event of the disease pathogenesis in neurodegeneration making synaptic markers a highly interesting group of proteins to investigate further in FTD (154). Neuronal pentraxins (NPTXs: neuronal pentraxin 1, 2, and neuronal pentraxin receptor) are a group of synaptic proteins modulating the function and pruning of synapses. NPTXs in CSF are lower in both genetic and sporadic FTD compared to presymptomatic mutation carriers and controls (155,156). Levels of NPTX2 inversely correlate to CSF NfL and data from a longitudinal analysis, although in a limited sample size, suggests that NPTX2 levels follow disease progression (157). Another protein found to be decreased in FTD is neurosecretory protein VGF (VGF) and the levels in CSF are correlated to the levels of NPTXs (158). VGF is suggested to be related to synaptic plasticity (159). On the other hand, even if NPTXs and VGF are reduced in FTD, other proteins linked to synaptic function in neurodegenerative diseases (for example neurogranin and synaptosome associated protein 25, SNAP-25), have not been observed to be altered in FTD (160).

### 3.4.4 Inflammation and glial activation

Neuroinflammation is a major contributor to the pathology in neurodegenerative diseases including FTD (161). The evidence supporting this statement is both from findings of microglial activation and astrogliosis in FTD, and GWAS studies identifying FTD risk genes related to the immune system (121,162). Moreover, autoimmune diseases are overrepresented in patients with FTD (163). Several proteins associated with inflammation have been suggested as potential neurochemical biomarkers in FTD including a) markers of glial cell activation, for example triggering receptor expressed on myeloid cells 2 (TREM2), chitotriosidase (CHIT1), YKL-40 and glial fibrillary acidic protein (GFAP) (164–168), b) cytokines and chemokines (160), c) complement system proteins (92,156). In general, findings of altered inflammatory proteins seem to be more frequent in FTD with a *GRN* mutation than in other genetic groups (160).

### 3.4.5 Gene-specific markers

Although the above-mentioned fluid biomarkers are found to be altered in FTD, many of them are general markers of neurodegeneration, axonal damage or neuroinflammation, pathways that are common in all neurodegenerative diseases. For example, neither Aβ, tau nor neurofilaments have performed well in predicting the underlying pathology or genetic causes of FTD (17). However, there are a couple of markers that are specific for certain genetic groups.

In *GRN* mutation carriers, there are lower levels of progranulin in both CSF and blood (87,169,170). The protein levels are reduced by approximately 50% in mutation carriers compared to non-carriers due to haploinsufficiency. This reduction is independent of clinical status (ie. progranulin levels are the same in presymptomatic and symptomatic *GRN* mutation carriers) (87).

Other genetic markers are dipeptides repeats, found in *C9orf72* repeat expansion carriers (65). As described in section 2.2.1, the repeat expansion mutation results in the formation of dipeptide repeats. Poly(GP) is detectable in CSF in *C9orf72* mutation carriers whereas individuals without the expanded repeat region have no poly(GP) in CSF (171,172). One thing to notice is that even if the specificity is 100%, the sensitivity is lower. In other words, poly(GP) is never found in non-carriers but there are *C9orf72* mutation carriers with no (or very low) levels of poly(GP) in CSF (172).

Mutation carriers are born with the pathogenic variant. As a consequence, progranulin protein levels are believed to always be reduced in *GRN* mutation carriers, although studies including children are lacking (173). Hence, DPRs and progranulin cannot be used as markers of symptom onset or disease progression. The potential use of measuring DPRs and progranulin could be as a screening tool for selecting individuals for further genetic analysis of the *C9orf72* repeat expansion mutation or a *GRN* mutation. In *GRN* mutation carriers, reduced progranulin could also be a valuable indicator that a novel variant is pathogenic. Furthermore, genetic biomarkers can be used for monitoring a treatment response (see section 4 Treatment).

# 4 TREATMENT

There is no available treatment that can prevent or halt FTD development and/or progression. Many of the clinical trials over the past years have focused on evaluating treatments that are established for other disorders, for example AD and psychiatric disorders (174,175). Acetylcholinesterase inhibitors, which are commonly used in AD to improve cognitive symptoms, do not have an effect on cognition in FTD (176). In contrast, some psychopharmacological drugs can be considered to manage specific behavioural or cognitive symptoms. For instance, selective serotonin re-uptake inhibitors may relieve apathy and disinhibition. Moreover, antipsychotic drugs are not seldom administered to patients with difficult behaviour but have scarcely been studied in FTD (174).

Over 20 different clinical trials have been conducted or are ongoing according to clinicaltrials.gov (Figure 5**)** (177). Promising mechanisms to target for disease-modifying drugs in FTD are:

1. **Blocking tau propagation** (in tauopathies such as CBD and PSP or FTD due to *MAPT* mutations). Studies of passive (and active) immunisation are ongoing (174). Antibodies targeting tau would prevent the spread from a neuron to another, thus hopefully blocking the disease progression. Immunotherapies against tau were originally developed for AD but antibodies towards different regions of tau are being explored (for example UCB0107).
2. **Suppressing translation of the *C9orf72* expanded repeat** (in *C9orf72* mutation carriers) (178). In ALS and FTD due to *C9orf72* mutations, there are ongoing clinical trials using an antisense oligonucleotide, ASO (WVE-004, BIIB078) (177). An ASO is a small molecule that, by binding to mRNA, can modify the translation of a given gene. In the case of *C9orf72* mutation carriers, mRNA including the repeat expansion is targeted, thus preventing the formation of RNA foci and toxic dipeptide repeats.

3. **Restoring extracellular progranulin levels** (in *GRN* mutation carriers). Since the extracellular levels of progranulin are reduced in *GRN* mutation carriers, the hypothesis is that FTD could be treated by restoring progranulin to normal levels (174). An antibody towards the SORT1 receptor has shown to succesfully increase plasma progranulin levels in *GRN* mutation carriers and the preliminary results from a phase 2 trial are promising (AL001) (152).

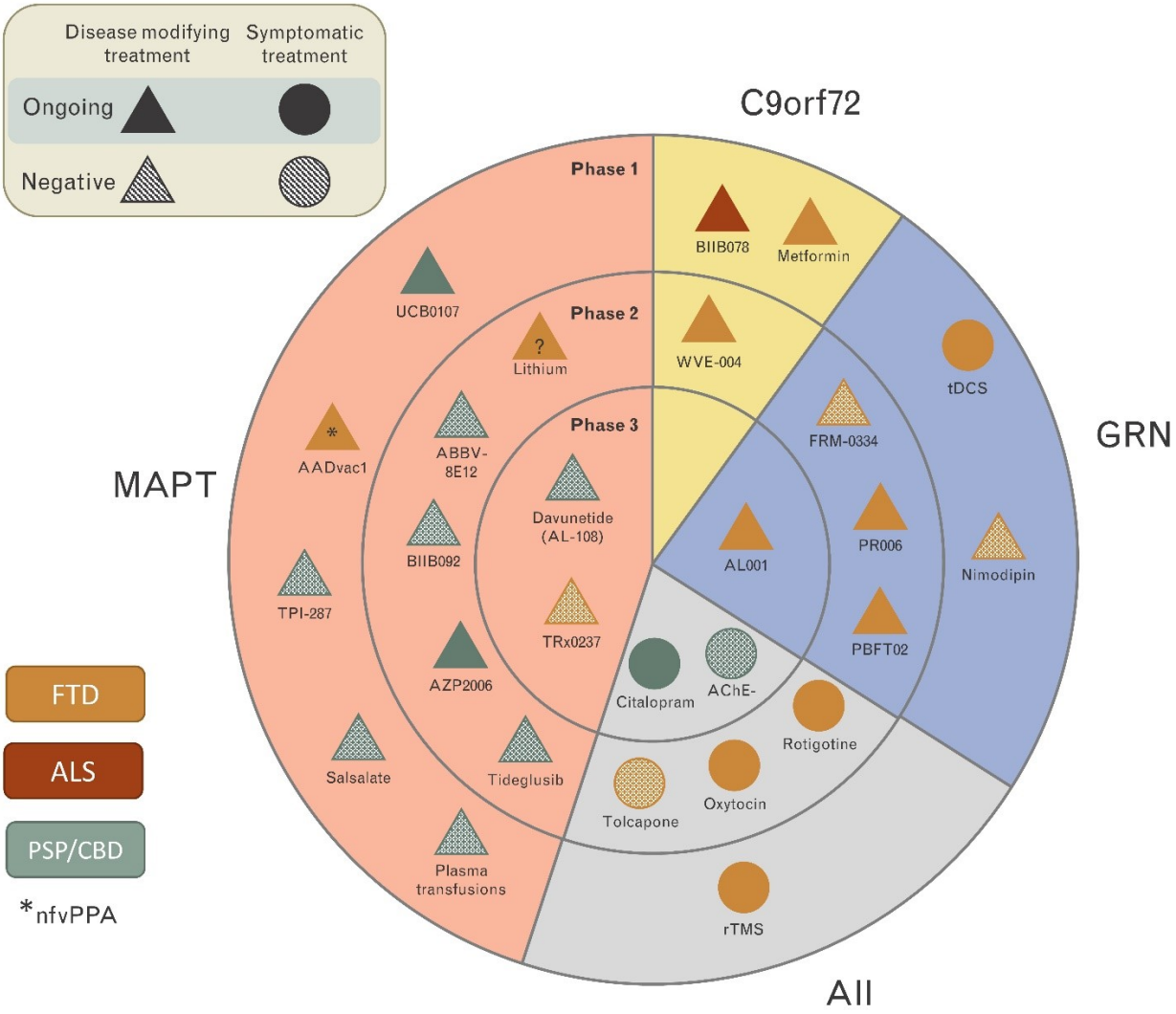


Figure 5. *Overview of previous and ongoing clinical trials in FTD. For details regarding the specific trials, please visit clinicaltrials.gov*

# 5 GENFI – PROJECT DESCRIPTION

The GENetic Frontotemporal dementia Initiative (GENFI) is a European-Canadian collaboration running a multicentre study initiated in 2012 by Professor Jonathan Rohrer and Professor Martin Rossor at the University College London (genfi.org) (179). GENFI recruits individuals with a 50% risk of FTD due to a pathogenic mutation in a first-degree relative, i.e. all participants are members of a family with autosomal dominant inherited FTD with an identified mutation. Using this study design, three different groups of participants will be included:

1. **Non-carriers (NC)**. Mutation non-carrier family members that serve as the control group,
2. **Presymptomatic mutation carriers (PMC)**. Mutation carriers that currently are asymptomatic but will present with FTD in their lifetime, and
3. **Affected mutation carriers (AMC).** Mutation carriers that have already developed clinical symptoms of FTD.

Besides being at 50% risk of FTD, the inclusion criteria are an age above 18 years, the participant must have an identified informant and be fluent in the language of assessment (prior to symptom onset). The exclusion criteria are another illness that could interfere in completing the assessment, or pregnancy. Research visits are performed annually using a standardised protocol (Figure 6).

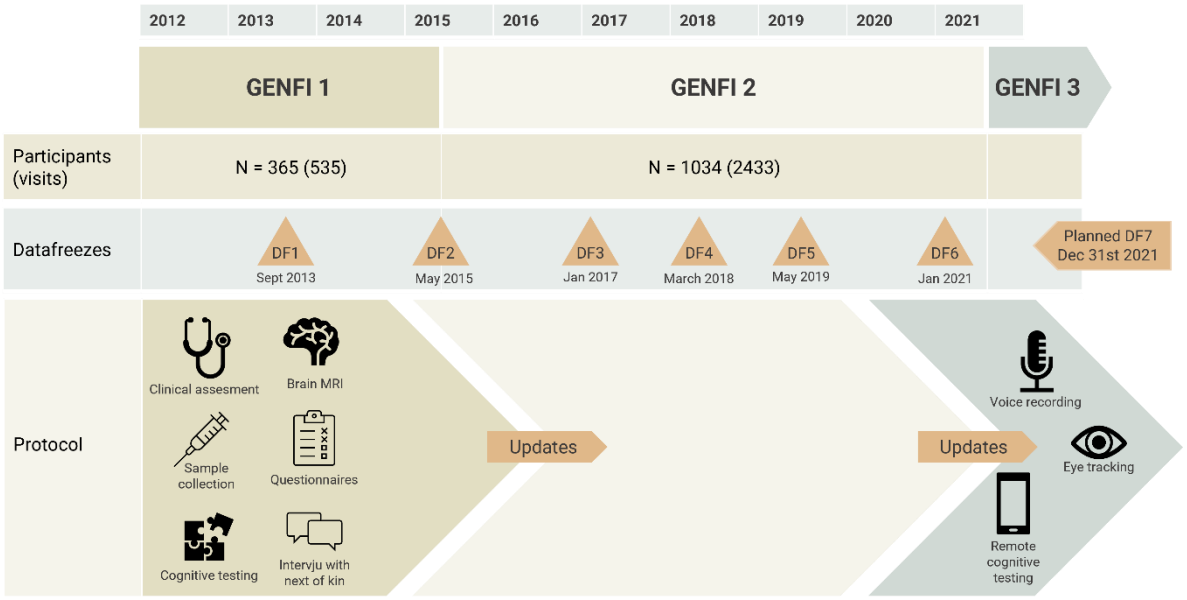


Figure 6. *Outline of the GENFI study including phases, number of visits (as of July 2021 for GENFI 2), data freezes and protocol updates. At the transition from GENFI 1 to GENFI 2, the protocol was updated but no additional parts were included in the visits. However, at the launch of GENFI 3, voice recording, eye tracking and an iPad-based cognitive test were included. CRF, case report form; DF, data freeze.*

Each visit involves a thorough clinical evaluation focused on symptoms and signs of dementia and related neurological disorders, MRI of the brain, neuropsychological assessment, questionnaires to a next of kin and collection of CSF and blood. Currently, over 1000 participants have been recruited across 25 different sites. The data is pseudonymised and uploaded to a common database where the GENFI researchers have access to the data collected at their respective sites. Access to data from the whole GENFI cohort is approved by the GENFI data access committee (represented by principal investigators from some of the sites) upon request. There have been six data freezes where all collected data was quality controlled and locked for subsequent change, and the final data freeze for GENFI 2 is planned for December 31$^{st}$ 2021. The work in this thesis is mainly based on data collected up to data freeze 4.

At the Stockholm GENFI site, in addition to the above-mentioned standardised protocol, we have also included genetic counselling, an extended neuropsychological test battery, electroencephalogram, questionnaires to the participant, skin biopsy and collection of saliva for DNA extraction. Each participant is reviewed at our regular multidisciplinary team conferences, and we collaborate closely with the Cognitive Unit at Karolinska University Hospital. The participants and the researchers are blinded to the mutation status, unless the participant has requested a presymptomatic genetic test. At present, we have enrolled 57 participants from 18 different families and performed 152 visits (Figure 7).

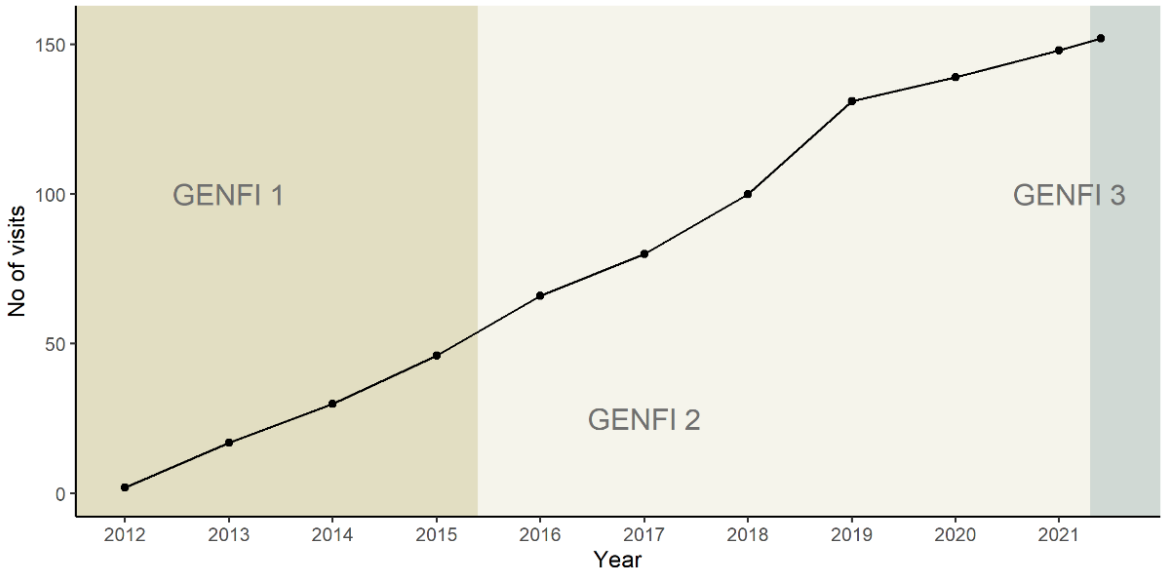


Figure 7. *Number of visits over time at the Stockholm site. Updated 2021.11.19.*

Enrolling presymptomatic mutation carriers in biomarker studies offers a unique opportunity to explore early pathological changes of FTD. Longitudinal data from the same individuals over time is ideal in this setting. However, in the case of cross-sectional studies, or longitudinal data with very few converters, PMC are at different stages of FTD, ie. on a continuum from presymptomatic to symptomatic (Figure 8). When the work of this thesis started, FTD researchers had adopted the calculation of Expected Years to symptom Onset (EYO) from the field of autosomal dominant AD. EYO represented the age of a participant minus the mean age at onset in the family which introduces a time aspect in otherwise cross-sectional data.

However, recent work from GENFI and Frontotemporal prevention initiative (FPI) shows that the age at onset can vary greatly within the same family, especially for *GRN* mutation carriers (61). Even if the individual age at symptom onset is associated with both the parental age at onset and the mean age at onset in a family, the correlations are weak. In this thesis, EYO was thus calculated based on the mean age at symptom onset in each genetic group estimated in Moore et al (58.2 years in *C9orf72*, 61.3 years in *GRN* and 49.5 years in *MAPT*) (61).

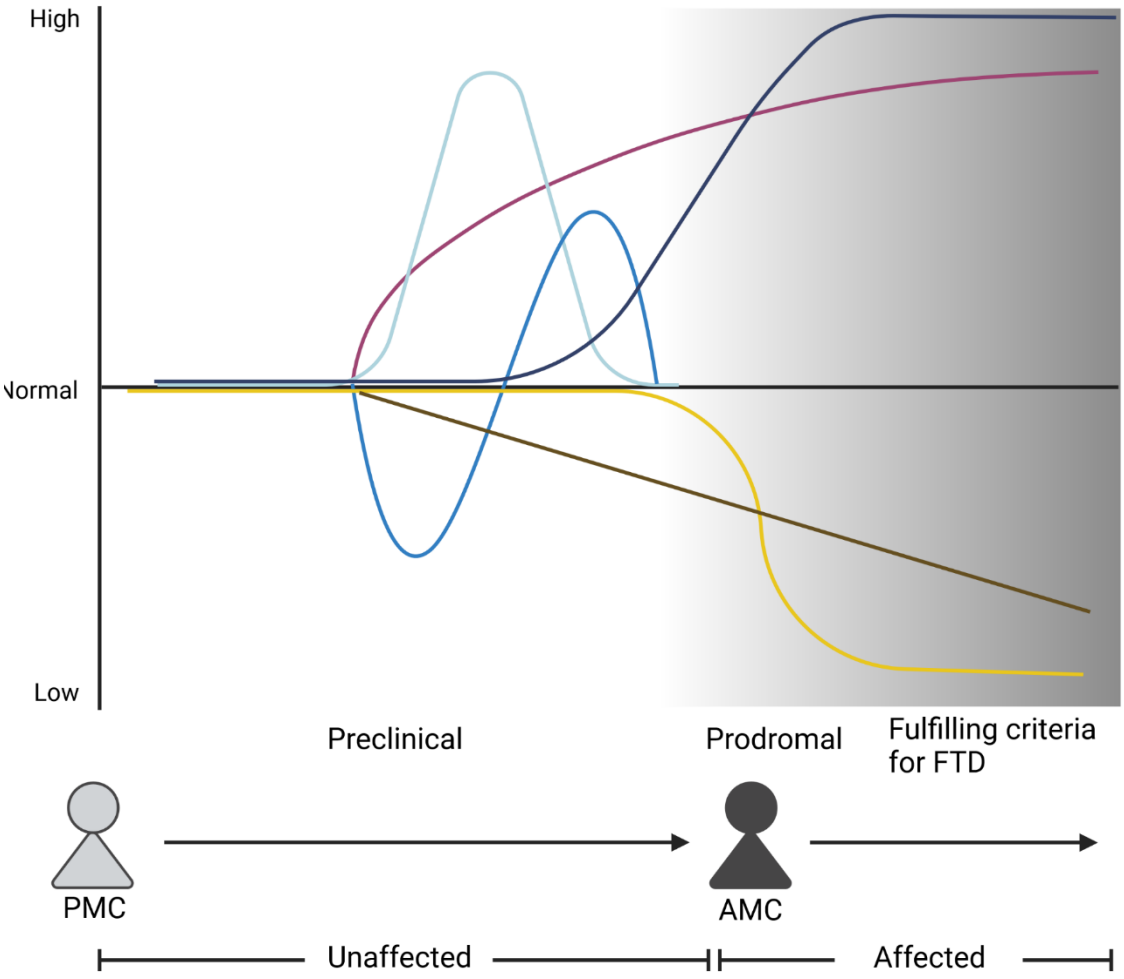


Figure 8. *Theoretical model for disease progression in FTD. A presymptomatic mutation carrier (PMC) will during time transition to a prodromal and then a fully symptomatic stage. The changes in different potential biomarkers will begin in a preclinical phase, i.e. when the pathology has started but the person does not express any clinical symptoms of FTD yet. Figure created with BioRender.com*

# 6 AIMS

The general purpose of GENFI is to study the early stages and progression of FTD in individuals with a genetic risk. In this thesis, different types of biomarkers (genetic, cognitive, and fluid) were explored with the focus of identifying differences between mutation carriers and non-carriers or between clinical statuses (unaffected and affected). Accordingly, the objective was to find biomarker candidates relevant in FTD but for different purposes.

Specific aims for each project were:

**Study I:** to determine the mutation frequency of the most common causative genes for FTD (*C9orf72*, *GRN* and *MAPT*) in an FTD cohort from Sweden. In addition, the distribution of mutations was assessed depending on the inheritance pattern in the family, and the results were compared to findings from other FTD cohorts.

**Study II:** to explore practice effects in the GENFI cohort and evaluate whether there is a difference in practice effects between PMC with mutations in *C9orf72*, *GRN* or *MAPT*, and NC.

**Study III:** to investigate differences in CSF protein levels between genetic and sporadic patients with bvFTD or PPA, PMC, and NC to discover novel protein biomarker candidates.

**Study IV:** to distinguish patients with genetic FTD from unaffected individuals based on a panel of CSF proteins using multivariate statistical analysis. Another aim was to identify a panel of CSF proteins with the potential to separate PMC from NC.

**Study V:** to investigate differences in plasma protein levels between genetic FTD, PMC, and NC. Furthermore, correlations between plasma and CSF protein levels were explored in a subset of the participants.

# 7 MATERIALS AND METHODS

A summary of the aims, applied methods, and participants included in studies I-V is found in Table 3.

## 7.1 PARTICIPANTS

### 7.1.1 Study I

The subjects included in study I were recruited via the Memory Clinic at Karolinska University Hospital in Huddinge between 1992 and 2013. All participants (n=132) had a clinical diagnosis of FTD or FTD-ALS and an available DNA sample.

### 7.1.2 Studies II, IV and V

The participants included in studies II, IV and V were all recruited through the GENFI study (see section 5). Following the design of GENFI, the participants can be categorised by for example mutation status and/or clinical status. Hence, we included affected individuals (AMC), i.e. with clinical symptoms of FTD, and unaffected individuals (PMC and NC).

In study II, we retrieved baseline and follow-up neuropsychological data from the GENFI participants enrolled between 2012 and 2018. In total, 803 participants had a baseline visit and we included 1670 visits in the analysis.

In the proteomics studies, all participants with one available baseline CSF and/or plasma GENFI sample collected between 2012 and 2019 were included (221 participants in study IV and 735 participants in study V).

**Table 3.** *Overview of the study design, aims, cohorts and statistical analysis for each project.*

| | Study I | Study II | Study III | Study IV | Study V |
|---|---|---|---|---|---|
| **Material** | Genetic screening<br>Family history | Neuropsychology | CSF | CSF | Plasma and CSF |
| **Design** | Cross-sectional | Longitudinal | Cross-sectional | Cross-sectional | Cross-sectional |
| **Aims** | - Determine the mutation frequency in an FTD cohort from Sweden.<br><br>- Explore the distribution of mutations depending on the inheritance pattern. | - Investigate practice effects in the GENFI cohort.<br><br>- Explore differences in practice effects between PMC and NC. | - Investigate differences in CSF protein profiles between patients with FTD, PMC and NC to discover novel protein biomarkers. | To identify panels of proteins and evaluate their potential to distinguish:<br>(I) affected individuals from unaffected individuals, and<br>(II) PMC from NC. | - Investigate differences in plasma protein levels between affected and unaffected individuals as well as PMC and NC.<br><br>- Explore correlations between plasma and CSF protein levels in a subset of the participants. |
| **Participants** | FTD = 132 (index) | **Visits (n)** / **N**<br>1 / 803<br>2 / 471<br>3 / 249<br>4 / 108<br>>4 / 39 | **Cohort 1**: NC = 8, PMC = 16, FTD = 29<br>**Cohort 2**: Controls = 18, FTD = 13, AD = 79 | NC = 76<br>PMC = 98<br>FTD/AMC = 47 | NC = 286<br>PMC = 298<br>FTD/AMC = 151 |
| **Statistical analysis** | - Nonparametric tests | - Linear mixed-effects model | - Nonparametric tests<br>- PCA<br>- Hierarchical clustering | - LASSO<br>- Random forest<br>- PCA and clustering | - Nonparametric tests<br>- Binomial regression<br>- Correlation analysis |

### 7.1.3 Study III

In study III, we included participants from our local GENFI Stockholm cohort assessed between 2012 and 2016, and patients with bvFTD or PPA recruited from the Memory Clinic at Karolinska University Hospital in Huddinge between 1997 and 2016. NC served as a control group and were together with PMC denoted as unaffected. GENFI participants and clinical cases with one available CSF sample and DNA were eligible. In addition, an independent cohort was selected from Uppsala University Hospital. This second cohort consisted of patients with bvFTD or AD from the Memory and Geriatrics clinic, and healthy controls. The healthy controls were recruited through advertisement in local newspapers and were determined cognitively unimpaired based on their medical history, a mini-mental state examination test, and the absence of abnormal radiological findings in the brain. In total, 163 participants (n=53 in cohort 1 and n=110 in cohort 2) were included in study III.

## 7.2 METHODS

### 7.2.1 Fluid sample collection

The biosampling at the different GENFI sites was according to an approved standardised protocol.

Peripheral blood was collected by venepuncture into ethylenediaminetetraacetic acid (EDTA) and serum clot activation tubes. For the genetic screening at baseline, genomic DNA was isolated from EDTA tubes with Gentra Puregene Blood kit (Qiagen) according to manufacturers' protocol and aliquoted into cryotubes. Participants recruited from the clinic (studies I and III) were screened for mutations in *GRN*, *MAPT,* and *C9orf72*. Mutations in *GRN* (13 exons) and *MAPT* (exons 2 and 9 to 13) were identified using Sanger sequencing while the *C9orf72* repeat expansion mutation was identified by repeat primed polymerase chain reaction and subsequently short tandem repeat assay to determine the number of repeats. Participants enrolled in GENFI (studies II-V) were only screened for the mutation segregating in their respective family using the same methods (including Sanger sequencing of *TBK1* when applicable). Serum tubes (study I) were left to clot at room temperature for at least 30 minutes before centrifugation.

Lumbar punctures were performed to collect CSF (studies III and IV). CSF samples collected within the GENFI study were obtained as per the standardised protocol and centrifuged immediately after collection. The clinical samples in study III were obtained according to local protocols (Karolinska University Hospital and Uppsala University, respectively).

All aliquots (DNA, plasma, serum, and CSF) were stored at -80°C in 0.5 ml polypropylene cryotubes.

### 7.2.2 Study I

Participant recruitment and genetic analysis have been described in previous sections (7.1.1 and 7.2.1). In addition to the genetic analysis, the family history of each index patient was reviewed. Clinical diagnoses (and neuropathological diagnoses if accessible), and age at symptom onset were investigated in three generations. Pedigrees were drawn and classified according to the described criteria in Wood et al. (180). Finally, the pedigree classification of the Swedish cohort was compared to results from other similar publications (180,181). Pearson's $\chi^2$ test or Fisher's exact test was used to assess differences in mutation frequency across clinical phenotypes in the index patients, pedigree categories, and cohorts.

### 7.2.3 Study II

The clinical and longitudinal neuropsychological data were obtained from data freeze 4 thus including both GENFI 1 and GENFI 2. The neuropsychological test battery was evaluated and updated at the transition to GENFI 2 resulting in some differences in the tests being used in the two phases of the study (Table 4). All test raw scores were converted into standard scores (z-scores). Instead of analysing all separate tests individually, they were combined into one composite cognitive score as well as domain-specific composite scores. Please see the supplementary materials attached to paper II for details in calculating standard and composite scores.

Table 4. *Neuropsychological tests assessed in GENFI 1 and GENFI 2 and to what cognitive domain they were included when calculating composite scores. FCRST, the free and cued selective reminding test; SEA, social cognition and emotional assessment.*

| | Language | Executive function | Attention and processing speed | Memory | Visuo-construction | Social cognition |
|---|---|---|---|---|---|---|
| GENFI 1 and 2 | ·Boston naming test<br>·Verbal fluency (animals) | ·Trail making test B<br>·Verbal fluency (letters)<br>·Digit span backwards | ·Trail making test A<br>·Digit symbol<br>·Digit span forward | ·Digit span forward/backward | ·Block design | |
| Only in GENFI 2 | ·Camel and cactus test | ·Stroop interference | ·Stroop ink and word naming | ·FCRST | ·Benson figure | ·Mini-SEA |

#### *7.2.3.1 Statistical methods*

Linear mixed-effects models were used to assess the presence, magnitude, and potential modifiers of practice effects. The final model included nine fixed effects: mutation group (AMC, PMC, NC) or mutated gene (*C9orf72*, *GRN*, *MAPT*, NC), visit, years from baseline visit, age, age^2, education, sex, baseline score, and the interaction between mutation group/gene and visit. The global composite score (or domain-specific composite score) was used as the outcome variable. Site and individual were included as random effects to account for within-subject correlations.

### 7.2.4 Study III, IV and V

#### *7.2.4.1 Antibody suspension bead array*

All samples included in the proteomics studies were analysed at the Science for Life Laboratory (SciLifeLab, Unit for affinity proteomics, Royal Institute of Technology) using a multiplexed suspension bead array (182). A detailed description of the method can be found in paper II. In summary, CSF and plasma samples were labelled with biotin, heat treated and subsequently mixed with an antibody suspension bead array together with a streptavidin-conjugated fluorophore (Figure 9). The readout was performed on a Flexmap 3D instrument (Luminex Corporation). Binding events were displayed as signal intensity (arbitrary units, AU or median fluorescence intensity, MFI) in cases where at least 30 beads per bead identity were measured.

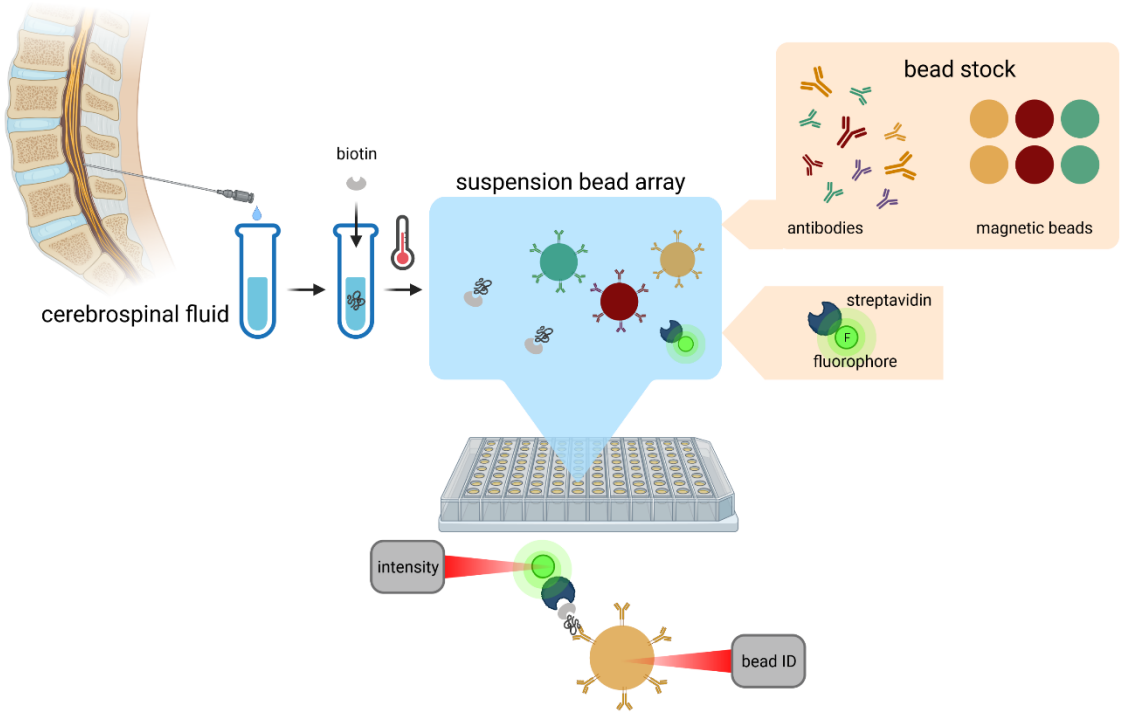


Figure 9. *Schematic overview of the suspension bead array. CSF was collected by lumbar puncture and handled according to protocol prior to analysis. After thawing, samples were labelled with biotin, heat-treated and mixed with the antibody suspension bead array and streptavidin. In the suspension bead array, colour-coded magnetic beads were coupled with antibodies, i.e. each bead identity (ID) is unique for the antibody bound to it. The samples were aliquoted onto plates in a structured distribution with regard to age, gender, mutation, and clinical status. In the Flexmap 3D, one laser detected the bead ID (antibody), and one detected the intensity of the fluorophore bound to that bead. The final readout was displayed in relative intensities. Figure created with BioRender.com*

The antibody selection was performed at the initial phase of each project. In the proteomics pilot study (study III), we chose already existing bead stocks developed in previous projects on ALS, AD and other neurodegenerative diseases. On the other hand, the bead stocks for studies IV and V were specifically designed for these projects. The selection of antibodies was guided by results from the pilot study and other published work. In addition, internal unpublished results and knowledge including for example antibody performance were also considered. In

study III, antibodies targeting 328 proteins were included in the experimental set-up (Figure 10). Of these, 70 proteins were selected for statistical analysis based on a very stringent antibody quality control with criteria of an intra-assay coefficient of variance below 10% and an inter-assay correlation above 90%. In studies IV and V, antibodies targeting 174 and 163 proteins were analysed and 111 and 158 were included in the statistical analysis respectively. The antibody quality control in the plasma study (study V) was not as rigorous as for the CSF studies (studies III and IV) allowing a more exploratory approach. The difference in the number of proteins between the experimental and statistical analyses is explained by the above-mentioned antibody selection process since the performances of the antibodies in the tailormade bead stocks (studies IV and V) are superior to the ones in the pilot study (study III).

| | Study III: Pilot | Study IV: CSF | Study V: Plasma |
|---|---|---|---|
| No of proteins in experimental analysis | 328 | 169 | 163 |
| No of proteins in statistical analysis | 70 | 111 | 158 |
| Intra-assay CV | < 10% | < 10% | < 20%[a] |
| Inter-assay correlation (rho) | > 0.8 | > 0.8 | > 0.8[b] |

[a] 97% of the antibodies had a CV < 20%
[b] 98% of the antibodies had an inter-assay rho > 0.8


Figure 10. *Comparison of the quality criteria in the different suspension bead array studies. Venn diagram showing the overlap of proteins included in the statistical analyses for the different studies. CV, coefficient of variance.*

### *7.2.4.2 Statistical methods*

Non-parametric tests. The output from the suspension bead array is a measure of relative intensities which does not fulfil the assumptions of parametric tests (normality and homoscedasticity). Instead, Mann-Whitney U tests were applied in study III (and in a sub-analysis in study IV) to investigate differences between groups (affected vs unaffected, bvFTD vs PPA, AMC vs PMC etc.).

Binominal regression. The method was used to explore plasma protein level differences between affected and unaffected individuals in study V. One model for each protein was built and the outcome was symptomatic status (ie. affected vs unaffected). We chose this model to be able to adjust for age and sex.

Multiple comparisons and selection of alpha. Adjustments for multiple comparisons were made when applicable by using the Benjamini-Hochberg procedure to decrease the false discovery rate (FDR) (183). In other words, this operation is applied to reduce the number of false positive result, i.e. the risk of type I errors or rejecting the null hypothesis when it is true. The alpha level (or probability of a type I error) is by convention usually set to 5% ($p < 0.05$).

However, the decision of how many false positive versus false negative results to allow is a matter of rigorousness. As the proteomics studies included in this thesis are exploratory, we have approached this issue differently depending on our purpose. In the pilot study (study III), we used bead stocks including a wide range of antibodies targeting proteins with increased RNA levels in brain tissue (184), and that in some cases have been implicated to be involved in neurodegenerative diseases (185). Hence, we sought to lower the probability of type I errors and chose an FDR adjusted alpha level of 1%. In study V on the other hand, we performed a large plasma screen including antibodies targeting carefully selected proteins. To our knowledge, that had never been done in genetic FTD before, and we thus chose a more allowing approach, setting alpha to 1% but with no correction for multiple testing.

Principal component analysis (PCA). For dimension reduction purposes and visualisation, we performed PCA in studies III and IV. This method allowed us to summarise a large set of correlated variables into a smaller number of variables (components) which explain the variance seen in the data. PCA was used in the data quality control steps in studies II-V to assess for example outliers and confounders. In studies III and IV, PCA was additionally applied to investigate combinations or panels of CSF proteins and visualise the separation between clinical groups.

Hierarchical clustering. Following the PCA, we performed clustering in studies III and IV. We chose this method to further interpret and visualise the discriminative performance of a selected number of proteins.

LASSO. In study IV, we applied least absolute shrinkage and selection operator (LASSO) which is a linear regression method primarily used for variable selection. LASSO is successful if there are many independent variables (proteins in our case) but just a subset of these variables is believed to contribute to the model (Figure 11A). If the number of observations is not much larger than the number of variables, using the least square fit will result in overfitting and thus poor performance on the test set. LASSO has the advantage over conventional least square fitting that it reduces irrelevant estimates to zero, which increases the prediction accuracy and makes model interpretation simpler. In addition, LASSO is particularly helpful when the independent variables are correlated which we can assume that many of the proteins included in study IV are.

Random forest. We applied this decision tree method in study IV as an additional variable selection approach to compare the outcome with the results from the LASSO. Since trees are intuitive and easy to visualise, the interpretation becomes simpler than for most multivariate analyses. Overall, a tree stratifies observations into different predictor groups, for example individuals with a) protein A levels above X, b) protein A levels below X and protein B levels above Y and c) protein A levels below X and protein B levels below Y (Figure 11B). In random forest, several trees are built using a training sample and the final outcome is based on the majority of the trees in the forest. The importance of each variable is expressed in mean decrease accuracy (mda) which is an estimate of the accuracy of the model including the variable minus the accuracy of a random model.

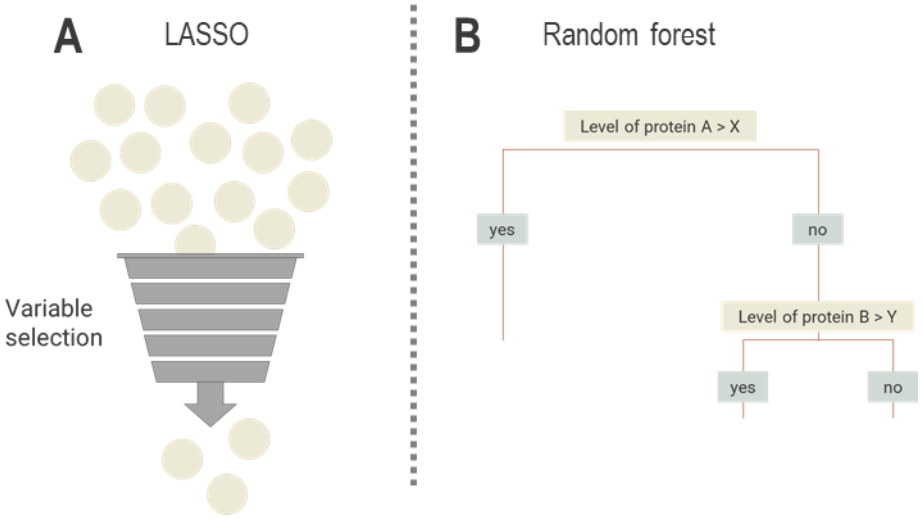


Figure 11. *Schematic illustrations of **A)** Least absolute shrinkage and selection operator and **B)** Random forest.*

## 7.3 ETHICAL CONSIDERATIONS

All studies were performed in accordance with the World Medical Association Declaration of Helsinki (186). The design, procedures, and data processing of the projects included in this thesis were approved by the Swedish Ethical Review Authority and a written informed consent was obtained from each participant. Studies involving individuals with a neurodegenerative disease should render extra care since the disorder itself may disrupt the possibility of self-determination and might impact the possibility to fully consent. Nevertheless, it is essential not to systematically exclude patients with cognitive impairment in research and deny them the same possibilities as other individuals. According to the EDCON Consensus Statements, patients with dementia should actively be involved in research where special consideration to the competence of the participant is taken into account (187). Patients with FTD often lack insight into their symptoms and might present with suspicious delusions and lack of trust. If so, this must also be considered when obtaining a consent to ensure that the research participant understands that participation is voluntary and without coercion etc. In GENFI, a majority of the Stockholm participants were recruited prior to symptom onset. In these cases, we can assume that the autonomy is fully intact when consenting to enrolment. However, even if the autonomy is preserved, other factors might impact the decision to participate in research. For example, the predicament of recruiting subjects from the same family, as the participants are not independent of each other and most certainly influence one another, should not be underestimated.

GENFI is a multicentre study and to enable research using the complete material, we share and store sensitive data in a common database. A unique code is generated for each participant and pseudonymised data is uploaded to the database. Since GENFI started, and during the work of this thesis, the General Data Protection Regulation has been implemented in the European Union and Sweden (Dataskyddsförordningen). Furthermore, we do not only handle health-

related data but also sensitive genetic information as the DNA for each participant is screened for mutations in either *C9orf72*, *GRN*, *MAPT* or *TBK1*. A genetic guardian is responsible for the genetic screening and uploads coded results to the database. The clinicians and coordinators meeting the participants are blinded to the genetic status and we never communicate research genetic results to the participants. However, in some cases where the participant has done a presymptomatic test in a clinical setting, they have chosen to share the result with us. It is important to know that the researchers involved in GENFI offer all possible clinical options available regarding genetic testing, but never persuade or even encourage a participant to make a specific choice. The close collaboration with the Unit for Hereditary Dementias at Karolinska University Hospital enables us to manage genetic issues, such as risk assessment, and a genetic consultation is included in the schedule at every GENFI visit.

# 8 MAIN FINDINGS

A detailed description of the results can be found in each constituent paper. A summary, highlighting the most important findings, is presented here.

## 8.1 STUDY I

In study I, 132 patients with FTD were screened for mutations in *GRN* and *MAPT* as well as the *C9orf72* repeat expansion mutation. A total mutation frequency of 34.1% was found and a novel pathogenic *GRN* mutation, not previously reported, was identified (Table 5, Figure 12A). We found a particularly high frequency of the *C9orf72* expansion in this cohort from Sweden (26.5%), which is more than three-quarters of all mutation carriers in the study.

Table 5. *Results from the genetic screening of C9orf72, GRN and MAPT performed in study I. VUS, variant of uncertain significance.*

| | ***C9orf72*** | ***GRN*** | ***MAPT*** |
|---|---|---|---|
| Frequency (%) of pathogenic variants | 26.5 (35/132) | 6.8 (9/132) | 0.8 (1/132) |
| Variants previously reported | GGGGCC | 6 pathogenic<br>2 benign | 1 pathogenic<br>1 likely benign |
| Novel variants | | 1 pathogenic | 1 VUS |

In addition, we assessed the family histories of all 132 cases, tracing back three generations. The pedigrees were classified according to already existing criteria (Wood et al) and the number of index cases assigned to each category is presented in Figure 12B. Please see the supplementary material in paper I for details on family history classification. The likelihood of finding a pathogenic mutation was highest in families with an autosomal dominant inheritance pattern (category "high", 76%). However, we identified a pathogenic mutation in three index cases (20%) with an apparent sporadic disease, two *C9orf72* expansion carriers and one *GRN* mutation carrier.

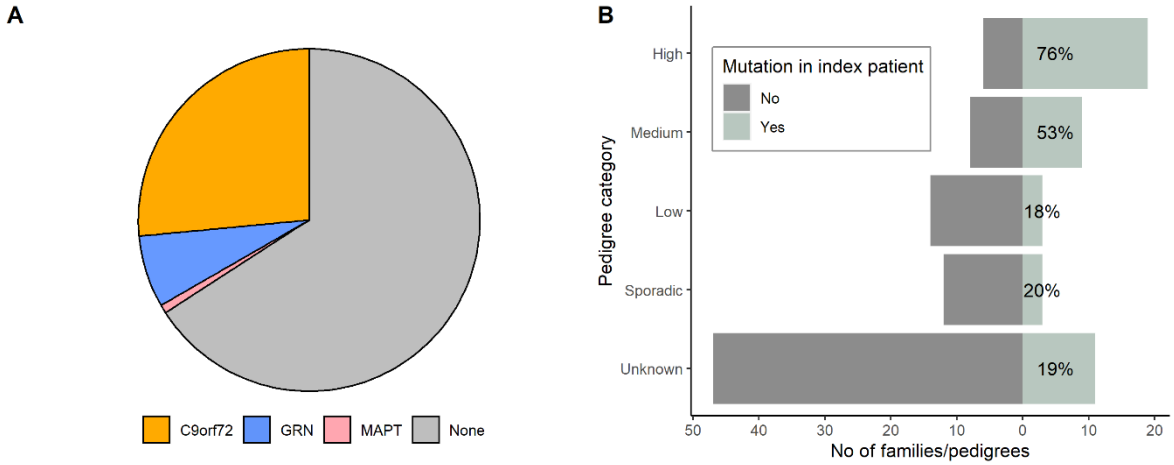


Figure 12. *Frequency of variants found by mutation screening of C9orf72, GRN and MAPT. **A)** Distribution of mutations **B)** Number of index cases in each pedigree category per mutation group. Pedigree categories (y-axis): High, medium, low likelihood of finding a mutation in the index patient, apparent sporadic disease, unknown family history.*

## 8.2 STUDY II

A potential bias in repeated cognitive testing is that participants get familiar with the tasks and test settings and improve their performances at follow-up test occasions, so-called practice effects. In study II, we confirmed that non-carriers within the GENFI study show a global practice effect over the first three visits (Figure 13). After the third visit, the neuropsychological performance remained stable and no significant improvement in global score was observed.

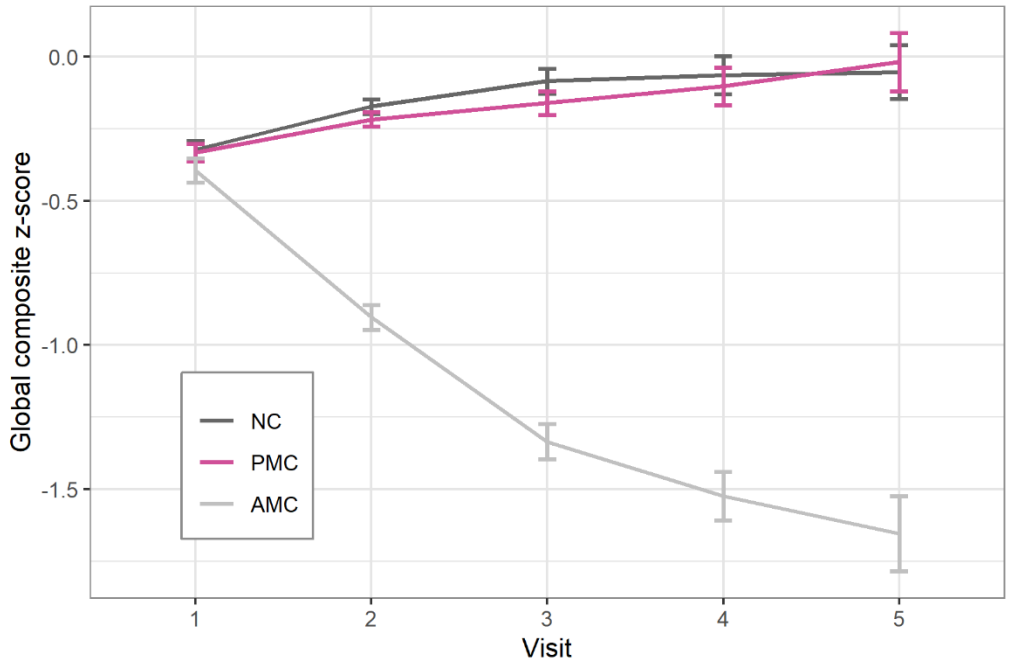


Figure 13. *Trajectories of global cognitive test scores, fitted line from mixed effect model. The model included mutation group (AMC, PMC, NC), visit (1-5), the interaction between mutation group and visit, years from baseline visit, age, age^2, education, sex and baseline score as fixed effects and site and individual as random effects. Error bars represent the standard errors of the means.*

As the number of participants with four or more visits was limited, we chose to focus the subsequent analyses on visits 1 to 3. Here, the global practice effect in NC was approximately 0.15 units per visit. When investigating the cognitive domains separately, practice effects were found in all domains except for visuoconstruction. The largest practice effect was observed in memory and social cognition, roughly 0.25 and 0.35 units per visit, respectively (Supplementary Table 1 in paper II).

Even if practice effects were observed also in PMC (purple line in Figure 13), the trajectories were different depending on the genetic group. Comparing the global test scores between visits 1 and 3, we found that presymptomatic *C9orf72* (PMC-C9) and *GRN* (PMC-GRN) carriers had lower practice effects than NC (Figure 14).

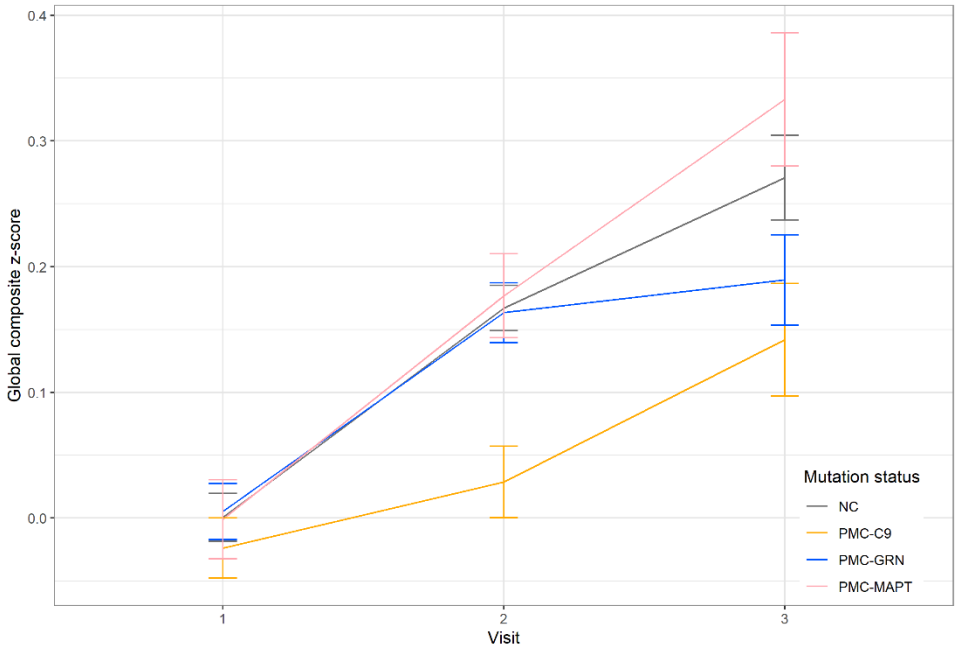


Figure 14. *Trajectories of global cognitive test scores across visit 1 to 3 in NC and PMC-C9, PMC-GRN and PMC-MAPT, fitted line from mixed effect model. The model included gene, visit (1-3), interaction between gene and visit, years from baseline visit, age, age^2, education, sex and baseline score as fixed effects and site and individual as random effects. Error bars represent the standard errors of the means.*

Moreover, PMC-C9 with less than 5 years to their expected symptom onset had overall a stable cognitive performance over time and thus no global practice effect (please see figure 1 in paper II). In this group of PMC-C9 in proximity to onset, an absence of practice effect was observed across all three visits for the cognitive domain's language, executive function, and memory. Furthermore, their visuoconstruction performance declined at each consecutive visit (unlike NC who were stable across all visits in this domain).

In summary, we confirmed the presence of practice effects in unaffected individuals (PMC and NC) in the GENFI cohort. Practice effects were lower in PMC carrying a mutation in either *C9orf72* or *GRN* compared to NC. The reduction in practice effect seems to be most apparent in individuals close to their expected symptom onset, suggesting that practice effects could potentially be a marker for imminent disease.

## 8.3 STUDY III

In study III, we report results from a pilot proteomic profiling of CSF samples from patients with FTD (sporadic and genetic), PMC and NC (in total 53 samples). We identified 26 CSF proteins with altered levels in FTD, implicating them for future investigation into their potential as biomarkers (Figure 15).

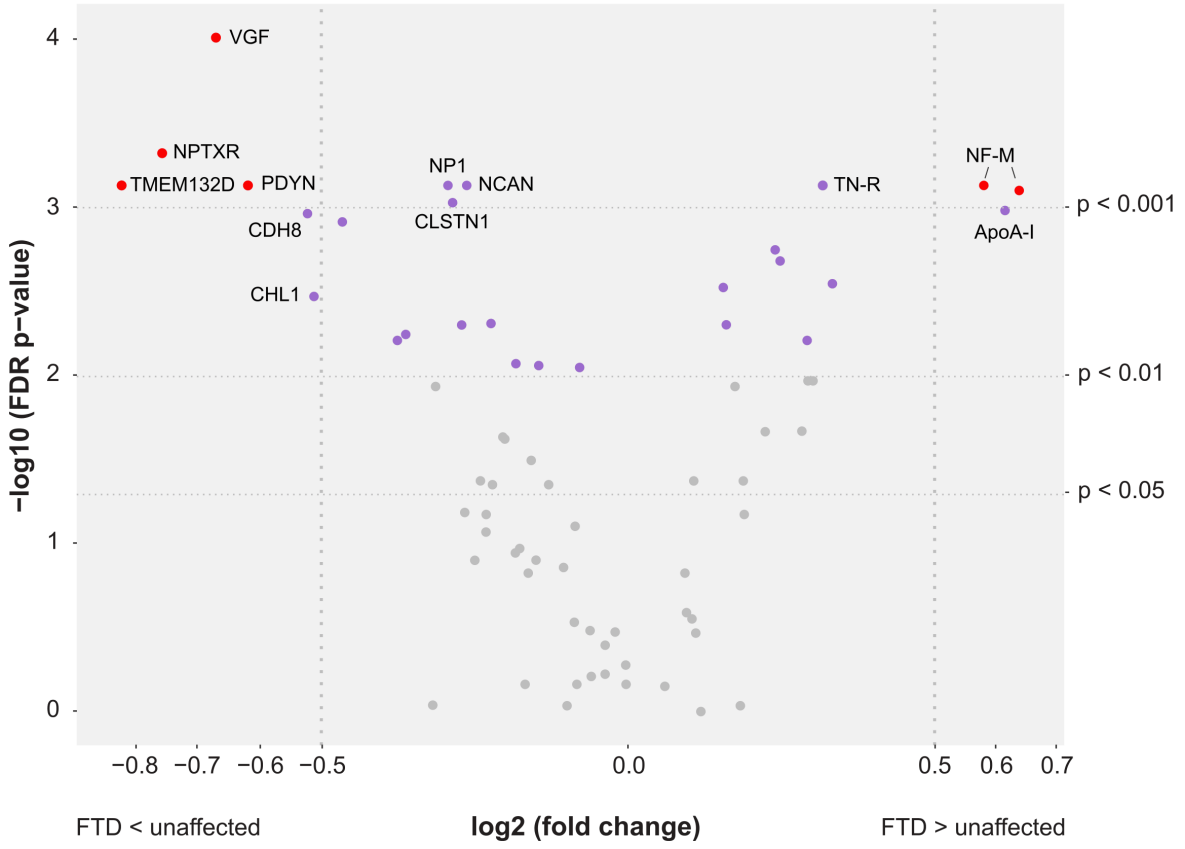


Figure 15. *Volcano plot of analysed proteins (n = 70). Differences in protein levels between FTD patients and unaffected individuals displayed by log2(fold change) and significance level displayed as -log10(p). All proteins with significant differences (FDR adjusted p < 0.01) are displayed in purple. Proteins highlighted with gene names have a p < 0.001 or an absolute log2(fold change) > 0.5 (corresponding to a fold change < 0.7 or > 1.4). The five proteins with both a p < 0.001 and an absolute log2(fold change) > 0.5 are displayed in red.*

PCA and hierarchical clustering showed that unaffected individuals (i.e. PMC and NC) mainly cluster together and that the protein profile of bvFTD seemed more uniform than in PPA. We observed a large variance in protein levels in PPA which might be contributed to an actual protein variability in this group or because of external factors such as the small sample size (n=13).

We compared CSF protein levels between affected vs unaffected individuals, bvFTD vs NC, bvFTD vs PMC, PPA vs NC, PPA vs PMC, PPA vs bvFTD and finally PMC vs NC (Figure 16).

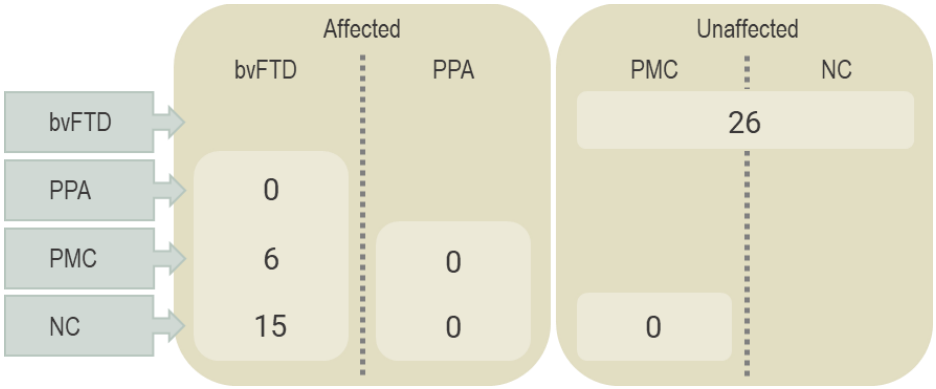


Figure 16. *Number of proteins with adjusted p<0.01 in the different group comparisons in study III.*

The proteins with the largest differences (lowest p-value and highest absolute fold-change) between affected and unaffected individuals were VGF, NPTXR, transmembrane protein 132D (TMEM132D), prodynorphin (PDYN) and NF-M/NEFM (red dots in Figure 15). Patients with FTD had higher levels of TN-R and NF-M/NEFM but lower levels of NPTXR, TMEM132D and PDYN compared to PMC and NC. Furthermore, patients with bvFTD could be separated from unaffected individuals with an accuracy of 85% by combining the CSF protein levels of VGF, TN-R and NF-M/NEFM (Figure 4 in paper III).

The results from the initial profiling were confirmed in an independent FTD cohort for four of the five top candidates (not TMEM132D). Additionally, levels of TN-R and NF-M/NEFM were significantly higher in FTD compared to AD.

## 8.4 STUDY IV

To follow up the results from the pilot CSF study, we performed proteomic profiling in a larger cohort with CSF samples from the GENFI cohort. Given the size of the cohort and the number of predictors (i.e. proteins, n=111), we chose a model-based statistical approach instead of comparing the different levels of each protein between clinical groups. This allowed us to explore patterns of protein profiles using machine-learning techniques. By applying LASSO and random forest, we identified four proteins that separated affected from unaffected individuals: NEFM, AQP4, NPTX2 and VGF (Figure 17A). Every model built with these proteins had an area under the curve (AUC) equal to or above 0.9. The fact that all affected participants in GENFI carry a pathogenic mutation gives a certain strength to study IV, compared to the pilot. Since no sporadic cases were included, the symptoms were highly likely contributed to an underlying diagnosis of FTD rather than another neurodegenerative disease. Even if the participants had different pathologies, they were probably more homogeneous than a cohort of both genetic and sporadic cases (as in the pilot).

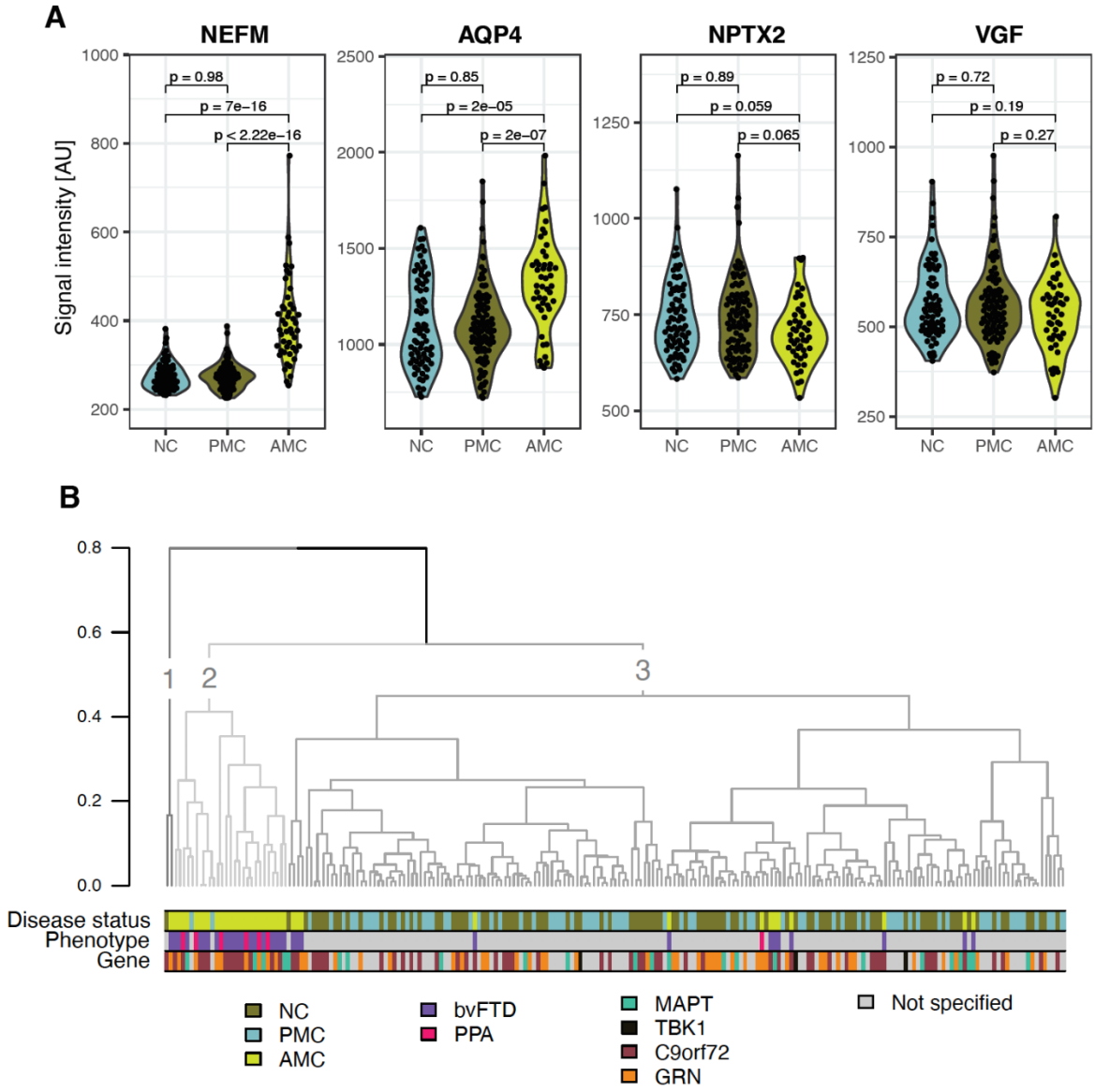


Figure 17. *Four proteins (NEFM, AQP4, NPTX2 and VGF) were selected by both random forest and LASSO when comparing affected and unaffected individuals.* ***A)*** *Violin plots for NEFM, AQP4, NPTX2 and VGF with p-values based on Wilcoxon rank sum tests.* ***B)*** *A hierarchical clustering based on principal component 1 and 2 from PCA. One bar indicates the disease status (AMC, PMC or NC), one bar indicates the clinical phenotype for the AMC, and a third bar that indicates which genetic group each individual belongs to.*

Subsequent analyses by PCA and hierarchical clustering including the four proteins selected by LASSO and random forest, confirmed the initial results and successfully separated affected from unaffected participants (Figure 17B, please compare to Figure 1B in paper IV where the results are annotated only for affected vs unaffected). However, no separation or clustering were observed by genetic group or phenotype. In other words, the protein profile could distinguish patients with FTD from unaffected individuals, but not *C9orf72*, *GRN*, *MAPT* mutation carriers from each other nor bvFTD from PPA.

In this study, we also aimed to assess potential differences between PMC and NC which had not been possible in the pilot study due to the limited sample size. When comparing PMC to

NC, only progranulin was selected by both LASSO and random forest. A subgroup analysis was then performed, comparing PMC with expected present or future TDP-43 pathology (mutation in *C9orf72* or *GRN*) and less than ten years to expected symptom onset (n=35), to age-matched NC (n=34). Six proteins were selected by either one of the models but again, only progranulin was selected by both. Interestingly, TDP-43 (or TARDBP) was selected in almost 86% of the LASSO models. As expected, the prediction accuracy was lower for these models (AUC ranging from 0.73 to 0.82) than for the models optimised for separating affected from unaffected individuals.

## 8.5 STUDY V

Several CSF studies in FTD have identified potential biomarker candidates, consequently raising the question of whether it is possible to find blood-based biomarkers as well. In study V, another proteomic profiling was performed but this time in a large collection of plasma samples (n=735).

Sixteen plasma proteins were found to be elevated in genetic FTD compared to unaffected individuals (PMC and NC) (Figure 18). Among the proteins with the lowest p-value and/or highest fold change were S100 calcium binding protein A12 (S100A12), exportin 5 (XPO5), chromogranin A (CHGA), PSAP, SORT1 and CHIT1 (Figure 19).

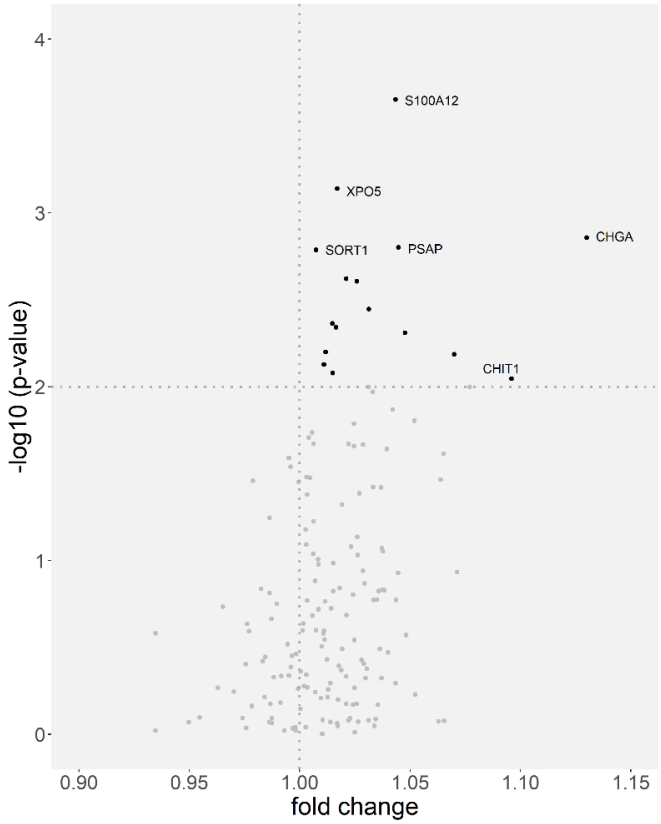


Figure 18. *Volcano plot showing results from comparison between affected (AMC) and unaffected individuals (PMC and NC). Black dots represent p-value<0.01. Dotted horizontal line = p-value 0.01, dotted vertical line = fold change 1.*

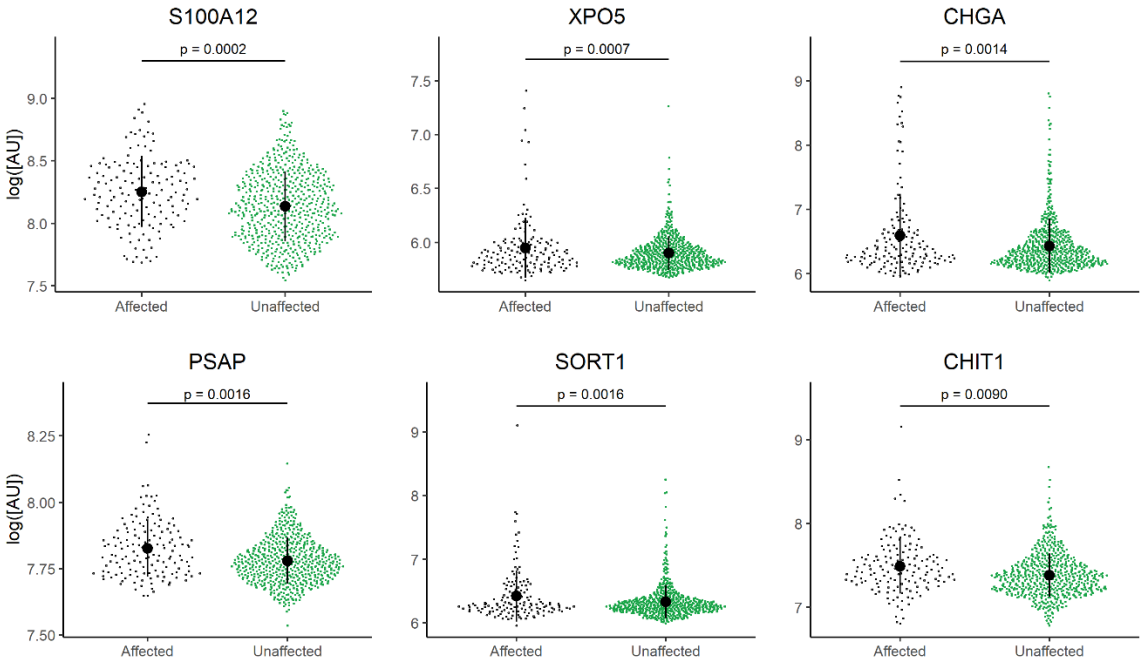


Figure 19. *Plasma levels of the six proteins with the lowest p-value and/or highest fold change when comparing affected (AMC) to unaffected individuals (PMC and NC).*

Next, the plasma protein levels in PMC were compared to the levels in NC (Table 6). Plasma progranulin was statistically significantly lower in PMC than NC which was due to the reduction of functional progranulin in *GRN* mutation carriers. Interestingly, when comparing PMC to NC, we observed a trend of altered levels of NPTX2 and MAPT in PMC highlighting these proteins for further analysis in plasma.

Table 6. *Results from comparison of plasma protein levels between PMC and NC. Proteins with p-value < 0.05 are presented.*

| Protein | Description | Antibody | p-value | Fold change |
|---|---|---|---|---|
| GRN[a] | progranulin | HPA028747 | 0.0001 | 0.9560 |
| NPTX2 | neuronal pentraxin 2 | HPA049799 | 0.0123 | 1.1198 |
| MAPT | microtubule associated protein tau | HPA069570 | 0.0425 | 0.9779 |

[a] a second antibody targeting GRN (AF2420) also showed lower levels in PMC compared to NC (p = 0.012). Correlation between the two GRN antibodies were 0.6 (p = 2.2e-16).

Some of the participants in study V were included in study IV (n=221) and CSF data was thus available from these individuals. Hence, in this subset of individuals, a correlation analysis was done comparing the plasma levels to the corresponding levels in CSF. In general, the correlations were weak, and only two proteins (zinc-alpha-2-glycoprotein, AZGP1, and VGF) had a statistically significant correlation between plasma and CSF.

# 9 DISCUSSION

The overall objective of this thesis was to study genetic frontotemporal dementia to identify biomarker candidates for different purposes. In the following discussion, important aspects regarding the studies will be included.

## 9.1 THE HETEROGENEITY IN FTD

Many of the challenges in research and clinical practice are related to the diversity in terms of phenotypic, genetic, and neuropathological heterogeneity in FTD. Currently, the diagnosis of FTD is based on consensus criteria including an evaluation of the symptoms (3). To reach a definitive FTD diagnosis, a confirmation of a genetic cause or post-mortem neuropathological findings of FTLD is needed. It has become obvious that the clinical presentation of FTD overlap with other diseases, both neurodegenerative and primary psychiatric disorders. Consequently, there is a risk that patients are misdiagnosed and therefore misinformed, stigmatised, and not properly taken care of. In addition, many of the symptoms are self- or next-of-kin-reported and clinicians naturally rely on observations from caregivers when evaluating them. The disease complexity and lack of objective symptom measurements specific for FTD underline the urge for multimodal diagnostic biomarkers.

In study I, we showed that members of families with genetic FTD present with variable phenotypes, from bvFTD to atypical parkinsonism. The phenotypic diversity might be due to the broad spectrum of symptoms in FTD or attributed to misdiagnoses meaning that symptoms are not recognised as FTD, and the patient is instead diagnosed with for example AD. Incorrect diagnoses are a problem in all generations but are probably more likely to occur in individuals diagnosed before the clinical consensus criteria of FTD were specified. We also showed that the likelihood of finding a pathogenic mutation was highest in families with an autosomal dominant inheritance pattern. However, we observed that even in individuals with apparent sporadic disease, 20% had a mutation (2 *C9orf72* and 1 *GRN*). The lack of a positive family history despite the presence of a mutation implicates the influence of other (genetic) modifiers

and reduced penetrance. Although the numbers are small in study I, these findings support a strategy of liberal genetic screening in FTD, regardless of family history.

## 9.2 BIOMARKERS OF SYMPTOM ONSET

In the fluid biomarker studies (studies III, IV and V), the focus was primarily on comparing affected versus unaffected individuals. Proteins with altered levels between individuals with and without clinical symptoms of FTD could potentially be valuable as diagnostic markers. One such protein previously identified is NfL. NfL in combination with the AD core biomarkers perform quite well in the differential diagnostics, i.e. a patient with suspected FTD could be distinguished from AD as they have normal levels of $A\beta_{42}$ and tau in CSF, and not mistaken for primary psychiatric disorder as they have high levels of CSF NfL (148). Nevertheless, since increased levels of NfL is not specific for FTD, there is a strong need for other biomarkers of symptom onset, both to aid in the clinical diagnosis but also to get insights into the pathological processes of conversion.

In study III, patients with FTD could successfully be separated from unaffected individuals based on the CSF protein profile and differences in protein profiles were greatest in this comparison (26 altered proteins). However, no statistically significant differences in protein levels were found within the unaffected group (PMC vs NC). This could be because the protein levels are indeed similar in PMC and NC, or due to the small cohort of presymptomatic subjects and controls (16 PMC and 8 NC). However, differences were found between bvFTD and NC (15 altered proteins) regardless of the small sample size in the NC group. Despite the limitation of sample size in the initial analysis, a strength in study III is the validation in a second cohort consisting of patients with FTD but also another neurodegenerative disease, namely AD. Here, the results could be replicated for a majority of the proteins.

It should be emphasised that there is an overlap in significant proteins between the two CSF studies (studies III and IV) namely NEFM, VGF and NPTXs. Although study IV is not a direct validation of study III, and the two studies use different statistical methods, the overlapping findings give further support to these proteins as attractive candidates for future investigation.

## 9.3 PRESYMPTOMATIC BIOMARKERS IN FTD

According to the established biomarker hypothesis in neurodegenerative diseases that pathological processes precede apparent clinical symptoms, we would assume that there are variables specific for FTD that are measurable prior to symptom onset (122). Indeed, finding a pathogenic mutation would tell us that this individual eventually will develop FTD, but it would not tell us when. Currently, there is no established definition of the early phases of FTD including a well-defined description of prodromal FTD, for example with subtle behavioural impairment (corresponding to cases of mild cognitive impairment progressing to AD). It is not clear when the actual time of conversion to FTD occurs, and if a period of "no disease" even exists in genetic FTD. A mutation carrier is born with the genetic variant and *GRN* carriers presumably have reduced progranulin levels from conception. How is this affecting the

mutation carriers up until the point of expression of clinical symptoms? Ideally, we want a biomarker allowing for estimation of risk of symptom onset within five, ten or twenty years (prognostic biomarker).

In study IV, differences in CSF protein levels between PMC and NC were explored. The only protein selected by both the statistical methods LASSO and random forest was progranulin. However, five additional proteins could potentially be of interest for further investigation, most importantly TDP-43. Since the identification of TDP-43 immunoreactive inclusions in FTD post-mortem brain tissue, the TDP-43 protein has been extensively investigated as a potential fluid biomarker in FTD. However, the results from CSF studies measuring different forms of TDP-43 are conflicting, and it seems especially difficult to establish accurate concentrations of pathological (phosphorylated) TDP-43 (188). Currently, TDP-43 in CSF does not reflect disease activity and is not useful to predict underlying TDP-43 proteinopathy (189). Our result from the LASSO model that TDP-43 is selected as one of the proteins contributing to the separation between PMC and NC is highly interesting since the analysis was done in "pathology filtered" PMC (section 8.4). In other words, only PMC with expected present or future TDP-43 proteinopathy were included, underlining the potential relevance of the finding. Forthcoming studies in larger cohorts will hopefully elucidate the intriguing value of measuring TDP-43 in CSF.

As per the longitudinal design of GENFI, one potential confounder in repeated cognitive testing is the presence of practice effects. A lack of practice effect has been suggested as a marker of early cognitive decline in studies of other neurological disorders which prompted the investigation of practice effects in GENFI (study II). We found that presymptomatic *C9orf72* expansion carriers had lower practice effect than other mutation carriers and non-carriers. This was most apparent when the PMC-C9 approached their expected symptom onset. We speculate that reduced practice effects might indicate that a person is approaching conversion to a symptomatic stage (proximity biomarker). More importantly, potential practice effects must be considered when evaluating cognitive outcomes in clinical trials. To our knowledge, study II is the first report of practice effects in FTD, and their full clinical implication are yet to be discovered.

## 9.4 BIOMARKER TRAJECTORIES IN FTD

Apart from progranulin and *C9orf72* dipeptide repeats, there are currently no biomarkers reflecting specific pathological processes in FTD. Neurofilaments are markers of axonal damage and as such associated to neurodegeneration in general and not only FTD. Likewise, synaptic proteins and markers of neuroinflammation are most likely portraying downstream effects of disease pathology and do not characterise FTD specific mechanisms. For that reason, the elevated levels of plasma PSAP and SORT1 in AMC found in study V are particularly interesting. Both PSAP and SORT1 are closely connected to progranulin processing and lysosomal function. However, these proteins were not found to be elevated in PMC and, similar to previous biomarker studies in FTD, most proteins identified as potential biomarkers in this

thesis were abnormal only in symptomatic cases. Since progranulin and DPR levels appear to stay unchanged throughout life in mutation carriers, there is still no fluid biomarker in FTD reflecting the onset of pathological process in the brain, equivalent to CSF $A\beta_{42}$ and tau in AD. We assume that the trajectories of protein biomarkers are linear or have a sigmoid shape (as for NfL). Yet, other protein patterns may occur over time such as a biphasic course, meaning that a protein can have a high peak and then subsequently be decreased, or even a multiphasic course including several peaks (Figure 8). If this is the case for at least some relevant proteins in FTD remains to be elucidated but it would complicate protein analysis in cross-sectional data. Large longitudinal datasets are essential to investigate the temporal changes in biomarker trajectories in FTD.

## 9.5 FROM EXPLORATIVE ANALYSIS TO VALIDATION

The suspension bead array technique offers the possibility to analyse multiple proteins in a large number of samples simultaneously (182). Furthermore, only a small volume of biofluid is required for each assay, making it an attractive choice considering the limited availability of CSF samples. However, using this targeted approach, we are limited by the selection and performance of the antibodies, including for example off-target binding and matrix effects. We chose this high-throughput analysis as an initial screening method, and replication of the findings is essential. This thesis contributes to the support of some important proteins in FTD. Whether these potential biomarkers will prove to be useful in a clinical setting, and if these results in genetic FTD can be translated also to sporadic FTD, remains to be investigated.

# 10 FUTURE PERSPECTIVES

The ultimate goal in FTD research is to reduce the suffering for people with FTD and their next of kin. This goal will hopefully be achieved when there is an effective treatment for FTD. Simultaneously with the pursuit of finding therapeutic targets and conducting clinical trials, a lot of effort can be put into supporting patients and families to help them cope with the disease. One such project is the development of national clinical guidelines by the Swedish FTD Initiative in 2019 (190). These guidelines were formulated to increase the awareness of FTD among health care professionals and to ensure that patients with FTD in Sweden receive equal and appropriate care and information. For example, the guidelines recommend that all patients with FTD should be offered genetic testing in a clinical setting and emphasise the value of a post-mortem diagnosis.

Apart from GENFI, several multicentre collaborations have been established over the last years such as the FPI and ALLFTD (191). These ongoing initiatives contribute to the understanding of FTD and help facilitate clinical trials. In addition, other collaborations are focused on finding novel genetic variants associated with FTD. There is also an increasing interest to apply machine-learning techniques and digital assessments to enable scientific efforts. Analysing research data with artificial intelligence, far beyond human capabilities, could potentially improve for example prediction and staging of FTD. Staging is a significant part of the diagnostic work-up of other diseases such as cancer, and useful to estimate the prognosis and select optimal treatment. If different treatment opportunities would be available in FTD, each with certain risks and side effects, the disease stage will be very important for the decision of treatment regime. In the coming years we will hopefully see some great discoveries in the field of FTD research

# 11 ACKNOWLEDGEMENTS

För det första vill jag börja med att tacka alla forskningsdeltagare som medverkat i dessa studier. Denna forskning hade inte varit möjlig utan ert generösa bidrag av både dyrbar tid och donation av provmaterial. Jag skulle särskilt vilja tacka **GENFIs eldsjälar** som även under svåra tider har investerat så mycket energi i projektet. Ni är en sann inspirationskälla för hela forskningsfältet och närhelst jag behöver lite uppmuntran så tänker jag på era familjer. Jag uppskattar er tilltro till vår förmåga och hoppas innerligt att vi framöver kommer att kunna erbjuda en botande behandling till alla drabbade.

Jag vill också rikta ett speciellt tack till:

**Caroline Graff**, min huvudhandledare. Tack för din handlingskraft och enorma kapacitet. Du har lärt mig att balansera misstänksamhet och tillit, framfusighet och taktkänsla, liksom värdet av att stå upp för mig själv. Tack för att du pushar mig att bli självständig men ändå finns där så fort jag behöver. Tack för att du är sann mot dig själv och för att du alltid säger vad du tycker. Du formligen strålar av entusiasm för din forskning och jag hoppas innerligt att ingenting någonsin ska kunna släcka din låga. Fortsätt kämpa för det du tror på och låt inte *någon* slå ned dig på vägen. You are a true fighter!

Min bihandledare **Vesna Jelic** för all klinisk guidning. Du har sannerligen hjärtat på rätt ställe. Jag beundrar den hängivenhet som du visar gentemot dina patienter. Någon gång får du inviga mig i hemligheten om hur du trollar med tider. Tack för din underbara humor och visad uppskattning.

Min bihandledare **Christin Andersson** för att du delat GENFI-resan med mig. Du var min klippa när jag kom in som ett blåbär. Tack för att jag fick luta mig mot dig tills jag var redo att stå på egna ben. Tack för ditt lugn och alla kloka reflektioner. Som jag saknar dig på ronderna!

Team translational genetics. **Abbe**, tack för att du funnits vid min sida, för att du uppmuntrat och utmanat mig. Jag kunde aldrig drömma om att få en så fantastisk kollega som du! Tack för

att du introducerade R i mitt liv, återuppväckte mitt forna matematikintresse och projicerade ditt hat mot Excel på mig. Tack för att alla våra möten haft en doft av kaffe och snus. **Charlotte** och **Emma**, tack för allt stöd och för möjligheten att tillsammans dela hela doktorand-upplevelsen. Tack för att ni varit mina bollplank, i stort och smått! **Catharina**, jag känner sådan tacksamhet för att du har blivit en del av GENFI-teamet. Det är tryggt att ha dig i båten och jag är övertygad om att du kan ta rodret och se till att vi inte styr ur kurs. **Behzad**, tack för ditt tålamod med att förklara en massa grundläggande cellulära mekanismer för mig. Jag önskar att jag hade haft mer tid att spela brädspel tillsammans med dig och Abbe, men allting har sin tid och jag hoppas att det kommer fler tillfällen framöver. **Jessica**, tack för att du sprider sådan trygghet och harmoni. **Helena**, tack för din positivitet och jag hoppas att vi får tillfälle att samarbeta mer inom SweFTDi i framtiden. **José** och **Kali**, thank you for your support. Tack **Eva Kallstenius** för allt stöd genom åren och för att du alltid ställer upp. Tack till all personal på Kognitiva mottagningen och speciellt Mottagningen för Ärftliga demenssjukdomar: **Nathalie**, **Maria** och **Ainoa**. Och **Mikaela** förstås, jag har väl inte riktigt släppt taget om dig ännu.

Swedish FTD Initiative och Schörlings stiftelse för generöst ekonomiskt bidrag och för att ha initierat detta samarbete. Tack **Peter**, du har blivit som en extra mentor för mig. This has been a *fantastic* journey! Tack **Anna**, **Jennie**, and **August**, för givande diskussioner och många minnesvärda middagar. **Julia** och **Sofia**, ni representerar liksom början och slutet av denna expedition. **Julia**, tack för långa, fina samtal och promenader. Tack för att du funnits där som vapensyster och konstnärlig inspiration. Din vänskap betyder mycket för mig. **Sofia**, tack för alla dina förlösande skratt. Slå vakt om din envishet och ditt självförtroende, du kommer att gå långt! Och tack till alla medarbetare i SweFTDi för ert bidrag till konsortiet: **Lars-Olof**, **Stefan**, **Olof**, **John**, **Hanna**, **Linnea**, **Ove, Maria LW**, **Alexander S**.

**Jonathan Rohrer (and the GENFI team)** for creating a beautiful collaboration at the forefront of FTD research. You breathe GENFI and that passion is indeed contagious. You are my greatest role model and I admire basically everything about you. You have a supernatural ability (or is it just a rare British gene?) to make everyone feel noticed and comfortable. I believe that your talent as a conciliator, in combination with your broad knowledge in the field, is the reason behind the success of GENFI.

**Team TG oldies** och särskilt **Håkan** som tålmodigt, om än tämligen motvilligt emellanåt, lärt mig om sekvensering och bedömning av genetiska variationer. Det är nog tack vare dig som jag finner tanken på ett bullrande fryshotell förvånansvärt trivsamt. Tack för att du har distraherat mig från jobb när det har behövts (och när det inte behövts). **Marie**, som la grunden för GENFI Stockholm och som visade på möjligheten att följa sin själ och ande. Du är min absoluta favorit crazy-cat-lady. **Anne** och **Hsin-hsin**, tack för att ni åkte med och ledsagade mig på mina första vetenskapliga konferenser. **Jenny**, tack för att du presenterade mig för Carro och för att du släppte in mig i din jobbvärld. Tack **Lotta**, **Lena**, **Anna-Karin**, **Steinunn**, **Annika**, **Antonio** och **Lina** för alla trevliga luncher och för den fina stämning ni skapade i

gruppen. När jag tänker tillbaka på tiden i Novum är det med värme och skratt tack vare alla er oldies. Jag minns framförallt upptåg såsom författandet av den galna stafettboken, Mästerkocks-turneringarna och Håkans flytande-kväve-glass.

Övriga kollegor i både gamla och nya samarbeten. **Una Smailovic**, thank you for being so perfectly professional and for being my PhD student inspiration. Thank you for generously welcoming me into the EEG-project. **Fredrik Sand och Per Östberg**, tack för att ni introducerat mig till ett nytt forskningsfält. Nu håller vi tummarna att det ger några spännande resultat! **Inger Nennesmo**, tack för gott samarbete och särskilt tack till att du med hjälp av kulturella inslag förgyllde min vistelse i Sydney. Med hopp om fruktbart kommande samarbete, tack till **Henrik Viklund** och **Christian Carlström**.

Tack till alla mina vänner och till hela storfamiljen som uppmuntrat och stöttat mig på vägen. **Mamma** och **pappa**, vad vore jag utan er? Tack för er gränslösa kärlek. Tack för att ni alltid trott att jag kan bli vad jag vill, för att ni ställer höga krav men ändå älskar mig förbehållslöst. **Sara**, tack för att du är min trygga hamn. Tack för att du ser upp till mig och för att du utmanar mina svårigheter. Tack för att du har fört **Linus** och **Tove** in i mitt liv.

Till **Malte**, **Ester** och **Svea**, mitt allt och meningen med min existens. Tack för att ni påminner mig om vad som är viktigt i livet och håller mig nere på jorden. Tack för era skratt, skrik, kramar och sparkar. Ni vet sannerligen hur man får mig att le. Jag älskar er!

Och slutligen, kära **Pierre**, mitt livs kärlek och allra bästa vän. Tack för ditt genuina intresse och aldrig sinande support. Tack för att du har så många känslor och för att du är en helt fantastisk manlig förebild. Tack för att du plockar fram mina bästa sidor och för förmånen att få skapa en familj med dig, en familj som jag längtar hem till varenda dag. Tack vare dig ser jag på framtiden med tillförsikt för vi möter den tillsammans, sida vid sida. Och kanske, kanske kan vi hoppas att mina sömngångande eskapader äntligen upphör nu en gång för alla?

"*You're the meaning in my life,*
*you're the inspiration.*
*You bring feeling to my life,*
*you're the inspiration*".

*Chicago*

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